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## SURVEILLANCE OF COLISTIN RESISTANCE PREVALENCE OF NOSOCOMIAL ORIGIN IN PERU

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Studying colistin resistance in nosocomial settings is crucial due to the growing threat of multidrug-resistant (MDR) Gram-negative bacterial infections. Low- to middle-income countries (LMIC) are particularly vulnerable to colistin resistance and understanding its epidemiology and risk factors can inform effective public health policies and interventions to combat antimicrobial resistance. A surveillance study conducted in Lima, Peru, collected 3703 bacterial isolates between January 2018 and October 2022. Clinical isolates were refrigerated and transported to the U.S. Naval Medical Research Unit No. 6 for identification and antimicrobial susceptibility testing. Bacterial isolates were identified using the MALDI-TOF Bruker and BD Phoenix automated system and tested for susceptibility using the Kirby-Bauer method and/or Phoenix. Colistin susceptibility testing (CST) was also conducted, and PCR was used to identify the mcr-1 gene associated with colistin resistance. Clinical and Laboratory Standards Institute 2022 guidelines were used for antimicrobial susceptibility testing (AST) interpretation. The most prevalent microorganisms subjected to CST were: Klebsiella pneumoniae (n=502), Pseudomonas aeruginosa (n=484), and Acinetobacter baumannii (n=203). AST revealed that over 85% (n=427) of K. pneumoniae, 83% (n=402) of P. aeruginosa and 96% (n=195) of A. baumannii were resistant to at least 2 different antibiotic families. CST analysis showed that 10% of K. pneumoniae were resistant to colistin, and 10% of those carried the mcr-1 gene. In addition, 5% (n=10) of A. baumannii and 3% (n=16) of P. aeruginosa isolates were resistant to colistin, and, interestingly, none carried the mcr-1 gene. Our findings demonstrate high levels of MDR among isolates, with observed resistance to colistin varying from 3 to 10%. Continuous surveillance of antimicrobial resistance to multiple classes of antibiotics is paramount in the nosocomial setting, with heightened importance in regions where antimicrobial treatment options are limited and colistin is an antibiotic of last resort.

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## THE LEPTOSPIRAL PROTEIN LIC12254 INTERACTS TO INTEGRINS VIA THE RGD MOTIF

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Leptospirosis is a zoonosis globally disseminated caused by pathogenic spirochetes of the genus Leptospira. Understanding leptospiral pathogenic mechanisms is critical for the efficient development of vaccines and diagnostic tests. Outer membrane proteins are considered potential antigens could mediate interactions with host components, such as integrins, that are involved in cell regulation, proliferation, adhesion and immune response. The leptospiral RGD protein encoded by gene lic12254 was selected for study. In silico analysis was investigated by LipoP, Interprot and CELLO web servers. Protein conservation analysis in different strains of Leptospira was performed by BLASTp and a 3D model was assessed by I-TASSER and analyzed by PyMOL. The recombinant proteins LIC12254 and LIC12254ΔRAA (RGD mutated) were cloned in pAE vector and expressed in E. coli strains. The evaluation of interaction of LIC12254 protein via RGD motif to human integrins was evaluated by ELISA. In silico analysis showed that protein LIC12254 is probably located on the outer membrane and has two conserved domains, Omp85 and DUF5982. 3D models showed a beta barrel structure and protein conservation analysis identified a high sequence similarity among pathogenic strains. In addition, the RGD is present only in pathogenic species. The motif RGD in LIC12254 protein sequence was replaced by PCR techniques and genes lic12254 and lic12254ΔRAA were cloned successfully into the protein expression pAE vector and recombinant proteins were obtained from insoluble form. Recombinant proteins were observed in the expected size of 54 kDa by SDS-PAGE and confirmed by western blot. The evaluation of interaction of LIC12254 protein to human integrins showed that rLIC12254 was capable to interact with αVβ8 and α8 integrins in a dose-dependent manner. As expected, the interaction with rLIC12254ΔRAA was inhibited. Our results described rLIC12254 outer membrane protein capable of interact to human integrins via RGD motif. Thus, rLIC12254 could be involved in bacterial pathogenesis by adhesion process.

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## CHARACTERIZATION OF ANTIGENIC SITES OF NEISSERIA GONORRHOAE USING HIGH-DENSITY PEPTIDE MICROARRAYS AND PSORALEN-INACTIVATED, WHOLE-CELL VACCINE IN MICE

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Neisseria gonorrhoeae infections are an urgent public health threat with over 100 million cases reported globally each year. The threat of N. gonorrhoaea, a sexually transmitted infection, has been further exasperated by the increase in drug resistant strains including against last line antibiotics necessitating the need for an effective vaccine. Vaccine development has been challenging due to a lack of clearly identified antigenic targets, a high degree of strain variability, and immune evasive mechanisms of the bacteria itself. Recent retrospective studies have indicated that vaccination against N. meningitidis, a closely related bacteria also of the Neisseria genus, using the protein-based meningococcal group B vaccine (4CMenB) may provide modest effectiveness of 23-53% against infection with N. gonorrhoaea—though the effect appeared short-lived. Further studies have sought to characterize cross-reactivity of different antigenic sites which may explain this protection. To assess different vaccine approaches to both known and unknown cross-reactive antigens we vaccinated mice with two different vaccines both with and without the mucosal adjuvant, dmLT. In the first case we utilized whole-cell, formalin inactivated N. gonorrhoaea (FA1090). In the second case Psoralen plus ultraviolet A light (PUVA) was used to create killed but metabolically active (KMBA) whole-cell vaccine. Antibody profiles were screened from four mouse serum pools using high-density peptide microarrays using proteins covering pre-selected membrane and intracellular proteins, where higher intensities indicated higher reactivity. We observed reactivity in all vaccines against 15 previously published antigenic sites in the 4CMenB vaccine. Further, we see reactivity equal to or greater than the suspected cross-reactive proteins against additional sites including other intracellular proteins and outer-surface membranes. These findings suggest that a multi-epitope vaccine can be developed using a PUVA-generated KBMA whole cell approach. Additional study is needed in mapping of possible epitopes for N. gonorrhoaea vaccine development.

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## ORAL CHOLERA VACCINATION CAMPAIGN COVERAGE SURVEY IN GARISSA, WAJIR, TANA RIVER, AND NAIROBI COUNTIES, KENYA

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Kenya confirmed cholera outbreaks on October 19, 2022. The outbreak progressed to 17 counties Garissa and Tana River Counties had the highest attack rates of Cholera at 239.1& 231.1/100,000 population. Kenya conducted its first-ever Oral cholera vaccination campaign in February 2023, in Garissa, Wajir, Tana River and Nairobi. The survey assessed Cholera vaccination coverage, Risk communication strategies, knowledge attitude and practices on cholera and water sanitation and

hygiene practices related to cholera. A household-based survey was conducted, targeting persons aged one year and above. WHO formula for calculating immunization coverage was used to calculate the households to be interviewed.Multi-stage proportionate-to-size cluster sampling strategy was used to determine the number of clusters and randomly selected 7 households per cluster. Data was collected using a standard questionnaire. Data was analyzed for continuous and categorical variables. A total of 122 clusters, and 858 households were visited with 5456 eligible participants. Of the 861 households targeted 99.7% (858/861) were interviewed. Coverage at individual level was 93.2% (2640/2834). Majority of the females 58.8%(1553/2640) were vaccinated. Those aged 6-14 years were the most vaccinated 26.9%(709/2640). Of those vaccinated, 7.7%(202/2640) experienced adverse events.A good number of the respondents were aware of the Oral Cholera vaccination campaign 90.2% (774/858) and 98.6% (846/858) said that those sick should seek treatment in Health facilities. Those who did not treat their drinking water were 62.6% (537/858) and 10.7% (92/858) lacked latrines. Water scarcity was also an issue with 51% (438/858) reporting not having water in the last month. The vaccine coverage was within the acceptable range to provide prevention and stop infection transmission. However, there is need to strengthen Water, Sanitation and Hygiene interventions across all the counties in addition to oral cholera vaccine in order to control cholera and reduce deaths.

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## LABORATORY EVALUATION OF THE IS2404 LAMP TEST FOR LABORATORY DIAGNOSIS OF BURULI ULCER DISEASE

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Buruli ulcer, caused by Mycobacterium ulcerans has emerged as an important public health problem in several rural communities in sub-Saharan Africa. Early diagnosis and prompt treatment are important in preventing disfiguring complications associated with the late stages of the disease progression. Presently there is no simple and rapid test that is appropriate for early diagnosis and use in low-resource settings where M. ulcerans is most prevalent. The study aimed to evaluate the use of crude DNA extract and the IS2404 LAMP test for rapid diagnosis of Buruli ulcer. We evaluated the LAMP method for detecting M. ulcerans in clinical specimens by investigating its performance with IS2404 PCR, a reference assay for detecting M. ulcerans. The effect of using crude and purified DNA extracts on the performance of the IS2404 LAMP test was also investigated. Seventy-five clinical samples from suspected BU cases were examined by LAMP and IS2404 PCR. A total of 49 positive samples were concordantly detected by both the LAMP and PCR tests. Additional two positive samples were detected by IS2404 PCR for which the IS2404 LAMP tests were negative. Also, both methods concordantly scored 24 samples as negative for Buruli ulcer while the LAMP assay discordantly scored two additional negative results. Taking the PCR results as a reference, the sensitivity and specificity of the LAMP test were 96.1% and 100% respectively. The lower detection limit of both the IS2404 LAMP and IS2404 PCR tests was 30 copies of IS2404. Nine of 30 samples were positive by both the IS2404 LAMP and IS2404 PCR when crude extracts of clinical specimens were used. Thus, the diagnostic sensitivity of the IS2404 LAMP test decreased with the use of crude DNA extract. In conclusion, the IS2404 LAMP test performed on purified DNA extracts d can be used as a simple and rapid test for the diagnosis of Buruli ulcer.

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## PHENOTYPIC CARBAPENEMASE DETECTION ADAPTED FOR RESOURCE CONSTRAINED SETTINGS

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WHO has estimated that by 2050 as many as 10 million people will die annually due to antimicrobial resistance. Carbapenems are broad-spectrum antibiotics often used as a last line of defense for resistant gram-negative bacterial infections. Resistance to carbapenems from transmissible carbapenemase enzymes is on the rise, though the scale of this issue in many locations remains unclear due to high costs and complexity of current testing methods. We modified the Carba NP phenotypic assay to quickly and economically differentiate the three classes of carbapenemases. Varied antibiotic concentrations, indicator solutions, and reaction volumes were evaluated in an iterative manner to adapt existing 'Carba NP test II' chemistry to a low-volume, plate-based format to objectively measure absorbance on various plate readers. Reaction speed and capacity to differentiate KPC, NDM, and OXA-48 enzymes (class A, B and D enzymes, respectively) were evaluated to optimize detection at ambient temperature, without dedicated incubation at 37°C. Changes in absorption at 560nm in the phenol-red-imipenem indicator solution were consistently observed before visible color change. Compared to the Carba NP version II, reliable results were obtained at ambient temperature with 3mg/mL of imipenem (vs. 6 mg/mL), 2 µL of bacteria (vs. 20µL), reaction volumes of 40µL (vs. 130 µL), and in 15 minutes (vs 2 hours). Three patients at Emory University Hospital with carbapenem-resistant gram-negative bacterial infections had positive cultures tested using the modified assay. All patients were found to have Class A (e.g., KPC) carbapenemase-producing bacteria within 15 minutes. In one patient, the emergence of a carbapenemase-producing bacteria while on systemic antibiotic therapy was observed. The modified Carba NP developed in this study is a rapid and economical method to screen for carbapenemases and is adaptable for use in resource constrained areas to inform clinical care and mitigate transmission in healthcare settings. Furthermore, the platform could be adapted to detect resistance to other key antibiotics, such as ceftriaxone resistant Salmonella typhi.

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## MOLECULAR CHARACTERIZATION OF EXTENDED SPECTRUM BETA-LACTAMASES (ESBLS) -PRODUCING KLEBSIELLA PNEUMONIAE AND ESCHERICHIA COLI ISOLATES FROM THE WESTERN REGIONAL HOSPITAL IN GHANA

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Extended spectrum beta-lactamases (ESBLs) are plasmid-mediated enzymes that hydrolyze beta-lactams except carbapenems and cephamycins but are inhibited by beta-lactamase inhibitors. Majority of ESBL plasmids also contain genes that confer resistance to a variety of non-beta-lactam antimicrobials. As a result, ESBL-producing isolates limit therapeutic options, contribute to treatment failure, increase morbidity and mortality, prolong hospitalization, and raise healthcare costs. This study determined antimicrobial resistance profiles and the presence of antibiotic resistance genes in K. pneumoniae and E. coli isolates from the Western Regional Hospital in Ghana. A total of 120 archived isolates recovered from blood, urine, wound, pleural aspirate, high vaginal, ear, and urethral swabs were used in the study. The Kirby Bauer agar disc diffusion method was used to determine the antimicrobial susceptibility profiles of the isolates. Multidrug-resistant strains were phenotypically screened for ESBL production by the double disc synergy test. All isolates with ESBL phenotypes were screened for blaTEM, blaSHV, and blaCTX-M genes by PCR. Very high resistance was observed for ampicillin (78.4%), cefazolin (59.3%), SXT (56.5%) and tetracycline (56.3%). Fairly high resistance was observed for nalidixic acid (46.4%), chloramphenicol (45.4%), cefpodixime (43.2%) and Cefuroxime (42.4%). Very low resistance was observed for imipenem (2.4%), doripenem (4.1%) and cefepime (4.2%) based on the CLSI inhibition zone size interpretation criteria (CLSI, 2012). Forty-two (34.7%) of the 120 isolates phenotypically expressed ESBLs. Of these, 33 (27.9%) were pure ESBLs and 8 (6.8%) were putative ESBL producers. Overall, 62.7% (n=69/110) of the ESBL genotypes expressed CTX-M types 1, 2 and 9 genes and followed by 60% (n=24/40) harbored in TEM (n=7), SHV (n=5), and OXA-1 (n=12) genes. The study showed a relatively high

level of ESBL-producing isolates at the Regional Hospital, largely the CTX-M type and underscores the need to routinely detect ESBL phenotypes and implement appropriate antimicrobial stewardship policies in health facilities.

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## IN SITU GROWTH OF ZIF-67 ON HALLOYSITE NANOTUBES EMBEDDED IN CHITOSAN HYDROGEL FOR THERAPEUTICS IN PARASITIC INFECTIONS

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Reconstruction for major bone defects caused by trauma, tumors and other diseases is a persistent clinical challenge. A successful bone treatment must take into account a number of factors, including the stability of the materials, stable microenvironments, an ample blood supply, and sufficient osteogenic activity. Finding biomaterials is thus required to assist the surgery to be completed. As a potential tactic, hydrogels could offer an internal environment with adequate moisture, comparable to natural extracellular matrices (ECMs). In this study, we synthesize a novel kind of nanocomposite hydrogel film by growing ZIF-67 nanoparticles in-situ on halloysite nanotubes (HNTs), which were then combined with chitosan matrix for biomedical application. Then, FT-IR, UV-DRS, XRD, and HRSEM were used to characterize the synthesized materials. Halloysite nanotubes added to hydrogel significantly improved its thermal and mechanical properties. Gram-positive and gram-negative bacteria were used to test the antimicrobial effectiveness of hydrogel, and the results showed that hydrogel film had maintained its strong antibacterial activity. The haemolysis assay demonstrated the haemocompatibility of the fabricated hydrogel film. Based on the research, it was concluded that fabricated hydrogel film could be applied for biomedical application.

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## ADHERENCE AND ACCEPTANCE OF ORAL AMOXICILLIN DISPERSIBLE TABLET FOR THE TREATMENT OF SICK CHILDREN IN KARACHI, PAKISTAN

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World Health Organization's Integrated Management of Childhood Illnesses (IMCI) guidelines suggest the use of amoxicillin for treatment of young infants with signs of Possible Serious Bacterial Infection (PSBI) where referral is not possible and for treatment of pneumonia in young infants and children. The suspension form of amoxicillin has been widely used for years; however, dispersible tablets (DT), a more user-friendly form of amoxicillin has been introduced in recent years. Experience from countries like Pakistan show mixed acceptance of DT formulation however adherence have been found to be better than oral suspension. In a cohort study of 535 caregivers presenting to primary healthcare centers in peri-urban Karachi, Pakistan children aged up to 5 years old were prescribed DT of Amoxicillin. Data was collected on day 8 of treatment with Amoxicillin DT. Frequencies and percentages are reported for responses. Mean age was 25 months (SD 18 months), indications included lower respiratory tract, ear and skin infections etc. At the time of prescription, 509 (98%) of mothers had never used dispersible tablets but 344 (69%) mothers thought that babies younger than 6month can easily be given DT. Post treatment, 383 (78%) mothers preferred Amoxicillin DT over Amoxicillin suspension for the following reasons: dissolves easily 423 (80%), can be stored without refrigerator 217 (41%), no special care needed for storage 310(59%), easy to understand dosage 229 (50%), freshly prepared medication every time 180 (34%), easy to store remaining tablets in blister packs 195 (37%), tastes better than suspension 264 (50%), and easy to carry during travel 151(28%). Very few complained about using DT form. Parents showed good adherence and acceptance of dispersible tablets. DT formulations can be introduced for general use in community settings.

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## GROUP B STREPTOCOCCUS ACUTE SUPPURATIVE PAROTITIS IN A YOUNG INFANT

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Acute suppurative parotitis is a rare condition in young infants aged 0-59 days. It is characterized by swelling of parotid glands, fever, and pain, caused by drainage of pus into the oral cavity from the Stensen duct. Staphylococcus aureus is the most common organism while other gram-positive and gram-negative organisms are also documented. Prevalence is higher in male gender. In this report, we describe the case of a 55-day-old preterm young infant, previously healthy, admitted in the emergency department of The National Institute of Child Health, Karachi, Pakistan with complain of fever, reluctance to feed, and irritability with unremarkable general and oral physical examination. On second day of hospitalization, pre-auricular edema was observed bilaterally close to the angle of the jaw along with signs of inflammation, greater on the right side. Ultrasound findings showed bilateral parotid gland enlargement. Lab investigations showed neutrophilia with left shift and thrombocytosis, raised C reactive protein and positive blood culture for Streptococcus Pyogenes. Empiric antibiotic therapy was initiated with injection Ampicillin and Gentamicin and adjusted with Injection Meropenem when culture reports were available. The infant showed steady improvement of symptom and swelling after 48 hours of treatment and got discharged after 7 days. The repeat ultrasound done on 10th day after diagnosis which was normal, with resolved parotid swelling. This case illustrates the need for consideration of disease among young infants and its likelihood in both genders. It also emphasizes on the early institution of appropriate therapy of acute suppurative parotitis for faster recovery and prevention of complications. Furthermore, there is a need to investigate the causes and treatment of this disease in infants and should be considered as a differential diagnosis within this age group.

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## EPIDEMIOLOGICAL BEHAVIOR OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) AT THE END OF THE COVID 19 PANDEMIC IN A HEALTH CARE CENTER IN MONTERÍA-COLOMBIA

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The epidemiological behavior of methicillin-resistant Staphylococcus aureus (MRSA) has been a major concern in healthcare centers worldwide. With the end of the COVID-19 pandemic, there has been an increased focus on other antibiotic-resistant pathogens, including MRSA.In Montería, Colombia, MRSA has been a constant problem in healthcare centers. With the COVID-19 pandemic, there has been an increase in demand for medical attention, which has increased the risk of MRSA transmission. Additionally, infection control measures, such as physical distancing and quarantine, may have been relaxed in some healthcare centers due to the need to prioritize care for COVID-19 patients. MRSA is a bacterial pathogen that is resistant to multiple antibiotics, making it difficult to treat. Moreover, it is highly contagious and can spread rapidly in healthcare settings if adequate infection control measures are not implemented. Therefore, the epidemiological behavior of MRSA at the end of the Covid 19 pandemic was evaluated in a health care center in Monteria, Colombia, during the period 2020-2022.A retrospective study was carried out, in which the records of bacterial isolates obtained in the different services of the health care center during the years 2020 and 2022 were consolidated. The biochemical identification tests and the definition of the antimicrobial susceptibility profiles for the isolated bacteria were performed with the automated MicroScan® system. An increasing prevalence of MRSA was found in the health care facility by year as follows: 2020 (9%), 2021 (51%) and 2022 (67%). In addition, the majority of MRSA cases were found to be acquired in the health care facility, suggesting that outbreaks were occurring in the health care facility.In conclusion, MRSA continues to be a major problem in health care facilities in Monteria, Colombia, and an increase in

the number of cases has been observed during the COVID-19 pandemic. It is important to implement adequate infection control measures to prevent and control the spread of MRSA in health care facilities.

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## ISOLATION AND MOLECULAR IDENTIFICATION OF A STRAIN OF PSEUDOMONAS AERUGINOSA XDR (EXTENSIVELY DRUG RESISTANT) IN POLYTRAUMA PATIENTS IN MONTERIA, COLOMBIA

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Pseudomonas aeruginosa is a Gram-negative bacterium commonly found in the environment and in hospitals. Although some strains are harmless to humans, others can cause serious infections, especially in patients with compromised immune systems. P. aeruginosa strains that are extremely resistant to multiple antibiotics are called XDR (Extensively Drug Resistant). The aim of this study was to report the isolation and identification of a P. aeruginosa XDR strain in patients with polytrauma attended at a health center in the city of Monteria.Samples were taken from patients with polytrauma attended at a health center in the city of Monteria. The samples were processed in the microbiology laboratory by culture techniques and the P. aeruginosa XDR strain was identified by biochemical and antimicrobial sensitivity tests. Molecular identification of the strain was performed by PCR.A P. aeruginosa XDR strain was identified in four polytrauma patients. The strain showed resistance to multiple antimicrobials, including carbapenemics, making it a high public health risk strain. Molecular identification confirmed the presence of the strain.The report of the isolation and identification of a strain of P. aeruginosa XDR in patients with polytrauma is important from a public health point of view because this bacterium is known for its ability to resist multiple antibiotics, which makes it a threat to the health of the general population, especially for those who are hospitalized or who have weakened immune systems.In this sense, it is essential to take preventive measures to avoid the spread of the bacterium, such as infection control in health centers, training of health personnel in the proper management of infected patients and the implementation of epidemiological surveillance protocols for the early detection of infections. In addition, strategies for the appropriate use of antibiotics should be promoted to avoid the development of bacterial resistance.

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## MYCOBACTERIUM LEPRAE ANTIGEN-SPECIFIC ANTIBODY PROFILING AND CYTOKINE ANALYSES REVEAL UNIQUE SIGNATURES OF LEPROSY AS WELL AS IMMUNE CHANGES WITH SCHISTOSOMA MANSONI CO-INFECTIONS

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The lack of commercially available laboratory tests for conclusive early diagnosis of leprosy has motivated the search for novel methods for accurate diagnosis and characterization of disease states. In addition, helminth infections appear to be associated with leprosy but immune mechanisms underlying these associations are not clear. We have developed a multiplexed 'Ab-omics' platform for deep biophysical characterization (both Fab & Fc ends) of a broad set of antigen-specific Abs including not only their isotype and subclass but also glycosylation and Fc receptor binding. Here, we apply the Ab-omics pipeline to sera from patients (n=35, from Minas Gerais, Brazil), clinically diagnosed with leprosy, using multiple Mycobacterium leprae antigens (Ag85B, GroES, Bacterioferritin, CFP-10, ML2567, PGL-1). Antigen-coated barcoded beads were incubated with serum and probed with various fluorescently labeled isotype and subclass probes, tetramerized Fc receptors and lectins. A feature selection model LASSO was used to identify biomarkers for leprosy. For T-cell mediated immune response, M. leprae stimulated PBMC were analyzed for cytokines and chemokines by flow cytometry. LASSO was able to identify signatures that distinguish leprosy+ and leprosy- samples with an AUC of 0.75. We also found statistically significant stronger FcR3A and FcR2B binding for the ML2567; stronger IgG, IgG2, FcR2A binding for GRoES; and stronger binding for FcR2A, FcR3B, IgG3 for the ML LAM in leprosy (without coinfection) compared to individuals without leprosy. T-cell response analyses revels higher TNF-α and CXCL8 associated with co-infection; IL17 and IFN-γ lower in co-infection. These immune profiles not only distinguish leprosy from non-leprosy but also suggest an impact of Schistosoma mansoni co-infection on both cell-mediated and humoral immunity. In addition, this Ab-omics approach can lead to discovery of biomarkers that complement clinical diagnosis as well as increase our understanding of the role of helminth co-infections in the susceptibility of clinical leprosy.

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## MAPPING CHOLERA HOTSPOTS IN THE ELIMINATION PROCESS IN CAMEROON, 2016-2022

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According to the "Ending Cholera: A Global Roadmap to 2030" initiative of the Global Task Force for Cholera Control (GTFCC) in which Cameroon was enrolled, one of the requirements for identified countries is to have an updated map of their hotspots for prioritization of elimination actions, hence the objective of this study to identify cholera hotspots in Cameroon. We conducted a retrospective study of cholera data from the 10 regions of Cameroon with risk analysis carried out over a period of 06 years from January 2016 to December 2021. A 5-step approach was used. A preliminary mapping based on calculation of the relative burden of disease according to WHO threshold calibration was done. A second mapping used the vulnerability criteria, namely: geographical accessibility, hygiene and sanitation, movement of people, access to drinking water and geographical location. The third prioritization was made by integrating the first two. A final prioritization and mapping were obtained after reviewing the vulnerability criteria with joint national experts. A classification of districts included "low priority (LP)", "medium priority (MP)" or "high priority (HP)". Data were extracted from DHIS2 and MAPE forms; GTFCC guideline and additional EXCEL-developed data tools were used to analyze vulnerability criteria and enable risk classification. QGIS software was used to present Health district (HD) hotspot prioritization. The years 2019 and 2020 recorded the highest number of cases with 1931(35.2%) and 1895(34.5%) respectively. There was a drop in the number of cases in 2017 (from 78 to 22) after which, an exponential increase in cases with a peak in 2019 (1931 cases). Mapping identified 101 (53%) priority HDs, of which 29 (15%) were of high priority, bringing the percentages of at-risk population to 63.3%. The Far North, North, South West, Littoral regions had respectively the highest number of hotspots, i.e. 73(72%). The North and Littoral regions have the largest number of HP HDs, respectively 11(39%) and 6(20.7%). We recommend taking into account the prioritization of hotspots for the elaboration of the cholera elimination strategic plan of Cameroon.

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## DESERT SORES: THE SCOURGE OF THE SAS "ROGUE HEROES" IN NORTH AFRICA, 1941-1943

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"Desert Sores: The Scourge of the SAS 'Rogue Heroes' in North Africa, 1941-1943" Ben Macintyre's 2017 book, Rogue Heroes, has sparked renewed interest in the operations of Special Air Service (SAS) personnel in the North African Campaign. Their unit became famous for swift, highly mobile raids on Italian and German posts along the Mediterranean coast throughout 1942. Their attacks, carried out on twin machine gun-kitted jeep laden with four well-armed men, played a significant role in the defeat of the Axis in North Africa. Desert life, however, took a physical toll on the SAS units. "Desert sores," vividly featured in an episode of the Netflix television series based on Macintyre's historical account, became a particular problem. Unable to bathe with any regularity, many men developed these painful ulcerated lesions. In severe cases, they became physically debilitating. Their precise aetiology, however, remained unclear. Was it a pathogen? If so, was it a bacterium or a parasite of some kind? Perhaps it was virtual absence of personal hygiene and chronic skin irritation the sand caused? What therapy worked best? It was not until the end of WWII that these puzzling questions were satisfactorily solved. This presentation, relying heavily on biomedical sources and historical accounts from WWII, will trace the evolution of the desert sores debate among British troops in North Africa. More specifically, it will examine their impact on SAS operations. The evolution of this debate reveals as much about competing schools of thought in tropical medicine as it does about the ever-expanding field of 20th-century microbiology.

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## MULTIFACETED REALITIES OF SCRUB TYPHUS: A CASE SERIES FROM SOUTHERN INDIA

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Scrub typhus is an acute febrile illness caused by Orientia tsutsugamushi, a gram-negative bacillus commonly occurring in Asia- Pacific region. It is transmitted to humans by the bite of an infected Leptotrombidium mite which then causes vasculitis with endothelial dysfunction resulting in widespread vascular damage. Scrub typhus is a serious disease that can cause a range of complications, including thrombocytopenia, meningitis, acute respiratory distress syndrome, and, rarely, myocarditis, underscoring the importance of early diagnosis and prompt treatment. We examine four cases of scrub typhus and review the literature to emphasize the importance of considering scrub typhus in patients of all age groups from endemic areas presenting with fever, thrombocytopenia, or transaminitis, regardless of typical clinical features. The characteristic lesion at the site of mite feeding, eschar, was present in two cases. Fever and thrombocytopenia or transaminitis were commonly noted. One of the cases involved a middle-aged woman who was diagnosed with typhus-induced myocarditis. ECHO showed global hypokinesia of the left ventricle, grade 3 left ventricular diastolic dysfunction and an ejection fraction of 30%. Post-treatment ejection fraction improved to 64%. In addition, a 23-day-old neonate with poor feeding and seizures was diagnosed with late-onset sepsis with meningitis due to bacteremia, confirmed by a lumbar puncture showing neutrophilic predominance. In all cases, scrub typhus was confirmed with a positive qualitative IgM ELISA. Though quantitative and paired titers are preferred, due to resource limitations, single qualitative titers were performed at the time of hospital admission. This case series demonstrated marked responses to doxycycline. It is important that healthcare providers evaluate the patient's exposure history, along with clinical presentation, to diagnose scrub typhus. Confirmation of the diagnosis is typically done through serological testing.

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## THE UNIVERSAL VITAL ASSESSMENT SCORE PREDICTS MORTALITY IN PATIENTS WITH COVID-19 IN RWANDA

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COVID-19 ranges from asymptomatic infection to the acute respiratory distress syndrome (ARDS). There are few data regarding clinical outcomes from COVD-19 from low income countries (LICs), including Rwanda. Accordingly, we aimed to determine 1) outcomes of patients admitted to hospital with COVID-19 in Rwanda, and 2) the ability of the Universal Vital Assessment (UVA) score to predict mortality. We conducted a retrospective study of patients aged ≥ 18 years hospitalized with laboratory confirmed COVID-19 at the University Teaching Hospital of Butare (CHUB), Rwanda from May-October 2021. For each subject, we calculated the UVA mortality risk score, which has been previously validated in patients hospitalized in sub-Saharan Africa. We used logistic regression to determine predictors of mortality and considered $P<0.05$ to be significant. Of the 150 patients included, 83 (55%) were female and the median (IQR) age was 61 (43-73) years. The median (IQR) of length of stay was 6 (3-10) days. Hypertension was identified in 36 (24%) of 150 and was the most common comorbidity. Respiratory failure occurred in 69 (46%) of 150 including 34 (23%) who developed ARDS. The case fatality rate was 44%. Factors independently associated with mortality included acute kidney injury (aOR 7.99, 95% CI 1.47-43.22, p=0.016), COVID-19 severity status (aOR 3.42, 95% CI 1.06-11.01, p=0.039), and the UVA score (aOR 7.15, 95% CI 1.56-32.79, p=0.011). The UVA score at admission had good discrimination for mortality with an area under the receiver operating characteristic curve of 0.85 (95% CI 0.80-0.92). At a UVA score cut-off of 4, the sensitivity, specificity, positive predictive value, and negative predictive value for mortality were 57.58%, 92.85%,86.36% and 73.58% respectively. Patients admitted to CHUB with COVID-19 had high mortality, which was predicted by the UVA score. Calculation of the UVA score in patients with COVID-19 in LICs may assist clinicians with triage and other management decisions.

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## THE PREVALENCE OF MENSTRUAL DYSFUNCTION FOLLOWING COVID-19 INFECTION IN THAILAND

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There is a lack of information in the literature describing menstrual dysfunction following infection with COVID-19. A recent systematic review found 12 studies evaluating menstrual cycle changes during the pandemic; only 3 specifically assessed the impact of infection. This case series aims to assess the prevalence and nature of menstrual changes following COVID-19 infection in Thailand. Ninety-six participants were recruited to the study. All had received inpatient care for COVID-19 at MedPark Hospital in Bangkok, Thailand. They completed a survey including menstrual history before and after illness. Basic demographic data and COVID-19 test results were collected from electronic patient records. To analyse menstrual disruption, we reviewed a subset consisting of female patients under the age of 50. Of 96 patients, 60 (62.5%) were female, of which 44 (73%) were under 50 years old. Mean age under 50 was 31.8, with a range of 15-47 years. Six (13.6%) reported menstrual dysfunction prior to COVID-19 infection; 38 (86.4%) reported no previous dysfunction. After infection, 11 (25%) reported a change in their menstruation, 6 of whom had

previously regular periods; 25 (56.8%) reported no menstrual dysfunction; and 8 (18.2%) were pregnant. Reported changes include irregular cycles or missed periods (n=5); shorter duration (n=3); heavy bleeding (n=2); and longer duration (n=1). This observational study found that while the majority of participants reported no menstrual disturbance following covid-19 infection, a proportion (11, 25%) reported a change. The nature of menstrual dysfunction was varied, with irregular/missed cycles and shorter cycles most common. Other studies with larger sample sizes have indicated that lighter bleeding and a prolonged cycle are commonly reported. Eight (18.2%) participants reported pregnancy. Although a small sample size, this suggests that short-term fertility was not affected in these women. Larger samples and prospective data collection will be valuable to further explore the impact of COVID-19 infection on menstruation patterns and its implications for patients.

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## PATTERNS AND PREDICTORS OF MORTALITY WITHIN THE FIRST 24 HOURS OF ADMISSION AMONG CHILDREN AGED 1-59 MONTHS AT A REGIONAL REFERRAL HOSPITAL IN SOUTH WESTERN UGANDA

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Most deaths among children under 5 years occur within the first 24 hours of hospital admission from preventable causes such as diarrhea, pneumonia, measles, malaria, and HIV/AIDS. This is worsened by socioeconomic factors such as delays seeking health care, delayed interventions, financial limitations, unavailability of life-saving equipment, and inadequate support services. The demographic and clinical predictors of death within 24 hours of hospital admission are not yet well documented in our setting. This study aimed to describe the patterns and predictors of mortality within the first 24 hours of admission among children aged 1-59 months admitted at Mbarara Regional Referral Hospital. We conducted a prospective cohort study among 208 children aged 1-59 months admitted at the Mbarara Regional Referral Hospital. Participants were consecutively enrolled and pre-hospital, clinical, and laboratory factors that predicted their mortality within 24 hours of in-hospital admission were studied. Patterns of mortality were described using proportions, means and median and statistical analysis of predictors of mortality was done using multivariate regression. The mortality rate within the first 24 hours of admission was 7.7% (16), the median time to death was 7 hours and death was higher among infants. Severe pneumonia, severe acute malnutrition, and malaria accounted for 26.4%, 23.5%, and 11.5%, respectively of deaths. Admission during the night (p-value 0.047, AHR 3.7 (95% CI 1.02-13.53)) and having an abnormal neutrophil count (p-value 0.034, AHR 3.5 (95% CI 1.10-11-31) were predictive of mortality. The most common causes of death within 24 hours of admission are pneumonia and severe acute malnutrition. The mortality rate was higher among infants <12 months (9, 56.2%) than older children (7, 43.8%). Children who are admitted at night or have an abnormal neutrophil count should receive extra monitoring and interventions due to a higher risk of mortality within 24 hours of admission.

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## RECONSTRUCTIVE SURGERY FOR THE NEGLECTED TROPICAL DISEASES (NTDS): GLOBAL GAPS AND FUTURE DIRECTIONS

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Several neglected tropical diseases (NTDs) are highly disfiguring, particularly those in resource-poor countries that lack access to basic surgeries. There has been a push to integrate surgeries into treatment programs for NTDs. In this paper, we provide an overview of the major disfiguring NTDs and discuss the processes and barriers that impede access to reconstructive surgical treatments or their integration into health systems. A review of the literature was conducted using the online database PubMed from 2008 to 2021 with the specific diseases listed as neglected tropical diseases either on the World Health Organization (WHO) or the PLoS Neglected Tropical Disease Web sites. Reference lists of identified articles and reviews were also searched, as were databases from the WHO's Weekly Epidemiological Record. Success in the surgical treatment and post-operative care of disfiguring NTDs would benefit from standardization and harmonization of surgical approaches and procedures. In some settings, reconstructive surgery should be used cautiously, emphasizing appropriate use of antibiotics, partnerships with global and local surgical teams, and local capacity building. Preventative hygiene approaches remain paramount in resource-poor areas. In conclusion, surgery is a promising treatment for NTDs that result in disfigurement and disability. The expansion of local capacity building, with medical trips and surgical training of local health workers, together with the development of universal surgical protocols remain essential cornerstones for NTD reconstructive surgery. Antibiotics and drug management should comprise key first steps before turning to surgery.

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## PATTERN OF OCCURRENCE, CLINICOPATHOLOGICAL PRESENTATION AND MANAGEMENT OF SALIVARY GLAND TUMOURS AMONG PATIENTS ATTENDING MUHIMBILI NATIONAL HOSPITAL, TANZANIA

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Tumours of salivary glands form one of most heterogeneous groups of lesions accounting for 2.8% and 10% of all head and neck tumours. The tumours have overlapping clinical and histological characteristics of benign and malignant salivary gland tumours which results in difficulty in diagnosis which mostly require experienced personnel. Delay in presentation has led to challenging management and poor outcomes. Authors set out to investigate pattern of occurrence, clinicopathological presentation and management of salivary gland tumours in Tanzania. A descriptive prospective hospital-based study was conducted at Muhimbili National Hospital between May 2021 and April 2022. Patient interview was conducted by investigator enquired about the socio-demographics main complaint(s), associated symptoms and examination findings. Tissue biopsies which were taken from all patients for histological investigation for diagnosis confirmation. Surgical and post-operative histology details were recorded. Data entry, cleaning, coding and analysis were done by using the SPSS 23.0 software. Ethical clearance was sought from the Ethical Board of MUHAS. A total of 597 patients with head and neck tumours were seen and among these, 63 patients were diagnosed with salivary gland tumours making prevalence of 10.56%. Majority (68.3%) of patients had salivary gland cancers, and most of patients (73%) first sought health care more than 3 months after initial symptom. Main reason for delay was neglect (41.3%). All benign salivary gland tumours were pleomorphic adenomas while adenoid cystic carcinoma was most common salivary gland cancer (39.5%). Patients with salivary gland cancers presented with ulcerated lesions, bleeding, tender, indurated, fixed to underlying structures, paraesthesia and facial nerve paralysis. Surgery was done on 46.5% of patients and the remaining were treated by palliative chemoradiotherapy. In conclusion, salivary gland cancers presented in late stages associated with patient's poor healthcare-seeking behaviour leading to treatment delays more palliative than curative therapy

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## AETIOLOGY OF ACUTE UNDIFFERENTIATED FEBRILE ILLNESS AT A TERTIARY CARE CENTRE IN EASTERN UTTAR PRADESH, INDIA

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Acute undifferentiated febrile illness (AUFI) a common presenting complaint, can cause significant mortality and morbidity if left undiagnosed. There is a regional variation in the aetiology of AUFI. Moreover, similarity in the symptoms makes an accurate clinical diagnosis difficult without laboratory confirmation. Thus, this study was done to identify the common aetiology of AUFI at a tertiary care centre in Eastern Uttar Pradesh, India. This cross-sectional study was conducted in SSL Hospital, Banaras Hindu University, Uttar Pradesh, India, between May 2021 and May 2022. All adult patients presenting with fever <14 days without any localizing sign were included in this study. ELISA tests were performed on all samples by Leptospira IgM ELISA, Chikungunya IgM ELISA, Scrub Typhus IgM ELISA , DENV IgM Capture ELISA, as per the manufacturer protocol. During the study period, 121 patients admitted with AUFI were included in the study. The most common aetiology found was Dengue comprising 41 (33.88%) of the total AUFI patients whereas 22/41 patients were found positive for dengue alone and rest were having co-infection in which Scrub Typhus was the most common co-infection. Second most common aetiology were Leptospirosis and Scrub Typhus comprising 29 (23.96%) patients each whereas 8/29 patients were positive for Leptospirosis alone and Scrub Typhus alone was positive in 10/29 patients. Remaining 21/29 patients of Leptospirosis had co-infections majorly of scrub typhus. Whereas in the remaining 19/29 patients of Scrub typhus, the most common co-infection was Leptospirosis. The patients found positive for Chikungunya were 9(8.49%) whereas only 6 were positive for Chikungunya only. Remaining 3 patients were having double co-infection of Scrub Typhus along with Leptospirosis. There were 13(10.74%) patients not found positive for any of the aetiology stated above and needed further clinical investigations. The most common cause of AUFI was viral. Availability of cost-effective serological test for dengue, scrub typhus and leptospirosis at primary health care setting would lead to early diagnosis and effective management of AUFI in this region.

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## IMPROVING THE REPEATABILITY OF A QUANTITATIVE G6PD POINT-OF-CARE DIAGNOSTIC THROUGH VARIATION OF TEST PROCEDURES

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymopathy worldwide and the main risk factor for 8-aminoquinoline (8AQ) induced haemolysis. The introduction of novel, short course, 8AQ-based treatment regimens for the radical cure of Plasmodium vivax (P. vivax) presents a good opportunity for more effective P. vivax control, however, widespread use will require stringent G6PD testing at the point-of-care (PoC). Diagnosing heterozygous women with intermediate G6PD activity at the PoC requires a quantitative G6PD measurement. In a controlled laboratory setting, the quantitative PoC diagnostic G6PD Standard (SD Biosensor, ROK) has good repeatability, however, anecdotal evidence suggests that this may not be the case in real-life settings. We assessed whether the repeatability of the G6PD Standard in real-life settings can be improved by varying test procedures. In a pilot study conducted in Australia, G6PD activity was tested in three volunteers using four different test procedures and the standard method; two methods used capillary blood, and three methods used venous EDTA blood. Each volunteer was tested ten times pairwise with each method. The pilot study found that using venous blood with a modified pipetting method (blood is absorbed from the pipette to the test membrane rather than dispensed) improved the repeatability of the G6PD measurements. The median difference between paired readings was 0.3U/gHb (IQR: 0.2-0.4) for the novel method compared to 0.5U/gHb (IQR: 0.2-0.9) for the manufacturer-recommended method. However, subsequent field studies in Indonesia (n=60) and Nepal (n=120) found no significant improvement compared to the standard manufacturer-recommended method. The field study in Nepal is ongoing with more than 60% of participants enrolled to date. We found that the repeatability of the G6PD Standard can be improved by using venous instead of capillary blood. The use of venous blood rather than capillary blood may improve repeatability since the variation in blood collection procedures is reduced, though we could not consistently replicate this improvement in field settings.

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## MISSED OPPORTUNITIES: SCREENING FOR CHAGAS DISEASE AND STRONGYLOIDIASIS IN LIVER AND KIDNEY TRANSPLANT RECIPIENTS BORN IN LATIN AMERICA

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Strongyloides stercoralis and Trypanosoma cruzi are common chronic parasitic infections in U.S. residents born in Latin America. Although immunocompetent individuals are often asymptomatic, patients receiving iatrogenic immunosuppression can develop life-threatening complications from disseminated strongyloidiasis and T. cruzi reactivation. We evaluated pre-transplant screening practices in liver and kidney recipients with epidemiologic risk factors for both parasites. We retrospectively identified all Hispanic/Latinx adult liver or kidney transplant recipients at MedStar Georgetown University Hospital in Washington, DC from January 1,2019 to December 31,2021. We included only patients born in Mexico, Central or South America. We recorded demographics, transplant characteristics, diagnostics, antiparasitics, and whether infectious disease (ID) consultation was performed. Sixty-five Latin-American born patients received transplants, including 8 (12%) from Mexico, 45 (69%) from Central America, and 12 (19%) from South America. Recipients from El Salvador accounted for 63%. Thirteen patients (20%) received Strongyloides screening, of which 2 (15%) tested positive. Liver recipients were more likely to be screened than kidney recipients (p<0.001). Eosinophilia was not associated with Strongyloides screening (p=0.09). Nine patients (14%) received T. cruzi screening, all with normal tests. There was no difference in screening based on the presence of EKG changes (p=0.1). All patients screened for T. cruzi or S. stercoralis had received pre-transplant ID consultation for an unrelated reason, most commonly latent tuberculosis infection. Despite epidemiologic risk factors, the majority of Latin-American born recipients of liver and kidney transplants at our institution did not receive pre-transplant Strongyloides or T. cruzi testing. Improved screening protocols based on geographic exposure history are needed to identify chronic parasitic infections prior to immunosuppression. Routine ID consultation for transplant candidates with prolonged residence abroad could increase screening uptake.

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## RETROSPECTIVE EPIDEMIOLOGICAL STUDY ON THE EFFECTIVENESS OF VISCERAL LEISHMANIASIS TREATMENT PROTOCOLS AND RISK FACTORS FOR RELAPSE IN TIATY EAST AND TIATY WEST SUB-COUNTIES, KENYA

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This project seeks to determine the effectiveness of current treatment protocols for visceral leishmaniasis (VL) in Tiaty East and Tiaty West Sub-counties, Kenya, and identify risk factors for relapse. The 2017 Republic of Kenya Ministry of Health's Guidelines for Prevention, Diagnosis, and Treatment of Visceral Leishmaniasis require patients to undergo follow-up examination six months after completing treatment to determine final cure, relapse, death, or loss to follow-up as well as assess for Post Kala-azar Dermal Leishmaniasis (PKDL). However, due to situational difficulties including lack of transportation, distance to health facilities, poverty, and low health-seeking behavior, follow-up is rarely done leading to a large gap in long-term treatment effectiveness data. Few studies have been done on VL relapse, but the rate has been shown to be between 1.4% and 14.4% globally with much higher rates in Eastern Africa. No study has been done on VL follow-up in Kenya with current treatment protocols. This study was conducted at Chemolingot Sub-county Hospital (CSCH) in Baringo, Kenya during February and March 2023 on patients living in Tiaty East and West that were treated before August 2022 at CSCH and Kimalel Health Centre. Patients were tracked down using contact information from medical records and Community Health Volunteer searches and referrals. Patients received a follow-up examination from a clinician including history taking, physical examination, and hemoglobin level to assess for the above outcomes. Compensation for the cost of travel to the health facility was provided. Preliminary results of 18 patients show all patients as fully cured. Preliminary analysis of relapse cases from medical records at CSCH show a low relapse rate as well, but further conclusions about relapse rate and risk factors cannot be drawn before formal analysis. From preliminary data, current treatment protocols in Kenya appear to be effective. Given the upcoming change to a new drug combination and shorter treatment period, study results should be compared to future treatment protocol follow-up data.

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## IMPLEMENTING LABORATORY QUALITY MANAGEMENT SYSTEMS IN GHANA - A BASELINE QUALITY AUDIT OF ACCREDITATION READINESS IN LOWER-TIER HEALTH FACILITIES

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In the last decade, the implementation of the Stepwise Laboratory Improvement Process Towards Accreditation (SLIPTA) in Africa has targeted laboratory performance to characterize error and improve patient safety but often targets the highest-tier laboratories. The current study implemented a SLIPTA quality audit in a representative sampling of Ghanaian health facilities. An audit using a modified SLIPTA checklist was carried out in health facilities randomly selected within districts stratified by ecological zone, remoteness from zonal Laboratories, and population density. The audit was conducted in regional and district hospitals, polyclinics, and sub-district facilities from February 2021 to July 2022. The Wilcoxon rank sum test and quantile regression were used to characterize the associations between laboratory characteristics and SLIPTA scores. Forty-nine health facilities were audited with the majority in the public health sector (81.6%). Laboratories performed a median of 30 (Interquartile range: 10 – 90) tests daily, with 40.8% of facilities only performing point-of-care testing. The median SLIPTA score was 44.1% (Interquartile range: 24.0% – 56.7%) with hospitals recording the highest (55.9%). Of the 12 SLIPTA quality systems essentials, performance was highest in Organization & Personnel, and poorest in Client Management and Occurrence Management & Process Improvement. In multivariable regression, the median SLIPTA scores reduced for facilities located in the Northern zone [-21.5(95% CI=-31.7- -11.3; p<0.001)], compared to those in the Southern zone and also for facilities that performed only point-of-care testing [-18.2(95% CI=-31.6- -4.7; p<0.01)] compared to moderate to high complexity testing. The majority of the laboratories audited were yet to attain international accreditation readiness. Quality compliance was equivalent to 1 star for hospitals but lower for the other facility tiers. There is a need to conduct a root cause analysis for laboratory quality with commensurate policy changes to enhance performance.

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## ASSESSMENT OF TREATMENT OUTCOMES OF HUMAN IMMUNODEFICIENCY VIRUS POSITIVES TRANSITIONED FROM TENOFOVIR/LAMIVUDINE/EFAVIRENZ TO DOLUTEGRAVIR REGIMEN COMBINATION IN A NIGERIAN TERTIARY HOSPITAL

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Due to the introduction of dolutegravir as a replacement for nevirapine or efavirenz in a fixed dose combination with Tenofovir/Lamivudine, as the preferred first-line option for the prevention and treatment of HIV infection, there is a need to assess the treatment outcomes of the new Tenofovir/Lamivudine/Dolutegravir (TLD) regimen in a Nigerian tertiary hospital. This retrospective study used data drawn from the treatment register of patients who transitioned from Tenofovir/Lamivudine/Efavirence (TLE) to TLD between January 2016 and January 2018. The data extracted were analyzed using SPSS statistical software version 20. Descriptive statistics were used to describe categorical and continuous variables. Chi-square test statistics was done to test for association between categorical variables and treatment outcomes and the level of significance was set at $p<0.05$. A total of 358 cases were reviewed. Their mean age was 44.29 ± 11.5 years and the majority 267 (74.6%) were females. Viral load suppression of ≤1000 copies/ml was achieved in 313 (87.4%) of cases while on TLE but increased to 339 (94.7%) when transitioned to TLD within the period of study. In addition, 36.3% had a high CD4 count while on TLE and this increased to 67.3% of those with high CD4 within the period of study. There was a statistically significant difference between the mean CD4 count while using TLE and then when transitioned to TLD (t=31.601; p-value-0.001). Treatment outcome was greatly improved in terms of virologic, immunologic, and clinical presentation among patients who transitioned from TDF/3TC/EFV to TDF/3TC/DTG in this study. The outcome of this study supports and encourages the use of TDF/3TC/DTG as the preferred first-line regimen in HIV treatment for the patient's maximum clinical benefit. This should be communicated to all stakeholders and policy-makers involved in the provision of effective healthcare service delivery in HIV management.

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## IMPACT OF THE INTRODUCTION OF A PACKAGE OF DIAGNOSTIC TOOLS, DIAGNOSTIC ALGORITHM, AND TRAINING AND COMMUNICATION ON OUTPATIENT ACUTE FEVER CASE MANAGEMENT AT THREE DIVERSE SITES IN UGANDA: RESULTS OF A RANDOMIZED CONTROLLED TRIAL

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Increasing trends of antimicrobial resistance are observed around the world, driven in part by excessive use of antimicrobials. Limited access to diagnostics particularly in low- and middle-income countries contributes to diagnostic uncertainty which may promote unnecessary antibiotic use. We investigated whether introducing a package of diagnostic tools, clinical algorithm, and training and communication messages could safely reduce antibiotic prescribing compared with current standard of care for febrile patients presenting to outpatient clinics in Uganda.This was an open-label, multi-center, two-arm randomized controlled trial conducted at three public health facilities (Aduku, Nagongera, and Kihihi health center IVs), between September 2020 to August 2021, comparing the proportions of antibiotic prescriptions and of clinical outcomes for febrile outpatients aged one year and older. The intervention arm included a package of point-of-care tests, a diagnostic and treatment algorithm, and training and communication messages. Standard-of-care was provided to patients in the control arm. A total of 2400 patients were enrolled with 49.5% in the intervention arm. Overall, there was no statistically significant difference in antibiotic prescriptions between the study arms; relative risk (RR 1.030, 95% CI 0.958-1.108). In the intervention arm, patients with positive malaria test results had a higher RR of being prescribed antibiotics (1.742 [1.517-2.000]) while those with negative malaria results had a lower RR (0.683, [0.625-0.748]). There was no significant difference in clinical outcomes. This study found that a diagnostic intervention for management of febrile outpatients did not achieve the overall desired impact on antibiotic prescribing at three diverse and representative health facility sites in Uganda.

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## PREVALENCE OF CHRONIC KIDNEY DISEASE IN A COHORT OF GUATEMALAN AGRICULTURAL WORKERS 2020-2022: THE AGRICULTURAL WORKERS AND RESPIRATORY ILLNESS IMPACT (AGRI) STUDY

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Chronic kidney disease (CKD) has recently been identified as a major public health concern in Central American agricultural workers and has been linked to physically stressful working conditions and dehydration. We measured estimated glomerular filtration rate (eGFR) annually from 2020-2022 among banana plantation workers in southwest Guatemala enrolled in the longitudinal AGricultural workers and Respiratory Illness impact (AGRI) study. The cohort is primarily comprised of otherwise healthy young workers (mean age 30 (range 18-62) years; 84% male) who are economically vulnerable (58% report food insecurity) and live within a geographically broad (2,600 km2) catchment area in southwest Guatemala. eGFR screening was completed for 1,879 workers in 2020, 1,085 workers in 2021, and 602 workers in 2022. The prevalence of eGFR < 90 ml/min/1.73m2 was 33.0%, 26.6% and 30.9% (2020, 2021, and 2022, respectively) within which the prevalence of eGFR < 60 was 2.3%, 1.9%, and 2.3% (2020, 2021, and 2022, respectively). Of the 1,233 workers that received at least two annual eGFR screenings in this period, 237 (19.2%; mean age 37.0 years, range 18-60 years) were "at risk" for CKD (defined as eGFR < 90 at least twice), and 13 (1.1%; mean age 37.5 years, range 23-59 years) also met the definition for moderate/severe CKD (defined as eGFR < 60 at least twice). Regression models adjusted for sex showed that age was associated with moderate/severe CKD (Relative Risk = 1.07, 95% Confidence interval: 1.01 – 1.12), indicating a 7% increase in risk with each increasing year of age. Comorbidities and modifiable risk factors are being collected and will be examined in expanded regression models. The high prevalence of CKD in this relatively young population suggests the need for regular screening and follow-up in agricultural workers in similar work environments, which can then lead to programs to reduce modifiable risk factors of disease. Ongoing assessments include follow-up testing of those workers with moderate/severe CKD screening and continued annual screening of workers enrolled in the AGRI study.

5577

## IMPACT OF IMPROVED DIAGNOSTIC TOOLS, PRACTICES, TRAINING AND COMMUNICATION ON ACUTE FEVER CASE MANAGEMENT AND ANTIBIOTIC PRESCRIPTIONS FOR PATIENTS PRESENTING AT OUTPATIENT FACILITIES IN UGANDA

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Antibiotic prescribing practices is one of the main causes of antimicrobial resistance. The study explored the key drivers and barriers to adherence to prescribing instructions by patients/caregivers.As part of a randomized trial, a qualitative assessment was done. This involved three arms of the study: pre-intervention, intervention and post-intervention. The intervention involved randomization of patients to either the intervention or control arm. A training and communication package was developed guided by the findings. Health workers were trained on the package for use with the intervention patients. It was pretested for clarity with sample patients. Meetings were held with policy makers to discuss the capacity, opportunity and motivational COM-B/TDF framework and behavior change techniqueThis study was conducted at three Health centres in Uganda; Aduku in Northern region, Kihihi in Western region, and Nagongera in the Eastern region.Focus groups discussions were conducted with patients who sought care from the health facility and in-depth interviews were conducted with health workers based on their role as clinicians and laboratory attendants. Content analysis identified two themes namely key drivers and barriers and the COM-B/TDF behaviour frameworks approach identified four themes.Results: 1) Key drivers included: drug availability, health worker knowledge and communication 2) Barriers included: use of treatment resorts and inability to buy drugs. Findings from the COMB-B/TDF showed that an opportunity like 1) good support network, 2) capability of the health workers with good knowledge, 3) cognition and interpersonal skills, 4) motivation and awareness from the training and communication package increased positive social and environmental factors to tackle poor adherence.

5578

## A SIMPLIFIED CAREGIVER DERIVED DIARRHEA SEVERITY SCORE (14DCODA) FOR USE IN SURVEYS WITH 14-DAY RECALL PERIODS: A VALIDATION STUDY NESTED WITH IN A VIRAL DIARRHEA SURVEILLANCE PROJECT IN AMAZONIAN PERU

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Diarrhea is a frequent syndrome in children in low- and middle-income countries (LMICs). While most diarrheal episodes are mild, some are severe and fatal, and the syndrome remains a leading cause of death in young children in LMICs. Nationally representative household surveys such as those conducted by DHS and MICS estimate diarrhea prevalence by asking caregivers about occurrence of diarrheal episodes in their children in the two-week preceding the interview. However, doubts have been raised about the reliability of illness recall over this period, leading to the exclusion of survey questions about the specific symptoms that might delineated severe from mild episodes. We address these questions by nesting a recall validation study within a larger diarrhea etiology study being carried out in Iquitos, a city in the Peruvian Amazon. Caregivers seeking care for children presenting with diarrheal illness were asked about symptoms for the 7 days prior to enrollment. At home visits 14 days later, they were then asked to recall the same illness features present on the day the child presented for care and on the days prior to presentation. CODA diarrhea severity scores were calculated for baseline ("gold standard") and at 14 day follow up (dCODA) and the latter compared for accuracy using ROC analysis. A dCODA score of 8 had an accuracy of 79.3% in classifying moderate to severe disease from mild disease as defined by the CODA score. Three symptom-specific questions that had a high accuracy included the maximum number of unformed stools in a 24-hour period (79.4%), days spent with fever (80.8%) and days of illness with vomiting (84.1%). We suggest that incorporating these three questions into household surveys may allow differentiation of episodes by severity across diverse contexts. This will be useful to improve estimates of health care coverage and understand the differential impact of disease specific interventions in reducing severe diarrheal disease.

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## HUMAN EXPOSURE TO ONCHOCERCA VOLVOLUS IN HIGH AND LOW RISK ONCHOCERCIASIS TRANSMISSION SETTINGS

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Onchocerciasis caused by Onchocerca volvulus is transmitted by blackflies. Simulium damnosum are primary vectors in Africa. Onchocerciasis is the second leading cause of blindness after Trachoma. Community-directed treatment with ivermectin (CDTI) is the strategy for onchocerciasis control. A recently published modelling study shows that onchocerciasis elimination time depends on connectedness between villages either by human mobility or migrating flies. This study was therefore conducted to generate data to substantiate/improve findings from the study by investigating onchocerciasis infection in mobile individuals at low (5 km from breeding sites) and high risk (15 km from breeding sites) communities frequenting both settlements. The study was conducted in three villages each in onchocerciasis low and high risk areas in the Nkwanta North District of Ghana. A total of 539 participants from low risk (225) and high risk (314) communities had questionnaire administered to them to obtain biodata, movement, CDTI history, etc. Dried blood spots and skin snips were collected for OV 16 testing and microfilariae detection respectively. The overall serological prevalence for the six communities was 29.1%, (high risk: Kone=52.63%, Abunyanya=50.0% and River View=22.86%; low risk: Lancha=14.94%, Badule=17.72% and Gborsike=8.77%). This was significantly higher (P<0.0001) in high-risk (41.50%) than in low risk (14.35%) areas. Skin snip prevalence was (high risk: Kone=5.15%, Abunyanya=6.25% and River View=5.71%; low risk: Lancha=4.5%, and Badule/Gborsike=0.00%). Prevalence in high risk (5.73%) was significantly higher (P<0.0001) than in low risk (0.92%). Overall skin snip prevalence was 2.9%. Negative skin snips are currently being screened for parasites using RT PCR. Questionnaire data shows skin snip positive participants from Lancha spend ample time in high risk communities and their farms where they experience blackfly bites. During onchocerciasis elimination mapping phase, information on human movements (questionnaire) and diagnostics with high sensitivity (PCR) must be considered to guide policies and strategies.

5580

## COST-BENEFIT ASSESSMENT OF SURGICAL INTERVENTION FOR FILARIAL HYDROCELE PATIENTS AT THE PRIMARY HEALTH CARE LEVEL IN BANGLADESH

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Lymphatic filariasis (LF) is the 2nd leading cause of disability, affecting 120 million people and endemic in 72 countries worldwide. One of the complications of chronic LF is hydrocele which reduces mobility, induces social stigma and depression, and drastically decreases the ability to work. Surgical correction of filarial hydrocele with minimal complications can help these people return to a socioeconomically productive life. The study evaluated the hydrocele-related lifetime economic loss and the surgical benefits compared to its cost based on the 838 hydrocelectomy surgeries conducted between March to June 2021. The data were collected before and one year after surgery. Assumptions included financial losses and gains for the patients. Area-based daily wage rates were used to determine average lifetime earning gain and discounted value from surgery to age 64 - the end of working life. We estimated the total costs and compared them with the benefit of the work capacity restored to determine the cost-benefit ratio. Further Incremental Cost-effectiveness ratio (ICER) was calculated to determine the intervention program's cost-effectiveness level. The total monetary benefit of the surgery was 3,892,118 USD (without discounting) and 2,700,505 USD (with discounting). The average lifetime income gain per patient was USD 4644 (without discounting) and USD 3222 (with discounting) and approximate cost-benefit ratio is 1:15. The ICER value (71 USD) makes the intervention highly cost-effective, as it is substantially lower than the per capita GDP of Bangladesh (1961 USD). The sensitivity analysis demonstrates that the results are robust to surgery cost variations. The colossal patient burden in Bangladesh warrants significant investment to reinstate these patients to a normal life. However, the study findings show that the required investment is minimal compared to the lifetime impact of the surgery on the patients, their families, and the country. Governments and international aid organizations should prioritize investing in similar projects to eliminate LF and help thousands by improving disability and averting poverty.

5581

## BASELINE EVALUATION OF ONCHOCERCIASIS TRANSMISSION IN FOUR DISTRICTS OF NORTHERN GHANA

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Onchocerciasis, a filarial disease transmitted by blackflies, is targeted by the World Health Organization (WHO) for elimination. The WHO-recommended serologic threshold for stopping MDA is Ov16 seroprevalence <0.1% in children <10 years; however, models suggest that a higher seroprevalence of ≤2% may be sufficient to indicate interruption of transmission. We sought to evaluate the 2% threshold using paired entomological and serological studies in 4 districts in Northern Ghana where transmission may have been interrupted. If the Ov16 seroprevalence in children 5–9 years is ≤2% and blackfly O-150 PCR positivity meets WHO stopping criteria, then MDA will be stopped, and the area monitored annually. Vector control began in Ghana in 1974 followed by mass drug administration (MDA) with ivermectin in 1998; baseline prevalence in the study area was 5–89%. In 2022, a baseline serosurvey of children 5–9 years was conducted. The districts were stratified by endemicity status and villages selected through probability proportionate to estimated size methodology. In villages, a multi-stage random sample of children were enrolled with parental permission. A separate convenience sample of children was selected from 5 first-line villages. Dried blood spots (DBS) were prepared from venipuncture specimens, then eluted for analysis with Ov16 IgG4 rapid diagnostic tests (RDT). Results were available for 2,087 children from 65 villages. RDT results were positive in 31 children from 18 villages. When adjusted for survey design, the overall positive percentage was 1.3% (95% CI 0.6%–2.0%) for the districts. District positive percentages ranged from 0.5% (95% CI 0.1%–1.7%) to 2.2% (95% CI 0.8%–4.6%). Mapping of the positive villages did not reveal clustering. Study results show the Ghana program has significantly reduced transmission and met the study serologic criteria for stopping MDA. Results from the ongoing Ov16 ELISA of DBS and O-150 PCR testing in blackflies will be needed for a stop-MDA decision. Demonstration that a higher serologic threshold is consistent with interruption of transmission would facilitate progress toward WHO 2030 goals.

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## EVALUATION OF HIGHER SEROLOGIC THRESHOLD FOR STOPPING MASS DRUG ADMINISTRATION IN ONCHOCERCIASIS ELIMINATION IN THE TUKUYU FOCUS, TANZANIA

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Mass drug administration (MDA) with ivermectin is the primary elimination strategy for onchocerciasis. MDA may be stopped if programs meet World Health Organization (WHO) entomological and seroprevalence (<0.1%) criteria, the latter of which is challenging and may be lower than necessary. A multi-year study to evaluate a serologic threshold of ≤2% for stopping MDA was conducted in Tukuyu focus, Tanzania. If seroprevalence was ≤2% and WHO entomological criteria were met, then MDA would be stopped, and annual follow-up would begin. We present baseline and year 1 follow-up results. In the baseline study, 2000 children 5–9 years old were randomly sampled in villages stratified by prevalence concurrent with an entomologic study. Additional 1st-line villages were added so that one associated with each breeding site was evaluated, but the results were excluded from the overall seroprevalence. In year 1 of follow-up, 800 children 5–9 years old were sampled in eight 1st-line villages and entomologic evaluation was repeated. After parental permission and child assent were obtained, questionnaires were administered, and dried blood spots (DBS) were collected. DBS were analysed by Ov16 enzyme-linked immunosorbent assay (ELISA) and Ov16 rapid diagnostic tests (RDT). Entomology results will be presented separately. In the baseline study, 2,561 children (51% male) were enrolled: 2,070 children (52% male) from randomly selected villages and 491 children (47% male) from purposively selected villages. The seroprevalence among the children in randomly selected villages was 1.21% by ELISA and 0.045% by RDT. In 1st-line villages, the seroprevalence was 0.61% by ELISA and 0% by RDT. In year 1 follow-up, 809 children (47% male) were sampled with seroprevalence 0.12% by RDT; ELISA results are pending. Demonstrating that MDA can be stopped at a threshold >0.1% is important to help countries achieve WHO 2030 roadmap goals. New diagnostics are being developed assuming that 1% will be the threshold. Serologic data presented here are consistent with the hypothesis that it is safe to stop MDA at a higher threshold, but more follow-up data are needed to demonstrate this.

5583

## PROVIDING EVIDENCE ON THE STATUS OF TRANSMISSION OF ONCHOCERCIASIS IN 5 COUNTIES IN LIBERIA

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In 2018, the Ministry of Health-Liberia conducted its first programmatic Ov16 serological survey (pre-stop) to provide evidence on the status of transmission of onchocerciasis in the Southwest Regions (five counties; Bomi, Cape Mount, Margibi, Grand Bassa and River Cess). These five counties had received 14th years of MDA with ivermectin. Specifically, the objective was to determine the village level sero-prevalence of Ov16 in children 5-9 years old. A target convenience sample size of 100 children from each of 30 frontline communities within 5km of a blackfly breeding site or onchocerciasis river basin was selected in line with WHO onchocerciasis technical subcommittee guidelines. All enrolled consented children were tested for Ov16 using RDT in the field, and DBS were collected to allow subsequent ELISA testing. Of children testing negative for rapid test, 10% was randomly selected for confirmatory testing using SD Ov16 ELISA (Abbott, South Korea). Out of the target sample size of 3,000, a sample size of 2,468 was achieved. 91 of these children tested positive for Ov16 via RDT in the field. Out of 30 communities tested with RDT, 19 communities had positive cases while 11 reported all negative tests. The overall seroprevalence rate of 3.7% (91/2432) was found in five counties (Bomi, Grand Bassa, Grand Cape Mount, Margibi and Rivercess) with rate of 3.7% (21/572) in onchocerciasis endemic county only. In addition, rates of 3.2% (10/314), 0.5% (3/595), 0.7% (3/455) was observed in onchocerciasis and lymphatic Filariasis co-endemic counties with a high rate of 10.9% (54/496), respectively. According to WHO, to proceed to a full stop MDA survey, the prevalence threshold for IUs to “pass” pre-stop is <2%. We realized only two counties have crossed this benchmark, onchocerciasis transmission is still ongoing in one of the counties (Rivercess) and there is a need for support to conduct similar testing in the remaining 10 counties.

5584

## HIGH PREVALENCE OF LOA LOA AND MANSONELLA PERSTANS IN NORTHERN GABON

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Loa loa infection is endemic in central African countries, such as Gabon, and in West Africa. In regions where onchocerciasis is co-endemic with loasis, L. loa infection represents a major obstacle to control of onchocerciasis. Subjects with Loa loa hypermicrofilaremia more than 8000 mf/mL are at risk of developing severe and/or serious adverse effects following treatment with ivermectin. In 2011, the distribution of filariasis due to L. loa and Mansonella perstans has been carried out in Gabon. Ten years later, we are interested in evaluating the evolution of the prevalence of these filariasis in a northern of Gabon. Participants were recruited in northern Gabon, from November 2021 to April 2022. Venous blood was collected in an EDTA tube for microfilaria detection using blood direct examination and leukoconcentration techniques. Sociodemographics, hematological and parasitological parameters have been recorded. During the study, 1342 participants over the age of 18 were screened for the L. loa and M. perstans detection in 36 villages. Prevalences were 30.0% (403/1342) for L. loa, 4.9% (66/1342) for M. perstans and 1.9% (25/1342) for the coinfection. A hypermicrofilaremia was found in 12.9% (52/403) of participants with L. loa infection. Age and gender were risk factors associated with Loa loa and M. perstans microfilariae carriage: men were twice more infected than women (OR = 1.9, 95% CI [1.49 - 2.41] ; pγ0.001) and older people (>55) (p=0.0012, OR = 1.48, IC95% [1.16; 1.90]) than age group varying between regarding loiasis. For M. perstans, same findings were observed for M. perstans for men (OR = 2.4, 95% CI [1.40; 4.20]; p<0.001) and older people (p= 0.003, OR = 2.15, IC95% [1.25; 3.74]). The filariasis distribution according to villages differed significantly for L. loa (p=0.0034) and M. perstans (p<0.001). In conclusion, the prevalence of L. loa in northern Gabon is higher than the global rate of 22.4% reported, 10 years ago unlike M. perstans, found two times less than the 10.0% of 10 years ago. There was heterogeneity between villages.

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## DIROFILARIA SP. HONG KONG AND BRUGIA SP. SRI LANKAN GENOTYPE ARE THE PRIMARY CAUSES OF FILARIAL INFECTION IN DOGS IN SRI LANKA

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The mosquito-borne Dirofilaria repens, Brugia malayi, B. ceylonensis, and flea- and louse-borne Acanthocheilonema reconditum are historically known to be endemic among dogs in Sri Lanka. Despite this, limited information on the prevalence, diversity, and predictors of filarial infections in dogs in Sri Lanka have resulted in suboptimal control and prevention of these parasites, some of which are known to be zoonotic. To address this, whole blood and metadata were collected and analysed from 423 pet dogs across three geo-climatic zones within Sri Lanka. Blood samples were screened using the Modified Knott's Test (MKT) and PCR followed by Sanger sequencing. Multivariable logistic regression models were used to assess predictors for canine filarial infections. Two novel genotypes, Dirofilaria sp. Hong Kong (Dirofilaria HK) and Brugia sp. Sri Lankan genotypes were identified infecting dogs. The overall prevalence of filarial infection in pet dogs by PCR was 37.1% (95% CI 32.5 - 41.9%, n=157), compared to 18.6% (95% CI 15 - 22.6%, n=78) detected using the MKT. More than 80% of filarial-positive dogs were infected with Dirofilaria HK, while the remaining dogs were infected with Brugia sp. SL genotype. Increasing age (p<0.001) and residing in the low-country wet zone (p<0.001), which include regions that were endemic for human filariasis in Sri Lanka were associated with filarial infections in the study subjects. No clear pathognomonic signs for filarial infection were identified, indicating that dogs may act as reservoirs for these potentially zoonotic pathogens. Given the morphologic similarity of Dirofilaria HK and Brugia sp. Sri Lankan microfilariae with those of D. repens and B. malayi, respectively, it is likely that these species have been misidentified in the past. Therefore, it is necessary to take measures to prevent and control canine filarial infections to safeguard both canine and human health.

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## DIFFERENCES IN VACCINE-SPECIFIC RESPONSES BETWEEN URBAN AND RURAL ENVIRONMENTS AND MEDIATORS OF THESE DIFFERENCES AMONG UGANDAN ADOLESCENTS: THE POPVAC TRIALS

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Geographic and urban-rural variation in vaccine immune responses has been observed. Helminth and malaria infections, more common in settings where immune responses are impaired, have been implicated due to their immunomodulatory roles. We aimed to assess urban-rural differences in vaccine responses in Uganda and to identify mediators (with a focus on parasites) of these differences. We conducted three linked randomised trials: POPVAC A assessed effects of intensive vs standard praziquantel treatment on vaccine responses in a high schistosomiasis burden rural area, POPVAC B effects of intermittent preventive malaria treatment vs placebo in a high malaria burden rural area, and POPVAC C effects of BCG re-vaccination in a low infection urban area. BCG, yellow fever (YF-17D), oral typhoid (Ty21a), HPV and tetanus/diphtheria (Td) vaccines were administered to the same schedule in each trial. Outcomes were BCG-specific IFN-γ 8 weeks post-BCG vaccination and antibody responses to vaccine-specific antigens 4 weeks post-vaccination (YF-17D, S. typhi lipopolysaccharide (LPS), HPV) and 24 weeks post-Td. Schistosomiasis and malaria infections at baseline were determined using CAA assay and PCR, respectively. Regression analysis adjusting for age and sex assessed differences in responses between studies, with comparisons restricted to standard treatment and placebo arms for POPVAC A and B, respectively. Vaccine responses from 239 (POPVAC A), 171 (B), and 151 (C) participants were compared. There were differences in responses between settings for all vaccines: tetanus IgG (p=0.004), yellow fever titers (p=0.005) and oral typhoid IgG (p<0.001) were lower in both rural settings compared to urban. However, diphtheria IgG (p<0.001) was lowest in POPVAC A (high schistosomiasis) but highest in POPVAC B (high malaria) and BCG-specific IFN-γ differences varied over time with POPVAC A participants having lower peak responses but higher waning responses. Differences were observed for all vaccines; causal mediation analysis to quantify the contribution of parasitic infections to these differences will be presented at the meeting.

5587

## HOW DOES THE PROPORTION OF NEVER TREATMENT INFLUENCE THE SUCCESS OF MASS DRUG ADMINISTRATION PROGRAMMES FOR THE ELIMINATION OF LYMPHATIC FILARIASIS?

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Mass drug administration (MDA) is the cornerstone for the elimination of lymphatic filariasis (LF). An important driver for achieving this goal is the proportion of the population that never received treatment (NT). We employ two individual-based stochastic transmission models to assess the maximum level of NT with which the 1% microfilaria (mf) prevalence threshold can still be achieved under different scenarios for coverage of annual MDA, the drug combination used and transmission settings. For Anopheles-transmission settings, we find that treating 80% of the eligible population annually with ivermectin + albendazole (IA) can achieve the target within 10 years of annual treatment when baseline mf prevalence is 10%, as long as NT is below 10%. Higher proportions of NT are acceptable when more efficacious treatment regimens are used. For Culex-transmission settings with a low (5%) baseline mf prevalence and DA or IDA treatment, elimination can be reached if treatment coverage among eligibles is 80% or higher. For 10% baseline mf prevalence, the target can be achieved when the annual coverage is 80% and NT is 15% or lower. Higher infection prevalence or levels of NT would make achieving the target more difficult. The proportion of people never treated in MDA programmes for LF can strongly influence the achievement of elimination and the impact of NT is greater in high transmission areas. This study provides policy-relevant quantitative insights into what levels of NT may be acceptable to achieve elimination in different epidemiological and programmatic settings. In addition, our results provide a starting point for further development of criteria for the evaluation of NT.

**5588**

## ASSESSING IMPACT OF IVERMECTIN AND ALBENDAZOLE MASS DRUG ADMINISTRATION ON TRANSMISSION OF LYMPHATIC FILARIASIS IN 24 DISTRICTS IN SENEGAL

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Mapping of lymphatic filariasis was conducted in Senegal in 2003-2010 and found 51 of 79 districts endemic with Wuchereria bancrofti parasite antigenemia prevalence in sentinel sites of 1.0 to 78%. Annual mass drug administration (MDA) with ivermectin and albendazole was initiated in 2007. In 2018-2021, 17 districts passed transmission assessment survey (TAS) for the first time and have since stopped MDA. In 2021, 24 districts with an average baseline antigenemia prevalence of 14% became eligible for pre-transmission assessment survey (pre-TAS) to assess impact of MDA on prevalence of W. bancrofti infection after achieving the required five effective annual MDA rounds with at least 80% program coverage. In the pre-TAS conducted in 2021, 300-350 persons over 5 years old were assessed for W. bancrofti antigenaemia in sentinel and spot-check sites in each district. W. bancrofti antigenaemia prevalence was fund to have reduced from 1-78% in sentinel sites and 3-32% in spot-check sites to ≤ 0.3% in all 24 districts. Subsequently, transmission assessment surveys were conducted in 24 districts in 2022 to determine if transmission of the parasite has been interrupted and MDA could be stopped. The 24 districts were grouped into 19 evaluation units (EU) based on similarity of baseline prevalence, ecological factors, proximity and population for the TAS. Between 1320 and 1548 randomly selected children in grades 1 and 2 (as proxy for 6-7-year-olds) were assessed for antigenaemia in 30 randomly selected clusters (primary schools) in each EU. In both pre-TAS and TAS, filarial test strips (FTS) were used to detect presence of W. bancrofti antigens in 75µl of finger stick blood. Two out of 19 EU recorded one positive case, significantly below the critical cut-off value of 18 positives, no positive case was found in the remaining 17 EU indicating that parasite transmission has been interrupted in all EU. MDA has consequently been stopped in the 24 districts and they are currently under post-treatment surveillance.

**5589**

## SIGNIFICANT ACHIEVEMENTS IN LYMPHATIC FILARIASIS ELIMINATION IN NORTHWESTERN ETHIOPIA

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Lymphatic filariasis (LF) was historically endemic in 104 (9%) of Ethiopia's districts. Efforts to eliminate LF as a public health problem started in three districts of West Gondar zone in Northwestern Ethiopia in 2012 with integrated annual mass administration (MDA) of ivermectin and albendazole for onchocerciasis and LF. Following the WHO guidelines, after five rounds of effective mass treatment with >65% epidemiological coverage and successful Pre-transmission assessment surveys, the first Transmission Assessment Survey (TAS-1) was conducted in 2016 in the three districts with total population of about 330,000 at the time: Metema, Gendewuha, and Quara, which were independent, contiguous implementation units combined into one evaluation unit. The TAS-1 tested 1899 children aged 6 and 7 sampled from randomly selected communities, only 3 of whom (0.2%) were positive for circulating filarial antigen (CFA), which was less than the critical cut-off of 18. Following the halt of MDA for LF and onchocerciasis in 2017, LF post-treatment surveillance surveys found zero CFA-positive children among 1877 tested in the second of the TAS series, TAS-2, in 2019. Finally, only 1 (0.06%) CFA-positive child (a 7-year-old lifelong resident) was found among 1611 children tested in the final TAS-3 in November 2022. This indicates that over 360,000 people are no longer at risk for LF transmission in these three districts of northwestern Ethiopia. Continued post-"elimination" surveillance should continue until surrounding areas also interrupt transmission as migration from endemic areas is common. The successful completion of the TAS series is the first of its kind in Ethiopia and has motivated other parts of the country to achieve LF elimination success.

**5590**

## NATIONWIDE RE-MAPPING SURVEY FOR LYMPHATIC FILARIASIS ELIMINATION IN THE DOMINICAN REPUBLIC

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The Dominican Republic aims to eliminate lymphatic filariasis (LF) as a public health problem and demonstrate elimination of transmission. Nationwide baseline mapping (1999-2003, 2007) using lot-quality assurance sampling for LF identified three endemic foci in need of mass drug administration (MDA). These foci are currently in post-treatment surveillance. Recognizing the gap since baseline mapping, the Ministry of Health (MSP) conducted an LF re-mapping survey in 2022 to confirm that active transmission does not exist in historically non-endemic areas. A nationwide cross-sectional household (HH) survey was conducted in 40 provincial and health area directorate evaluation units (EU). Within each EU, 30 communities were randomly sampled; within each community, 16 HHs were selected where one member ≥6 years of age was asked to participate. A blood sample was tested for circulating filarial antigen (CFA) by Filariasis Test Strip (FTS). The prevalence and spatial distribution of LF antigenemia and LF morbidity was evaluated. Case investigations, treatment, and night blood sample collection were conducted per MSP guidelines. Provisionally, 19 of 15379 participants tested were CFA-positive (age range: 21-68 years). All 14 of

those tested to date for microfilaremia by night blood sample were negative; follow-up testing is pending for 5 individuals. Lymphedema was self-reported by 166 of 15358 adults and hydrocele by 19 of 6001 adult males. Weighted CFA prevalence estimates by EU ranged from 0-3.6%, with five EUs having an upper confidence limit &gt2%, the proposed threshold for starting MDA in re-mapping assessments. Additional investigation is needed to determine whether these results represent evidence of past or current transmission and if interventions such as MDA or focal MDA are needed. This re-mapping approach contributes to the conversation and possible methodologies for establishing verification of elimination of LF transmission.

**5591**

## MOLECULAR EPIDEMIOLOGY OF CIRCULATING DIROFILARIA IMMITIS AND D. REPENS IN CULICIDAE MOSQUITOES FROM REYNOSA, TAMAULIPAS

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Dirofilariasis is a disease caused by a nematode of the genus Dirofilaria, as D. immitis and D. repens. This disease affects both dogs and other vertebrate hosts and involves the parasite lodging in the heart or other organs. The parasite is transmitted to another host by mosquito bites. The aim of this study was to assess the prevalence of D. immitis and D. repens in dogs and mosquitoes in Reynosa, Tamaulipas, Mexico. An assay was performed to identify the presence of Dirofilaria in dog blood samples and mosquito abdomen to determine the prevalence of both filaria's species. For the capture of mosquitoes, 25 colonies distributed in Reynosa city were sampled using Sentinel traps, whereas for dog blood samples we had the support of veterinaries that treat dogs from different parts of the city and adjacent areas. An nested PCR assay was performed for the detection of species of Dirofilaria. In addition, a second PCR assay was performed to determine the mosquito species that fed on human blood to determine a possible zoonosis. A 2.64% prevalence of D. immitis infection was found in dogs from 188 samples tested, while no presence of the parasite was found in female mosquitoes from 250 specimens. Four of the five dogs positive for the presence of Dirofilaria spp. belonged to another US cities whose neighboring areas have a higher prevalence, specifically localized in the Rio Grande Valley; only one was found to be from Reynosa. Two hundred fifty engorged Culicidae mosquitoes were captured, of which 98.5% were fed with human blood. The mosquito's species with the highest population density are Aedes aegypti and Culex quinquefasciatus and both species are competent as a vector for Dirofilaria. All samples of Ae. aegypti and most samples of Cx. quinquefasciatus reveals at least having fed on humans' blood, making this finding a possible risk of Dirofilaria zoonosis towards humans in the city of Reynosa. Another culicid species was identified were An quadrimaculatus (not showing human blood feeding), and in the case of Cx. erraticus and Ae. epactius, not exist too much information regarding their vector competence for Dirofilaria.

**5592**

## GENETIC DIVERSITY WITH THE EMERGING ZOONOSIS OF AN ONCHOCERCA SPECIES OF HUMAN POPULATIONS IN TARABA STATE, NIGERIA

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A better understanding of Onchocerca population genetic processes in a specific biogeography is needed to support the onchocerciasis elimination goals. The study investigated the genetic diversity of Onchocerca microfilariae using a fragment of 16S rDNA genes in some onchocerciasis endemic communities of Taraba State, Nigeria. The investigation was in eight selected communities comprising six endemic communities with a high prevalence of infection despite receiving mass drug administration with ivermectin for between 10 and 16 years of annual treatments and two non-endemic areas. Skin snips were obtained from 321 consenting participants after community engagement and who were more than five years of age and had resided within the communities for more than ten years or since birth. DNA was extracted from the microfilariae that emerged from the normal saline and the residual skin snip samples and preserved in RNAlater® in a 1.5 ml centrifuge tubes. A Polymerase Chain Reaction (PCR) amplification with generic and species-specific primers targeting the 16S gene of the DNA extracted. The PCR product was eluded and cleaned for sequencing, while the resultant sequences were analysed to identify the Onchocerca species. The multiple sequence alignment protocol showed the distinct diversity of two sequences G49_O.v. Gashaka and Y02_O.v. Yorro samples with 100% similarity that matched sequences of O. volvulus from Cameroon that has been deposited in the GenBank, indicating the emergence of a new polymorphic strain of O. volvulus species. Similarly, two O. ochengi sequences isolated from human samples, O. ochengi G44_Gashaka; O. ochengi G01_Gashaka and O. ochengi Y04_Yorro, matched with an O. ochengi sequence in the GenBank from Cameroon and is being reported from the study communities for the first. The study has identified O. volvulus as the cause of onchocerciasis and O. ochengi with the potentials of an emerging zoonotic onchocerciasis in the cattle (animal)-Simulium(vector)- human interface characteristic of the communities.

**5593**

## BENCHMARKING AN ACCESSIBLE METHOD FOR GENERATING COMPLETE GENOMES FROM PARASITIC NEMATODES

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Parasitic nematodes, including filarial worms and soil-transmitted helminths, are etiological agents of both acute and chronic disease. Recent technological and methodological advances in genomics have revolutionized the study and treatment of parasitic nematodes. Though remarkably impactful, these advances have also underscored a critical issue: Generating short-read DNA sequence data (and building and maintaining the infrastructure needed to do so) is expensive, making genomic resources largely inaccessible. The Oxford Nanopore Technologies (ONT) MinION is an inexpensive and portable next-generation sequencing platform capable of producing ultra-long DNA sequences ideal for whole genome assemblies; however, poor read level accuracy has hindered widespread adoption of the platform. Recent advances in ONT chemistries have produced more accurate and reliable read level data. To determine if whole genome assemblies using only ONT data reflect current gold standard hybrid assemblies, we generated complete genome assemblies for the parasitic nematodes Brugia malayi and Trichuris trichiura using only ONT MinION Q20+ sequence data. We compared each ONT MinION assembly to reference genomes generated using a hybrid assembly approach, and assessed contiguity, completeness, and accuracy using QUAST, BUSCO, and MUMmer4, respectively. For B. malayi, we generated an 88 gigabase (Gb) assembly in 85 contigs that covers 99.8% of the reference genome. The two assemblies both contain >99% of BUSCOs known for the phylum Nematoda and are >99.9% identical when considering only single base pair (bp) mismatches. For T. trichiura, we generated a 71 Gb assembly in 158 contigs that covers 99.4% of the reference genome. The two assemblies both contain ~46% of BUSCOs known for the Nematoda and are >97.8% identical when considering only single bp mismatches. These results highlight the ONT MinION and its Q20+ platform update as an accessible stand-alone alternative to traditional sequencing approaches for the generation of whole genome assemblies for parasitic nematodes.

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## BMA-LAD-2 AS A NOVEL ANTIBODY TARGET FOR THE TREATMENT OF LYMPHATIC FILARIASIS

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Efforts to control the global burden of Lymphatic Filariasis focus primarily on Mass Drug Administration (MDA). While highly effective against microfilariae, current MDA regimens have little to modest effects on adult worms. Thus, MDA typically has to be repeated over several years. Additionally, the most efficacious MDA regimen is contraindicated in areas co-endemic with Loa loa and Onchocerca volvulus infections due to adverse effects caused by rapid killing of microfilariae. The long-term goal of this study is to develop a novel macrofilaricidal agent that would allow for fewer MDA treatments and safe use in areas with L. loa and O. volvulus. Previously, we showed that Bma-LAD-2, an intestinal protein in Brugia malayi likely involved in tight junction formation, is essential for adult worm fecundity, metabolism, and motility. In this study, we are testing whether antibodies targeting Bma-LAD-2 can function as macrofilaricidal agents. We have recombinantly expressed a soluble form of the outer binding Ig domains of Bma-LAD-2 (Bma-LAD-2 Ig1-4) and have created stably transfected HEK293 cells expressing full-length transmembrane Bma-LAD-2 protein. Bma-LAD-2 transfected cells were found to have higher electrical resistance across a monolayer than non-transfected HEK cells by TransEpithelial Electrical Resistance (TEER) assay. Addition of polyclonal rabbit antibodies against Bma-LAD-2¬ Ig1-4 to the transfected cell line decreased TEER values to those of non-transfected cells. Finally, we have found that anti-SARS-CoV-2 antibodies in human sera cultured with adult B.malayi worms were still functional after ingestion by adult worms. Together, these results suggest that adult filarial worms ingest antibodies into their intestinal tracts, and that antibodies against Bma-LAD-2 have the potential to interrupt intestinal tight junctions of filariae. We are currently working to determine the effect of treating adult B.malayi worms with anti-Bma-LAD-2 antibody. It is possible that if the binding domains of the Bma-LAD-2 are conserved across filarial species, these antibodies could be used as a pan-filarial macrofilaricide.

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## IMPACT OF WUCHERERIA BANCROFTI INFECTION ON CERVICAL MUCOSAL IMMUNITY OF WOMEN IN LINDI, TANZANIA

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Lymphatic filariasis is a mosquito-transmitted parasitic infection in tropical regions. Our group had previously described an increased HIV incidence in individuals infected with Wuchereria bancrofti in Southwest Tanzania. Most HIV transmissions in sub-Saharan Africa occur through heterosexual contact. However, W. bancrofti is not known to cause lesions or ulcers in the genital mucosa as other diseases are known to do. The aim of this work is to analyze cervical mucosal T cell phenotypes in women infected with W. bancrofti and compare the results with uninfected women from the same area. Women (aged 18-45) from the Lindi region of Tanzania were screened for W. bancrofti infection using filarial test strips (FTS, Abbott). HIV status was determined using HIV 1/2 3.0 (Standard Diagnostics Inc) and positive results were confirmed using Uni-Gold Recombigen HIV - ½ (Trinity Biotech). From each woman, a cervical mucosal sample was taken for T cell phenotyping and Pap smear and HPV typing were performed. Samples were stained for flow cytometric measurement of activation, cell lineage and maturation markers, as well as HIV receptor and facilitator markers (HLA-DR, CD38, Vdelta2, CD45, CD27, CD25, FoxP3, CCR5, beta7) on CD4 and CD8 T cells. Between October and December 2020, 38 HIV-uninfected female study participants (mean age 24.6 years) were recruited; 26 of them were infected with W. bancrofti. None had abnormalities in the Pap smear and 12 women were found to have high-risk (HR) HPV types. Two of 12 filarial-uninfected women had HR HPV (17%) detected in the mucosal sample versus 10/25 (40%) HR HPV in W. bancrofti infected women (p=0.2). An increased expression of HIV co-receptor CCR5 on memory CD4 T cells as well as increased number of gamma-delta2+ T cells (p=0.0042) were found in the cervical mucosa of W. bancrofti-infected women. The systemic infection with W. bancrofti infection, which, unlike other helminthic infections, has no known genital lesions, was associated with T cell changes of the cervical mucosa in a small number of women. We additionally found an augmented prevalence of HPV in W. bancrofti infected women.

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## EFFECTS OF WUCHERERIA BANCROFTI INFECTION ON CD4 T CELL RESPONSES TO SPECIFIC AND NON-SPECIFIC ANTIGENS

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Lymphatic filariasis, primarily caused by Wuchereria bancrofti, is a mosquito-transmitted disease, which is affecting people living in tropical regions. Infection with W. bancrofti is associated with chronic inflammations that may cause lymphedema and hydroceles. The adult worm of W. bancrofti lives for many years in the human host and even without disfiguring consequences leads to modulation of the adaptive immune response. This study aims to determine whether chronic W. bancrofti infection affects CD4 T cell responses to specific and non-specific antigens. Blood samples were collected from 141 participants living in two W. bancrofti endemic regions in Tanzania: Kyela district in Mbeya region, n=106 and Lindi district, n=35. Samples were stimulated with the whole lysate of Mycobacterium tuberculosis (Mtb), Staphylococcus Enterotoxin B (SEB) or PBS (control) for 16 hours. The frequency of CD4 T cells responding to stimulation by secreting interferon gamma (IFN-γ) or interleukin 2 (IL-2) cytokines was measured by flow cytometry. W. bancrofti-infected individuals had significantly fewer Mtb specific IL-2 producing CD4 T cells compared to uninfected individuals (p =0.010), while IFN-γ responses to Mtb specific stimulation were comparable between W. bancrofti infected and uninfected individuals. Interestingly, W. bancrofti infection showed significant reduced frequencies of IFN-γ (p<0.0001) and IL2 (p<0.0001) CD4 T cells responses upon stimulation with SEB compared to uninfected individuals. Our findings show diminished CD4 T cell responses to SEB in W. bancrofti infected individuals. Stimulation with Mtb showed differences for only one cytokine, IL-2, otherwise comparable results between the infected and the control group. These results indicate that chronic infection with W. bancrofti suppresses CD4 T cell responses, most likely as part of the immune evasion strategy of the parasite. These reduced immune responses might have a deleterious impact on the capability of the host to fight other infections.

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## MULTIPLEXED HIGH THROUGHPUT POINT-OF-CARE BIOSENSING OF ONCHOCERCIASIS ANTIBODY MARKERS

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Neglected tropical diseases (NTDs) disproportionately affect marginalized populations, often in resource-poor settings. The critical lack of cost-effective diagnostic tests devices for NTDs was highlighted in WHO's 2021-2030 Road Map. Current serological tests for onchocerciasis, which aid diagnosis and are important for disease mapping and mass drug administration decisions, detect only one antigen-specific antibody, limiting their sensitivity and specificity. Here, we report inexpensive yet multiplexed and high-throughput optical and electronic biosensing methods for point-of-care (POC) detection of antibodies against several onchocerciasis antigens from a single drop of serum. The optical method uses enzymatic silver metallization on standard glass slides and a custom cellphone app that measures the optical readout. The electronic method uses similar assay chemistry, but on microelectrode chips and generates a direct electronic readout. Recombinant onchocerciasis antigens are immobilized using laser-cut polydimethylsiloxane microwells. After sample incubation with onchocerciasis-positive and control samples, a horseradish peroxidase labeled probe is added. Finally, silver substrates are added to generate silver metallization related to the measured biomarker concentration. To demonstrate the POC detection, onchocerciasis patient (n=56) and control (n=35) sera were measured for IgG4 against multiple antigens (FABP, OV-16, Ov33, OvMSA) from single drops (3µL) of diluted sera. Onchocerciasis+ samples exhibited dark metallization decreasing with serial dilution, while controls showed low metallization. These findings were cross-referenced against an FABP ELISA, which demonstrated similar results. Multiple logistic regression with the onchocerciasis antigens improved the diagnostic accuracy (AuROC>0.95), indicating the value of multiplexed detection. This inexpensive multiplexed POC diagnostic approach holds promise for onchocerciasis detection as well as other NTDs.

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## CYTOKINE IN UTERO PRIMING IS ASSOCIATED WITH DETRIMENTAL BIRTH OUTCOMES AND CHILD INFECTIONS

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Infections during pregnancy are associated with foetal immune imprinting that impacts birth outcomes and susceptibility to childhood infections. This study investigated how anti-parasitic cytokine profiles in newborns' cord blood mononuclear cells (CBMCs) relate to low birth weight (LBW) and childhood infection. A total of 311 Kenyan mothers were followed during pregnancy and were tested for parasitic infections at each ante-natal clinic visit. At delivery, cord blood was collected and newborns' cytokine responses to Plasmodium falciparum, S. hematobium, and W. bancrofti antigens were assessed. The baby's birth weight was also recorded. Infants were followed to 18 months of age and blood, urine and stool samples were collected at every visit and tested for parasitic infections. Cytokine profiles of each child were classified by principle component analysis and termed as positive or negative response. We observed a positive association between IL-10, TNF-a and Th-1 responses in babies born to mothers with maternal malaria infection but negative response in those born to mothers co-infected with malaria and helminths. LBW was associated with IL-5 and IL-10 sensitized babies but not with any maternal infection. We also observed increased risk of malaria infection in children whose CBMCs had a predominant Th-1 profile particularly IL-2. Childhood malaria was also associated with increased risk of having severe anaemia, hookworm infection and any co-infection. This study suggests that maternal sensitization of either Th-1 or Th-2 cytokine profiles may contribute to poor pregnancy outcomes and increased susceptibility to childhood infections. Additionally, differential imprinting trajectories in utero were associated with either mothers were mono- or co-infected.

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## IMPACT AND COST EFFECTIVENESS OF ANNUAL VS. TWICE ANNUAL MASS DRUG ADMINISTRATION FOR ELIMINATION OF LYMPHATIC FILARIASIS AND CONTROL OF ON CHOCERCIASIS IN COTE D'IVOIRE

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Lymphatic filariasis (LF) and onchocerciasis (oncho) are coendemic in many parts of Cote d'Ivoire, and can cause severe economic and physical burdens on populations and health systems. Mass drug administration (MDA) of ivermectin and albendazole is a key intervention strategy to eliminate these diseases in Africa. While most country programs implement MDA annually, some have posited that a twice per year MDA may be more effective, potentially reducing the time to elimination. Despite these potential benefits, very few studies have examined the costs and program implications for switching to a semi-annual schedule. We compared the relative impact and cost effectiveness of annual versus twice-yearly MDA in the two districts Abengourou (1x MDA) and Akoupe (2x MDA) in south-eastern Côte d'Ivoire. The Ministry of Health and other implementing partners collected cost data associated with the inputs and activities needed to run MDA programs. Data was collected from January - December 2014 and used to project costs for the full 3-year study period. The annual financial costs were 20% higher for 2x/year MDA ($34,012USD vs. $22,839USD). One year after the third round of 1x and the fifth round of 2x MDA, the LF microfilaria (Mf) prevalence decreased from 8.4% to 2.5% in the 1x MDA area and from 8.1% to 2.3% in the 2x MDA area. The oncho skin Mf prevalence decreased from 22.7% to 8.4% and from 23.1% to 7.5% in these areas, respectively. In conclusion, 2x MDA was more costly but no more effective than annual MDA in this study. Based on these results, we recommend that country programs focus limited resources to conduct high quality annual MDA, in accordance with current WHO guidance.

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## INVESTIGATING KNOWLEDGE, ATTITUDES, AND PRACTICES OF HEALTH WORKERS ON THE MANAGEMENT OF FEMALE GENITAL SCHISTOSOMIASIS IN THE SOUTH REGION OF CAMEROON

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Schistosomiasis is an acute and chronic disease caused by parasitic worms that can take two main forms: intestinal or urogenital. If left untreated, the urogenital form can lead to female genital schistosomiasis (FGS) in women and girls; frequently resulting in severe reproductive health complications which are often misdiagnosed as sexually-transmitted infections. This study assessed healthcare workers' knowledge, attitudes and practices of the management of FGS in the South Region of Cameroon.Using quantitative research methods, a questionnaire was administered to 104 health workers from all levels in the health system (Health facility, district and Region) from all the 10 health districts of the South Region. Over half of the participants

had heard about FGS but very few had clinical knowledge that could enable them to diagnose, treat and prevent FGS. Most healthcare workers did not have any confidence in their ability to effectively manage cases. Healthcare workers in the South Region of Cameroon present significant gaps in knowledge about FGS especially when it comes to its diagnosis and treatment. There is a great need for the National NTDs programme to develop training manuals and organize capacity building workshops to equip healthcare workers with knowledge on FGS. Also, it is important to take advantage of other campaigns like free distribution of Ivermectin and Albendazole in communities against lymphatic filaria to educate the community on FGS and also improve upon water sanitation and hygiene to break the chain of transmission of the infection.

**5601**

## INVESTIGATING THE INFLUENCE OF PATHOGENIC LEPTOSPIRE SHEDDING BY RAT POPULATIONS ON HUMAN LEPTOSPIRA INCIDENCE IN SALVADOR, BRAZIL

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Leptospirosis is a neglected zoonotic disease responsible for 1 million cases and 60,000 deaths annually, making it a major global health concern. Environmental distribution and concentration of pathogenic leptospires shed from rats are speculated to affect infection risk, but little is known on this association. Our objective was to investigate whether variations in leptospire shedding influence human Leptospira infection risk. We explored a novel approach of combining several statistical models to elucidate links between shedding and human Leptospira incidence, and the interplay with other risk factors. We performed eco-epidemiological studies in an urban slum environment in north-eastern Brazil. During 2013-14, we sampled roughly 500 Rattus norvegicus and quantified their shedding status and load, identified 700 unique points for R. norvegicus presence, and performed sequential surveys of residents to evaluate potential environmental and socioeconomic risk factors. We integrated data from these populations by building three statistical models. Models were built based on the Rattus norvegicus data for two outcomes: 1) shedding by individual rats and 2) the relative abundance of rats. The estimated 'total shedding' variable, obtained by multiplying the predictions from those two models, was used in a third model, as a risk factor for human Leptospira infection. Our results suggest that 'total shedding' by rat populations is an important risk factor for human infections (odds ratio [OR]=1.4; 95% confidence interval [CI]: 1.0-1.9), however, rat abundance, rather than shedding by individual rats, is the main driver of this association. Among factors pertaining to surroundings and the environment, infection risk was higher in areas with higher vegetative land cover i.e., more rural areas (OR=2.1; 95% CI: 1.1-3.9), and when rainfall entered the house (OR=1.9; 95% CI: 1.4-2.6). Effective prevention strategies will require control of the reservoir population in addition to addressing the structural features of slum settlements that promote transmission.

**5602**

## MAINTAINING ELIMINATION OF TRACHOMA AS A PUBLIC HEALTH PROBLEM: POST-VALIDATION SURVEILLANCE PLANS IN VALIDATED COUNTRIES

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Trachoma is targeted for global elimination as a public health problem by 2030. Repeated infections with Chlamydia trachomatis lead to inner eyelid scarring, causing the eyelashes to turn in and rub the cornea (trachomatous trichiasis, or TT) which can lead to blindness. The WHO-endorsed SAFE strategy (Surgery, Antibiotics, Facial cleanliness and Environmental improvement) is used to eliminate trachoma. To date, 15 countries have been validated by WHO as having eliminated trachoma. The epidemiological targets for validation are: trachomatous inflammation – follicular (TF) <5% among children 1-9 years and TT <0.2% in adults 15 and above in all districts. National Program submit dossiers to WHO to be validated. In these dossiers, plans to conduct post-validation surveillance (PVS) are described. We conducted a literature review to understand the organic landscape of PVS plans and activities. A search was conducted using the PubMed and Google Scholar databases using the terms "trachoma" and "post-validation surveillance." A Google search using "trachoma elimination dossiers" and country names was conducted. Abstracts were reviewed, and elimination dossiers, documents describing programmatic plans, or operations research on PVS were retained. Three dossiers were found (Ghana, Iran and Mexico). Programmatic documents with PVS plans were available from Oman and Morocco; a peer-reviewed publication was available for The Gambia. All countries plan to use a passive surveillance approach (case reporting via health information systems), though populations targeted and methods vary. Four countries also had an active surveillance approach: sentinel sites, contact investigations, or periodic surveys. All countries rely on clinical indicators; one country is considering syndromic and biological indicators. Two countries (Ghana and Morocco) have conducted operations research on PVS using clinical and biological indicators. In these, clinical signs remained under the elimination threshold. Age-adjusted seroprevalence varied by study site but seroconversion rates were low. Where detected, current infection prevalence was low.

**5603**

## INTEGRATED SURVEILLANCE FOR LYMPHATIC FILARIASIS, VISCERAL LEISHMANIASIS AND DENGUE ARE DIFFICULT PROPOSITION

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Lymphatic Filariasis (LF), Kala azar and Dengue are the public health concerns in India. These diseases are included in 20 identified NTDs. The surveillance of clinically manifested LF cases is done annually and updated once a year whereas, microfilaria prevalence is done in sentinel and random sites @ 1 site per million population as per WHO guidelines. Visceral leishmaniasis or Kala azar case search is also done in endemic areas for both with symptoms or for PKDL ones unlike malaria where surveillance is done routinely from all areas mostly at weekly or fortnightly intervals. Similarly, the surveillance of dengue cases is also institution (hospitals/labs) based. The integration of such type of surveillance would have various challenges like skill of the technicians for all the three diseases, different diagnostic labs and partial verticality in human resource etc. The various challenges including control command of human resource, difference in skill development and vertical implementation specific to surveillance, case detection and management in respect of these three NTDs namely LF, Kala azar and dengue prevalent in India need to be carefully looked into before considering integrated surveillance, however, vector surveillance can be undertaken in an integrated manner so that evidence generated can be used for action.

5604

## IMPLEMENTATION OF A SUSTAINABLE AEDES AEGYPTI CONTROL STRATEGY: A COMMUNITY-BASED MODEL

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Dengue, Chikungunya, and Zika viruses pose significant challenges for public health disease management in the tropics. The control of these arboviruses has traditionally relied on strategies focused on the mosquito vector, Aedes aegypti, through insecticides or more recently with the use of suppression or replacement strategies. Traditional efforts have failed to contain arbovirus transmission or control the vector, and while novel technologies have shown promising results they are still being tested. We implemented a sustainable community-focused Participatory Action Research methodology for Ae. aegypti control based on the Care Group and DengueChat programs that have been previously evaluated in high-risk communities for dengue in Managua, Nicaragua. Between 2017 and 2020, house-to-house visits were made to promote container management. These were carried out jointly between a community "brigadista" (volunteer) and a household participant. All containers with water inside and outside of the household were checked to promote resident-led elimination of breeding sites. Our team promoted the implementation without carrying out the removal of positive containers. Subsequently, the "brigadista" coordinated meetings with neighbors to build rapport based on the evidence of the control activities and generate collective achievements with neighbors to avoiding stigmatization. Throughout the study, the Ministry of Health carried out routine control measures in the area. In the baseline wet season of 2017, we found 161 pupae per household, 38 pupae per container, and 0.35 pupae per person. At the baseline of the dry season in 2018, we found 60 pupae per household, 20 pupae per container, and 0.13 pupae per person. After the implementation of our control strategy, we observed a Reduction of 81% for pupae per household, 64% pupae per container, and 81% pupae per person. With a sustained reduction of productive containers in the area. Our results show that the inclusion of communities in the management and control activities of Ae. aegypti can be maintained and run by community members and sustained over time.

5605

## COST EFFECTIVENESS OF COMPARATIVE SURVEY DESIGNS FOR HELMINTH CONTROL PROGRAMS: POST-HOC COST ANALYSIS AND MODELLING OF THE KENYAN NATIONAL SCHOOL BASED DEWORMING PROGRAM

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Soil-transmitted helminths (STH) and schistosomiasis comprise the most wide-spread NTDs globally. Preventative chemotherapy is a cost-effective approach to controlling morbidity of both diseases, but relies on large scale surveys to determine and revise treatment frequency. Availability of detailed information on survey costs is limited despite recent methodological surveying innovations. We micro-costed a survey of STH and schistosomiasis in Kenya, and linked results to precision estimates of competing survey methods to compare cost-efficiency. Costs from a 2017 Kenyan parasitological survey were retrospectively analyzed and extrapolated to explore marginal changes when altering survey size, defined by the number of schools sampled and the number of samples taken per school. Subsequent costs were applied to simulated precision estimates of model-based geostatistical (MBG) and traditional survey designs. Cost-precision was calculated for a range of survey sizes per method. Four traditional survey design scenarios, based around WHO guidelines, were selected to act as reference cases for calculating incremental cost-effectiveness ratios (ICERs) for MBG design. MBG designed surveys showed improved cost-precision, particularly if optimizing number of schools against samples per school. MBG was found to be more cost-effective under 87 of 92 comparisons to reference cases. This comprised 14 situations where MBG was both cheaper and more precise, 42 which had cost saving with precision trade off (ICERs; $8,915-$344,932 per percentage precision lost); and 31 more precise with increased cost (ICERs; $426-$147,748 per percentage precision gained). The remaining 5 comparisons represented extremes of MBG simulated site selection, unlikely to be applied in practice.

5606

## SEROSTATUS OF ANTI-RABIES TITER VACCINE LEVELS IN IMPOUNDED DOGS IN MUNTINLUPA CITY, PHILIPPINES, 2021

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Rabies is a zoonotic disease transmissible thru bite by an infected rabid animal. It is a vaccine preventable disease wherein a titer of 0.5 IU/ml is considered on protective level based on the WHO and WOAH (World Organization for Animal Health, formerly known as OIE). This study aims to investigate the serostatus of anti-rabies titer vaccine level that is impounded dogs in Muntinlupa city. Two hundred and fifty-two (252) serum samples were collected from dogs that is impounded in Muntinlupa city pound with unknown history of vaccination, out of these 86 (34.13%) dogs showed antibody titer above 0.5 EU/ml. all dogs collected with serum samples were processed and tested using ELISA with manufacturer's graphpad prism software to check all computation of the vaccinal immune response. Out of 252 serum samples, 166 (65.87%) impounded dogs showed anti-rabies antibody titer below 0.5 EU/ml/ indicating susceptibility to rabies infection and thereby posing possible threat to surrounding human and animal population in the area.

5607

## ASSESSING HEALTH SYSTEM'S PERFORMANCE FOR NEGLECTED TROPICAL DISEASES (NTDS) THROUGH WHO'S DATA QUALITY ASSESSMENT (DQA) TOOL IN FOUR WEST AFRICAN COUNTRIES

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The WHO's Data Quality Assessment (DQA)for Neglected Tropical Diseases (NTDs) is an important Monitoring and Evaluation (M&E) tool that can be used after Mass Drug Administration (MDA) to analyze data quality. The tool evaluates two parts: 1) Verification of MDA data, which includes verification factor (VF, ratio of recounted and reported numbers) for up to five indicators, and 2) An assessment of data management and reporting system through a. M&E structure, b. Indicator reporting guidelines, c. Data-collection, reporting tools, d. Data management processes, and e. Links with the national reporting system. Act to End NTDs | West is a five-year USAID-funded program that aims to eliminate or control five NTDs (LF, Trachoma, OV, SCH, and STH) in 11 West African countries. It routinely implements DQAs in its portfolio. We summarized data across seven DQAs (84 service delivery points) across four West African countries. The average verification factor was 94.4 (SD-20), demonstrating potential over-reporting for indicators assessed ("Number of pills administered" and "Number of people treated.") The scores were high for all five system assessments, particularly Indicator reporting guidelines (3.0 out of 3.0) and Links with national reporting system (2.9 out of 3.0). We explored the association between system scores and the VF to identify ways of enhancing data quality using DQAs. The VF and total system scores had a moderate positive correlation, r= 0.42. (pr<0.05). Additionally, we built a linear regression model with VF as the outcome variable and five system scores, border districts, and MDA

rounds as independent variables. The analysis showed positive associations between VF and M&E structure, data collection, and reporting tools, where VF increased by 0.10 (95% CI: 0.01, 0.20), and 0.45 (95% CI: 0.27, 0.63) respectively per unit increase in the system score. We observed that the number of MDA rounds and border districts had an inverse relationship with VF. These findings indicate that improving system scores (a & b) would enhance the accuracy of MDA data (VF). The programmatic response could also include focusing on improving MDA at border districts.

5608

## MEASURING THE OUTCOME OF THE MASS DRUGS ADMINISTRATION OF LYMPHATIC FILARIASIS THROUGH SENTINEL AND SPOT SITES SURVEYS FROM 2012 - 2021

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The results of the survey show that the disease is endemic in 13 of the 15 counties. The programme began implementing the first LF MDA, using a community distribution method, in 2012 in all 13 endemic counties. The programme has subsequently treated in 2013, 2015-2019 and 2021 in all endemic counties. Based on WHO guidelines to monitor the progress and impact of MDA, Sentinel sites must be identified in each implementation unit for evaluation after every 3 rounds of MDA. The program has conducted 3 destined Sentinel and Spot Check Sites Surveys in the Counties. The objective is to showcase the progress of MDA in reducing micro filarial in the communities through monitoring and Evaluation of Sentinel and Spot sites survey at the county level. To showcase results and data indicating a downward trend of the impact of MDA only lymphatic filariasis with the use of Albendazole in Liberia. To demonstrate the strategies in determining the impact of the Mass Drug Administration in controlling and eliminating Lymphatic Filariasis in Liberia. The Sentinel and Spot Check sites survey were conducted with a cross-sectional approach whereby people ages 5 years and above were sampled. Samples collected from participants were tested with Filarial Test Strips and counting chambers. The method of sample collection and testing were recommended by WHO. The NTD programme has conducted three sentinel site surveys; the first, baseline, was conducted in 2012 in 11 sites and the second was conducted in 2016 in 11 sites across the same 11 counties. The second survey was conducted in 4spot-check sites and 7 sentinel sites. The county of River Gee had an increase from 0% to 0.99%Mf. Maryland, the only county with a MF rate over 1% showed a reduction from 11.37% to 8.36%.The slow rate of reduction is likely attributed to receiving 2 rounds of MDA instead of 3 rounds.

5609

## INCIDENCE OF SNAKEBITES IN RURAL POPULATIONS OF REPUBLIC OF CONGO

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Snakebites are a real social, economic and public health problem in the world. Few studies on snakebites have been conducted in Congo. The objective of this study is to report data on the risk and incidence of snakebites in rural populations in the Republic of Congo. A household survey of rural populations was conducted in 5 districts. This household survey was conducted using a questionnaire that took into account the characteristics of snakebite victims, the circumstances of the bite (location, season, time of day, activity) and the victim's recourse to treatment (traditional practitioners, dispensaries, self-medication). For the calculation of incidence, the average age of the respondents was used as a proxy for the period covered by the survey, thus completing the denominator. The collected data were entered into Microsoft Excel 2013, exported, and analyzed using Package for Social Science (SPSS) version 22 software. A total of 133 snakebite cases were recorded during the study period. In Okoyo District, 80 snakebite cases were reported and 2 deaths recorded. In Enyéllé District, 35 cases of snakebites were reported and 5 deaths were recorded. In Gamboma, 13 cases of snakebites were reported and no deaths were recorded. In the districts of Tchiamba-Nzassi and Mokéko, 1 case and 4 cases of snakebites were respectively recorded with no deaths. The incidence measured during this survey was 2857 bites per 100,000 inhabitants per year in Okoyo, 3535 bites per 100,000 inhabitants per year in Enyellé and 1032 bites per 100,000 inhabitants per year in Gamboma. More than half of the victims sought traditional care in Okoyo, Enyelle and Gamboma districts. While in Enyelle, 28.57% of cases used traditional-modern care and 7.69 of cases in Gamboma used modern care.This study highlights the need to intensify snakebite surveys throughout Congo by conducting surveys at three levels: health facilities, households and traditional healers in order to have a more general knowledge of snakebite victims. A program to raise awareness among rural populations about the dangers involved should be put in place.

5610

## UTILITY OF THE LOOP-MEDIATED ISOTHERMAL AMPLIFICATION ASSAY FOR THE DIAGNOSIS OF VISCERAL LEISHMANIASIS FROM BLOOD SAMPLES IN ETHIOPIA

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Rapid and accurate visceral leishmaniasis (VL) diagnosis is needed to initiate prompt treatment to reduce morbidity and mortality. Here, we evaluated the performance of loop-mediated isothermal amplification (LAMP) assay for the diagnosis of VL from blood in an endemic area in Ethiopia. LAMP was positive in 117/122 confirmed VL cases and negative in 149/152 controls, resulting in a sensitivity of 95.9% (95% CI: 90.69-98.66) and a specificity of 98.0% (95% CI: 94.34-99.59), respectively. The sensitivity of the LAMP assay was 95.0% (95% CI: 88.61-98.34) in HIV-negatives and 100% (95% CI: 85.18-100.0) in HIV-positives. Compared with microscopy, LAMP detected 82/87 (94.3%, 95% CI: 87.10-98.11) of the microscopy1 cases and was negative in 11/27 (40.7%, 95% CI: 22.39-61.20) of the microscopy2 cases. Compared with the rK39 serology, LAMP detected 113/120 (94.2%, 95% CI: 88.35-97.62) of the rK391 cases and was negative in 149/154 (96.8%, 95% CI: 92.59-98.94) of the rK392 cases. However, when compared with microscopy only, rK39 detected 83/87 (95.4%, 95% CI: 88.64-98.73) of the microscopy1 cases and negative in only 12/27 (44.4%, 95% CI: 25.48-64.67) of the microscopy- cases. There was an excellent agreement between rK39 and LAMP (Kappa 5 0.91, 95% CI: 0.86-0.96). Furthermore, an algorithm using rK39 followed by LAMP would yield a sensitivity of 99.2% (95%CI: 95.52-99.89) and a specificity of 98.0% (95% CI: 94.34-99.59). The findings demonstrate that LAMP assay is an accurate and rapid molecular assay for VL diagnosis, including in HIV-1 co-infected patients, in an endemic setting.

5611

## VISCERAL LEISHMANIASIS: IMPROVED MOLECULAR DIAGNOSIS USING THE MINI DIRECT ON BLOOD PCR NUCLEIC ACID LATERAL FLOW IMMUNOASSAY (DBPCR-NALFIA)

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Accurate and early diagnosis of Visceral Leishmaniasis (VL) is important to install proper treatment, because of the fatality of the condition and the high toxicity of available treatments. Current diagnostic methods include parasitology and serology (with rK39 dipstick test and direct agglutination test). These methods do have limitations (patient safety or diagnostic accuracy), and molecular testing is proposed to improve diagnosis. Current molecular tools, in particular PCR, have high accuracy for detecting VL, however their complexity and high costs make their use

unsuitable for endemic areas with limited resources. Consequently, there is a need for a simple molecular diagnostic test that can be implemented in resource limited setting. We have developed a miniaturized direct-on-blood PCR nucleic acid lateral flow immunoassay (mini-dbPCR-NALFIA) as an innovative, easy-to-use molecular assay for the diagnosis of VL in these particular settings. Unlike other simplified molecular methods, such as LAMP, the mini-dbPCR-NALFIA does not require DNA extraction and utilizes a handheld, portable thermal cycler powered by a solar-charged power pack enabling to perform the test without any laboratory infrastructure. Reading of results is done using a rapid lateral flow strip. In the present study we have conducted a laboratory evaluation on the mini db-PCR-NALFIA to determine its diagnostic accuracy. Patient samples (N=146) with suspected VL were tested using the mini db-PCR-NALFIA and compared to conventional PCR (reference test). Sensitivity and specificity represented the accuracy. Cohen's k determined the degree or agreeableness between the mini db-PCR-NALFIA and other diagnostic tests (PCR and rk39 rapid test). Compared to qPCR, the mini db-PCR-NALFIA for VL had a sensitivity of 95.83% (95% CI, 88.30%-99.13%) and a specificity of 97.22% (95% CI, 90.32% - 99.66%). The agreement between both tests was excellent (k-value: 0.93). The Limit of Detection of the platform is around 10 parasites per microliter of blood (spiked with promastigotes).

**5612**

## COST EFFECTIVENESS ANALYSIS OF CONGENITAL CHAGAS DISEASE SCREENING METHODS IN BOLIVIA

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Of the 6 to 7 million global cases of Chagas disease, Bolivia is the country with the highest prevalence, many due to congenital transmission, which is one of the routes of transmission of Chagas disease. The WHO calls for the elimination of congenital Chagas disease by 2030. This study compares the cost-effectiveness of three methods for a national screening intervention: qPCR, Western Blot, and microhematocrit (all utilized at ages zero, one, and nine months). We used decision tree analysis to compare the cost effectiveness of three screening methods: i) microhematocrit, ii) qPCR, and iii) Western blot, considering microhematocrit as the standard of care. Transition probabilities were taken from previous studies or derived as assumptions. Previous studies and primary data collection from Bolivia provided cost data. We performed one-way sensitivity analysis to test the model's uncertainty, represented through a tornado diagram. Parameters included were the methods' respective sensitivities; the probability of being tested at zero, one, and nine months; and the costs of the microhematocrit, qPCR, and Western Blot tests. The model indicates that screening intervention using qPCR is more cost-effective than using microhematocrit, considering a willingness to pay (WTP) threshold of three times the GDP per capita of Bolivia. National implementation of qPCR would result in earlier diagnosis of congenital Chagas disease, resulting in effective treatment for infants and prevention of future costs. We continue to develop our model, comparing Western Blot data with that of qPCR and microhematocrit. We also continue to analyze the effectiveness of these interventions, using DALYs to consider long-term effects. The most common method to diagnose congenital Chagas disease in Bolivia, microhematocrit, has low sensitivity: more than 40% of infected infants are misdiagnosed due to false negatives. qPCR's high cost and Western Blot's long learning curve have resulted in less frequent use. Considering the direct and indirect costs for Chagas disease patients, the two methods' overall cost-effectiveness must be re-evaluated.

**5613**

## POTENTIAL BIOMARKERS FOR ASYMPTOMATIC VISCERAL LEISHMANIASIS AMONG DEPLOYED U.S. MILITARY PERSONNEL

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Visceral leishmaniasis (VL) is caused by infection with Leishmania (L.) donovani or L. infantum (syn. L. chagasi) parasites. Despite infection, most individuals immunologically control the parasite and remain asymptomatic. However, some people progress to symptomatic VL, potentially leading to death if untreated. Although several immune biomarkers of symptomatic VL have been described; our objective was to determine new biomarkers capable of identifying asymptomatic visceral leishmaniasis (AVL), in addition to interferon gamma. Levels of chemokines/cytokines in supernatants of peripheral mononuclear blood cells (PBMC) from 35 AVL+ healthy Iraq-deployed participants, stimulated in vitro with soluble Leishmania antigen (SLA) for 72 hours, were assessed by a bead-based assay that allows the measure of multiple analytes. Additionally, we evaluated the chemokine/cytokine levels in supernatants from whole blood of 18 subsequently-immunosuppressed AVL+ Iraq and Afghanistan deployers (tumor necrosis factor α inhibitor users, n=7; renal transplant, n=4; or HIV infection, n=7), stimulated in vitro with SLA for 24 hours and tested by the same assay. We also studied blood of AVL negative controls, both healthy (n=14) and immunosuppressed (n=22). Three biomarkers: monokine induced by gamma interferon (MIG) (AUC 0.87, 71% sensitivity), monocyte chemoattractant protein-1 (MCP-1) (AUC 0.79, 91% sensitivity) and interleukin (IL)-8 (AUC 0.80, 66% sensitivity) were detected at high levels in AVL+ supernatants from stimulated cultures from healthy deployers compared to uninfected controls. MCP-1 was detected at high levels in AVL+ persons living with HIV (AUC 0.92, 100% sensitivity). In conclusion, chemokine profiling is a useful strategy for identifying cellular immune responses in AVL+ individuals. Our results show that MCP-1 is a candidate biomarker in assessing AVL in stimulated whole blood of persons living with HIV and in PBMC of healthy adults and MIG (and IL-8 to a lesser extent) is also possible biomarkers in assessing AVL+ in healthy deployers.

**5614**

## OPTIMIZATION AND VALIDATION OF RECOMBINANT ANTIGEN BASED INDIRECT ELISA FOR CUTANEOUS LEISHMANIASIS

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Cutaneous leishmaniasis (CL) in Sri Lanka is caused by Leishmania donovani, a parasite having a potential for visceralization. Even though CL is not generally considered as a disease that induces humoral responses, several recent studies have reported antibody responses against CL. This study was designed to assess the antibody responses of CL patients in Sri Lanka by optimizing and validating a recombinant rK39 and KMP11 antigen-based ELISA. Optimization of the ELISA included the checkerboard titration method to determine the optimum antigen concentration and sample dilution. The ELISA was conducted using a standard protocol after optimization. The optimum antigen concentration of rK39 ELISA was 3.0 µg/ml and the optimum sample dilution was 1:400. The optimum antigen concentration for KMP11 ELISA was 2.0 µg/ml and the optimum sample dilution was 1:50. The optimized ELISAs were conducted for 140 serum

samples from confirmed CL patients (both treated and non-treated) and for negative samples taken from healthy individuals from non-endemic areas. The established ELISA protocol using parasite crude antigen was also performed for comparison. The results were analyzed using SPSS V26.0 software and cut-off values for each antigen were determined by Receiver-Operator-Characteristic (ROC) curve. ELISA for rK39 showed 51.4% sensitivity and 60% specificity. ELISA for KMP11 showed 71.4% sensitivity and 52% specificity. ELISA for parasite crude antigen showed 84.3% sensitivity and 85.7% specificity. Therefore, the parasite crude antigen ELISA was a better assay than recombinant antigen based ELISAs for the detection of ani-leishmania antibodies. The low sensitivity and specificity of the tests are the major limitation when developing these into a reliable diagnostic tool for CL.

**5615**

## EVALUATION OF NOVODIAG® STOOL PARASITES TEST, A HIGH-PLEX STOOL TEST, AGAINST TRADITIONAL METHODS IN A HIGH-RISK TRAVELLER AND MIGRANT POPULATION AS A POTENTIAL FOR QUICKER AND MORE ACCURATE IDENTIFICATION OF INTESTINAL PARASITES

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Introduction. Identification of intestinal pathogens via stool microscopy requires highly trained personnel, multiple samples and is labour intensive. Early identification is key to limiting spread and informing treatment to prevent clinical complications. We aimed to compare traditional methods with the Novodiag® Stool Parasites test (Mobidiag, Espoo, Finland), a CE-marked high-plex test that combines real-time PCR (rt-PCR) and microarray assays for the identification of 25 pathogens. Methods. Stool samples from traveller and migrant populations were run on the Novodiag® Stool Parasites test (NSP) and a Giardia intestinalis in-house rt-PCR assay. Positive samples were analysed using traditional microscopy staining of formalin-ethyl acetate faecal concentrates for ova, cysts and parasites (OCP). Results. Between September 2022 and February 2023, 454 tests for 448 patients were run on the NSP. One assay failed due to sample inhibition. 48 patients had positive results; 4 patients had dual infection (3 on NSP and 1 on OCP). Stool OCP was performed on 46 samples, results were concordant in 25 out of 46 samples. Hymenolepis nana was identified in two OCP samples which were not identified by NSP and on two NSP samples not seen on OCP. NSP detected more Enterobius vermicularis, Schistosoma spp, Strongyloides stercoralis, Ancylostoma duodenale, Enterocytozoon bieneusi, Necator americanus and Taenia spp than OCP. Detection of Giardia was similar between standard rt-PCR and NSP; there were 19 positive Giardia samples in NSP, 17 of which were also detected by rt-PCR. One sample was detected by rt-PCR and not detected by NSP. Discussion. NSP had a greater detection rate for a wider range of pathogens than OCP and is equivalent to rt-PCR for detection of Giardia. However, as it cannot identify all intestinal parasites, it cannot replace standard microscopy, but can provide an appropriate additional diagnostic tool for identification of common pathogens with a more rapid turn-around time, greater number of pathogens identified and requires less skilled personnel.

**5616**

## A SEROLOGICAL 'TEST OF TREATMENT RESPONSE' FOR CHAGAS DISEASE

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Chagas disease, caused by the protozoan Trypanosoma cruzi, is the most prevalent parasitic disease in the western hemisphere. Treatment with available antiparasitics is lengthy, often with adverse events, and efficacy varies depending on age and stage of disease. Determining if treatment is effective is critical, as ~30% of infected individuals will develop cardiac or gastrointestinal complications that can be fatal. There is currently no standardized assay to measure treatment response and guide clinical decision-making. Accordingly, we sought to develop a 'Test of Treatment Response' (TTR) to measure post-treatment serological decline as an accepted surrogate for decreasing parasite burden. To identify potential biomarkers, we probed a peptide array comprising ~8000 sequences from 41 known T. cruzi antigens with IgG from Chagasic subjects and healthy controls. Four peptides were selected based on sensitivity against a geographically diverse panel of Chagasic sera and on responsiveness to serological decline in post-Rx samples. A prototype ELISA using the 4 peptides was tested on a longitudinal treatment cohort from São Paulo, Brazil that included samples pre-Rx and 6,12, and 36-months post-Rx. When tested on samples collected 12m post-Rx, each peptide biomarker detected serological decline (defined as ≥20% decline in IgG titer) in a larger proportion of individuals (45-71%) than conventional ELISA (22%). Using a simple algorithm to combine individual peptide results, the peptide ELISA detected serodecline in 72/76 patients at 36m post-Rx compared to 16/76 by conventional ELISA. Our results demonstrate that synthetic peptides may be more sensitive serologic indicators of treatment response than full-length recombinant or native proteins, revealing serodecline faster and to a greater magnitude than conventional ELISA. However, further studies are needed to determine the exact relationship between serodecline and parasite burden. Development and clinical evaluations of the peptide TTR assay are ongoing with the ultimate aim to provide commercial ELISA and point-of-care versions.

**5617**

## EVALUATION OF TOXOPLASMA GONDII EXCRETORY/SECRETORY AND MEMBRANE ANTIGEN FOR THE DETECTION OF INFECTION IN ACUTE PHASE BY WESTERN BLOTTING

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One of the most common zoonoses affecting humans is toxoplasmosis, which is caused by Toxoplasma gondii and represents a danger because of the significant morbidity and mortality levels in at-risk populations: immunosuppressed patients and pregnant women. Since at-risk populations need to be constantly monitored, a confirmatory test that is sensitive and specific enough to detect and differentiate the different stages of the disease is needed. To meet this objective, 25 Holtzman rats of 30 days old were infected intraperitoneally with 105 T. gondii tachyzoites of the RH strain and followed up to 90 days post infection. Blood was collected and used for disease detection using immunological tests to detect proteins and antibodies generated during infection (acute and chronic phase). The antigens used were total antigen lysate (TLA), excretory secretory antigens (E/S) and membrane antigens. Western blot results showed that using the excretory secretory (E/S) antigens, 25 kD and 30 kD bands were recognised that only appeared in the rats of the 30 and 60 day post infection (chronic phase) group. When the antigens of the membrane fraction were confronted against the sera of rats from the 60 days post infection group (chronic phase), the most significant bands detected were 80 kD, 19 kD, 15 kD, as these bands were not evident in the cytoplasmic fraction when confronted with the same sera. The T. gondii total antigen lysate (TLA) showed too many bands when confronted with rat sera from the chronic and acute group, and it was not possible to establish significant

differences between the two phases. This study concludes that excretory secretory antigens (E/S) and membrane antigens are the most optimal to differentiate the chronic and acute phases of infection because the bands can be observed better separated and with higher specificity (30 and 60 kDa in the E/S antigen) compared to TLA, where too many and in many cases non-specific bands are reported, making the diagnosis of the phase more difficult. These results will serve as a basis for studies in at-risk human populations to differentiate between chronic and acute phases of infection.

**5618**

## DRIED BLOOD SPOTS: A SUITABLE ALTERNATIVE TO USING WHOLE BLOOD SAMPLES FOR DIAGNOSTIC TESTING OF VISCERAL LEISHMANIASIS IN THE POST-ELIMINATION ERA

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Serum or whole blood collection, processing, transport and storage still represent a significant challenge in low resource settings where mass surveillance is required to sustain disease elimination. Therefore, in this study, we explored the diagnostic efficacy of dried blood spots (DBS) as a minimally invasive and potentially cost-effective alternative sampling technique to whole blood sampling procedures for subsequent detection of Leishmania donovani antibodies or DNA. Archived serum, DNA samples from whole blood of visceral leishmaniasis (VL) cases and healthy controls, and DBS from corresponding cases and controls, were used. Both molecular and serological assays were optimized to detect L. donovani antibodies or DNA in DBS elute and results were compared against those obtained with whole blood. Serological assays (both rK28 ELISA and rK39 ELISA) of DBS samples showed sensitivity and specificity of 100% and had excellent agreement with results from whole blood samples (kappa value ranged from 0.98-1). Bland-Altman analysis of OD values from rK28-ELISA with DBS elute and patients' serum showed an excellent agreement (ICC=0.9) whereas a good agreement (ICC=0.8) was observed in the case of rK39-ELISA. However, qPCR and RPA of DBS samples had a diminished sensitivity of 76% and 68 %, respectively, and poor agreement was observed with the whole blood samples.Our results demonstrate that DBS offer excellent diagnostic efficiency for serological assays and represent a viable alternative to whole blood sampling procedures.

**5619**

## ACCURACY OF THE "TESA-BLOT RAPID TEST" FOR THE DIAGNOSIS OF CHAGAS DISEASE

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A common form of Trypanosoma cruzi transmission is vertical mother-to-child transmission, which is the main form of transmission in nonedemic countries in addition to being a significant -but neglected-contributor in endemic regions. Lack of awareness and timely diagnosis in rural endemic areas, exacerbate high infection rates, with more than one million Latin American women of childbearing age are infected with Chagas disease. Only approximately 10% of these women are aware of their infection leaving thousands of newborns at risk of being born infected. The lack of access to rapid and accurate diagnostic assays makes it difficult to detect positive mothers, especially since many of these women have limited contact with healthcare workers prenatally and only come to the hospital for delivery and then return to a rural area. Conventional diagnostic tests- such as HAI, IFA, and ELISA-. lack reproducibility and reliability of results and take hours to process. Therefore, the test results are often not available until after the mother has left the health centre. In order to address this issue, we developed a rapid Western blot (using the trypomastigote excretory-secretory antigen), which has a sensitivity of 100% and a specificity of over 94%. For the evaluation of the standardized "TESA Blot rapid test", 35 samples from mothers and samples from their babies (34 samples) were tested, comparing the standard TESA to the new reapid TESA. In conclusion, it was possible to detect patients in the acute phase in 25 minutes and the chronic phase in 35 minutes, compared to the 14 hours needed for the original TESA blot. This allows for timely follow-up and treatment for the mother and newborns.

**5620**

## EVALUATION OF TRYPANOSOMA CRUZI AMASTIGOTE ANTIGENS IN CARDIAC TISSUE AT DIFFERENT POST-INFECTION TIMES

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Chagas disease is caused by Trypanosoma cruzi and affects between 6-7 million people mainly in Latin America. Many people in the acute phase have no symptoms and in some cases only general symptoms are present, which makes diagnosis difficult. It is known that 30% of infected persons develop cardiac manifestations after 20-30 years of infection. The development of chronic cardiomyopathy in infected persons increases the risk of death by 20% compared to other types of cardiomyopathy. Currently, government programs are not efficient in monitoring and diagnosing the disease. Therefore, it is important to develop better tools in in vivo models to understand the development of the pathogenesis of the disease. It is difficult to identify the presence of amastigotes when tissue parasitism begins to decline. The availability of specific polyclonal antibodies, conjugated with fluorophores or HRP, against amastigote would be a good alternative to identify them in a more efficient way. Therefore, axenic amastigotes, free of cells and other stages, were obtained by amastigogenesis from trypomastigotes of strain Y, cultured in LL-CMK2 cells and RPMI medium at 2% SFB. Trypomastigotes were incubated in medio Dulbecco's Modified Eagle's medium alto en glucosa (hgDMEM) with 20 mM MES buffer at pH 5 with 0.4% BSA (condition I) and without BSA (condition II). The evaluation of the transformation kinetics was performed by counting in Neubauer hemocytometer where it was observed that at 4 h of incubation 75% and 95% of the incubated parasites were transformed to amastigotes, in condition I and II, respectively. While at 8 h of incubation 97% and 100% were observed for condition I and II respectively, however with condition I at 12 h, 100% transformation was also observed. According to these results, successful amastigogenesis was obtained in both conditions, with faster transformation in condition II. These results contribute to the production of polyclonal antibodies from immunizing rabbits with the lysate of these amastigotes. In order to identify more specifically, by immunofluorescence or immunohistochemistry, the presence of tissue amastigotes in animals.

**5621**

## COMPARATIVE ANALYSIS OF A CHAGAS DISEASE RAPID DIAGNOSTIC TEST (RDT) FOR THE DETECTION OF ANTI-TRYPANOSOMA CRUZI ANTIBODIES AMONG SERUM COLLECTED FROM MULTIPLE REGIONS OF COLOMBIA

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Chagas disease (CD) is known to effect those living in impoverished and vulnerable circumstances, placing those at risk for vector-borne and oral transmission of Trypanosoma cruzi within endemic regions. Rapid Diagnostic Tests (RDT) for CD can be utilized in the field, local clinics and health facilities with limited access to a laboratory which requires specialized equipment and trained technicians. Serum collected and stored (-80C°) from individuals living within the CD endemic regions of

Sierra Nevada de Santa Marta (SNSM), Urabá, Boyacá, and Chocó, regions of Colombia from 2003 until present day, were tested with a lateral flow immunochromatographic assay (DPP® Chagas System; Chembio Diagnostic Systems, Inc.) and compared with confirmed positive and negative stored samples. Among these serum samples, CD was confirmed positive (n=102) with positivity to both T. cruzi IgG ELISA and Immunofluorsecence Antibody (IFA) assays developed at the Universidad de Antioquia (UdeA) in MedellÍn, Colombia, from a T. cruzi isolate cultured from SNSM region of Colombia. Sensitivity and specificity of the UdeA T. cruzi IgG ELISA and IFAT among Colombian serum samples is 98% and 97%, respectively. DPP® Chagas System RDT utilizes a Micro Reader to interpret the immunochromatographic results with either, non-reactive, indeterminate, or reactive. Following manufacturer's protocol, 5µl of serum from both positive and negative serum samples were tested with DPP® Chagas System and results were available within 15 minutes. Results of testing reveal sensitivity of 98.88% [95% CI 93.96-99.97% (n=89/90)] were true negative and specificity of 97.05% [95% CI 91.65-99.39% (n=99/102)] were true positive. Failure rate of testing was 1.04% (n=2/192) in which testing had to be repeated due to non-functioning cartridge or invalid result. The DPP® Chagas System RDT was easy to use and provided both highly sensitive and specific results among those living with CD from these regions of Colombia. The use of RDTs for CD screening and diagnostics can help reach populations with limited resources in Colombia and other endemic regions.

5622

## DETECTION OF A TOXOPLASMA GONDII ANTIGENIC PROTEIN AND ITS POTENTIAL USE IN THE NONINVASIVE DIAGNOSIS OF TOXOPLASMOSIS

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Toxoplasma gondii is an intracellular parasite that can infect all warm-blooded living beings, including man. In immunocompetent patients the process is asymptomatic, however cysts persist for life in tissues, when the individual is immunosuppressed (transplants, pregnancy, HIV) reactivation of the active stage of T.gondii can become fatal. Non-invasive and confirmatory techniques are needed in order to detect active T.gondii infection in immunosuppressed individuals. A total of 20 urine samples from HIV patients separated into 2 groups were used: Group I: 10 HIV patients with suspicion of active toxoplasmosis with Cq values (35-20) in CSF samples in the amplification of a REP529 fragment, IgG positive and with a clinical diagnosis of Toxoplasmosis, Group II: 10 HIV patients negative for T.gondii. Hydrogel nanoparticles will be used in order to concentrate small proteins ≤30kD in urine, followed by dot blot detection of a dense granule protein (GRA1) of T.gondii. In GRA1 positive patients, a black spot was observed on the PDVF membrane, detecting GRA1 in 40% (4/10) patients in Group I, and in 0% (0/10) of patients in Group II. The use of nanoparticles in urine samples allowed the detection of GRA1, a dense granule protein of Toxoplasma gondii involved in the development of an active stage or reactivation stage due to immunosuppression, making this technique a promising tool for the non-invasive diagnosis of toxoplasmosis.

5623

## ASSESSING THE TSETSE FLY MICROBIOME COMPOSITION AND THE POTENTIAL ASSOCIATION OF SOME BACTERIA TAXA WITH TRYPANOSOME ESTABLISHMENT

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Tsetse flies are biological vectors of trypanosomes which cause African trypanosomiasis. No vaccine is available, and drugs are toxic with increasing emergence of resistance. Reducing vector competence can be additive tools to stop disease transmission. Some bacteria have been shown to be used as paratransgenic organisms capable of blocking trypanosome's development in flies. Understanding the role of tsetse microbiome in disease transmission could improve knowledge in initiatives to develop new vector control strategies. We aim to determine the microbiome composition of tsetse flies and their association with trypanosome establishment. Tsetse flies were collected from Campo, southern Cameroon and total DNA was extracted from fly bodies and heads separately. Trypanosome species were identified by PCR. Amplification of the V3-V4 region of the 16S rRNA gene followed by sequencing on Illumina miseq with subsequent metagenomic analyses were performed to identify the different bacteria communities. PCR analysis of 2186 Glossina p. palpalis revealed 20.08% trypanosome infections with Trypanosoma congolense (13.73 %,) the predominant species; 0.17 % were T. b. gambiense. 21.27% of infected flies produced mature infections. From 192 samples randomly sequenced, a total of 31 bacteria genera were identified with the primary symbiont Wigglesworthia displaying 47.29% abundance. Globally, significant differences were observed in the microbiome diversity among tsetse species, between teneral and non-teneral flies and between flies displaying or not displaying mature trypanosome infections. In addition, differential abundance testing showed several bacteria taxa such as Dechloromonas, Ralstonia and Serratia associated with trypanosome maturation in tsetse flies.This study has shown some bacteria associated with trypanosome infection maturation in flies, which therefore need further studies to investigate an understanding of their mechanism of action and alternatively, transformed and used to block trypanosome development in tsetse flies.

5624

## SIMILARITIES BETWEEN GENES FOR TRYPANOSOMA CRUZI MICROTUBULE ASSOCIATED PROTEINS AND HUMAN INTERFERONS

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Trypanosoma cruzi Microtubule Associated Proteins (MAPS) are encoded by a family of homologous genes. We have previously shown that one of these genes, coding for the T. cruzi human serodiagnostic antigen Ag36, is similar in gene sequence to human immune Tripartite Motif (TRIM) proteins, such as TRIM21. Since TRIM proteins are intrinsic to human innate immunity, we examined several cytokines key to innate immunity; for example, human Interferon and Interleukin genes. The Needleman-Wunsch Algorithm, which measures and scores the extent of similarity of two gene sequences, was used to compare Genbank M21331 (the Ag 36 gene) with these two genes, respectively. The tools and gene sequence analysis were performed and the results stored at https://usegalaxy.eu. Human Interferon gamma showed 17.9% identity, Human Interferon alpha displayed 13.6% identity, and Human beta Interferon indicated a 12.6% identity to M21331. Human Interleukin genes showed no significant similarity to M21331. The possible implications during T. cruzi infection and manifestation of Chagas' disease of these similarities to human Interferon genes and the 33 T. cruzi MAP genes will be discussed.

5625

## HYBRID ASSEMBLY OF THE LEISHMANIA (VIANNIA) PERUVIANA GENOME

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Leishmania (Viannia) braziliensis complex encompasses two closely related species (L. (V.) braziliensis and L. (V.) peruviana) with different geographical distributions and disease phenotypes. Unlike L. (V.) braziliensis which has been extensively studied, L. (V.) peruviana is still largely uninvestigated. Furthermore, this latter species lacks a reliable or complete reference genome which is key for population genetics, functional studies, and surveillance. In this study we report a high-quality genomic sequence of L. (V.) peruviana obtained from combining long and short reads generated by high-throughput sequencing. For this purpose, genomic DNA from the MHOM/PE/87/PAB2880 L. (V.) peruviana strain was sequenced on

MinION nanopore and Illumina NovaSeq. The resulting Nanopore reads were used for de novo assembly using Flye 2.9.1 to generate contigs and chromosome scaffolds. Then Illumina reads were aligned onto these scaffolds with Minimap 2 2.17 to perform error correction, close gaps, reduce sequencing errors, and generate a consensus sequence. Nanopore sequencing resulted in 84.33Mb estimated bases contained in 19.24K unpaired reads (Length N50=10.89K) whereas Illumina sequencing resulted in 3.56Gb paired reads contained in 23.5M reads (Length N50=151bp). A BLAST search against the NCBI database showed that all reads aligned with at least one species of the Leishmania (Viannia) braziliensis complex. The kinetoplast genome (kDNA) was contained in a single 23.1Kb scaffold whereas the nuclear genome was contained in 197 scaffolds with a total length of ~35Mb (N50=36Kb). This version of the genome constitutes an improved assembly compared to the one previously reported for this species which was limited to 25Mb fragments into 27,873 scaffolds (N50=1.3Kb). Referenced-based, de novo gene annotation and a deeper analysis of chromosome structure, copy number, and single nucleotide polymorphisms are currently being conducted on this improved assembly. Our results will provide a foundational backbone for comparative genomics, phylogenetics, and surveillance of Leishmania (Viannia) peruviana.

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## MECHANISM OF INTESTINAL BARRIER REPAIR IN GIARDIASIS

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The protozoan parasite Giardia duodenalis is the cause of the common diarrheal disease, giardiasis. Diarrhea is one of the most severe acute symptoms, however, infections can be subclinical. It is transmitted via ingesting infectious cysts in contaminated water or food. Giardiasis is one of the main causes of growth stunting in children under the age of two in developing countries. It has been demonstrated that alterations in intestinal barrier integrity contribute to reduced nutrient absorption, which leads to growth stunting in children. Although intestinal barrier abnormalities have been associated with infection, nothing is known about how the intestinal barrier repairs itself after an infection. Previous studies have demonstrated that Giardia infection is associated with intestinal dysbiosis in humans and animals. We are investigating the function of aryl hydrocarbon receptor (AHR) in barrier repair after a Giardia infection because AHR signaling has been demonstrated to promote intestinal repair in other systems. We measured the levels of particular AHR ligands originating from the microbiome in the plasma of infected C57BL/6 mice and saw a decrease in indole-3-ethanol and indole-3-pyruvic acid after 21 days of infection. As IL-22 signaling can also enhance barrier repair, we quantified IL-22 transcripts by RT-PCR and found significantly elevated levels of IL-22 mRNA in infected animals. Our findings suggest that Giardia can inhibit barrier repair by altering the microbiome's ability to produce AHR ligands, but that in some situations, IL-22 can reverse this defect. This study will give better insight into developing effective dietary interventions to prevent growth stunting in infected children.

5627

## GENOMIC ANALYSIS DEMONSTRATES EXTENSIVE DIVERSITY AND SUBTLE POPULATION STRUCTURE IN PLASMODIUM VIVAX ACROSS 9 DISTRICTS OF ETHIOPIA

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Ethiopia suffers the greatest burden of Plasmodium vivax in the African continent with infections contributing as much as 40% of malaria morbidity. Efforts to contain and eliminate P. vivax are constrained by limited knowledge of the major adaptations and epidemiological drivers sustaining local endemicity. We conducted genomic analysis of 155 P. vivax genomes across 9 districts in Ethiopia. The genomic data was generated within the MalariaGEN P. vivax Community Project, using isolates sourced from cross-sectional surveys conducted in Ethiopia from 2012-2016. Measures of within-host and population diversity and structure were conducted using scikit-allel, ADMIXTURE, hmmIBD and custom scripts. Signatures of selection were detected using REHH software with cross-country comparisons against MalariaGEN data from sites in Thailand (n=129) and Indonesia (n=191) with respective low and high levels of chloroquine resistant P. vivax. A high proportion of Ethiopian infections were polyclonal (26%), with 44.7 % comprising clones with high relatedness (identity-by-descent >25%), indicating frequent co-transmission and superinfection. Several infection networks comprised isolates from neighbouring as well as more distal districts, indicating complex patterns of infection spread between communities. Amplification of the Duffy binding protein gene (pvdbp1) was observed at high frequency in 8 districts (range 16%-75%), with up to 5 copies, suggestive of an important local adaptive function. Cross-population comparisons of haplotype homozygosity found evidence of positive selection in a region proximal to the chloroquine resistance transporter gene (pvcrt-o), which has been implicated in chloroquine resistance. The genomic patterns in Ethiopia highlight adaptations of potential public health concern in an endemic setting with moderately high and stable transmission within and between districts.

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## IN VITRO TRANSCRIPTOMIC REMODELING OF CARDIOMYOCYTES CAUSED BY TRYPANOSOMA CRUZI

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Chagas disease can lead to cardiac dysfunction or fatal arrythmias. while these manifestations occur more frequently in geographic areas more prevalent with the TcI/II circulating genetic strains of Trypanosoma cruzi; strain-specificity in the expression of disease is influenced by yet-to-be-determined factors. To define the cardiomyocytes differential transcriptomic responses resulting from infection with different T. cruzi strains and explore their relationships with pathogenesis, HL-1 cardiomyocytes were infected with TcI/II or TcVI T. cruzi trypomastigotes. RNA was serially isolated post-infection for microarray analysis. Enrichment analyses of differentially expressed genes highlighted the most affected biological pathways. We found that Oxidative stress-related GO terms, 'Hypertrophy model', 'Apoptosis', and 'MAPK signaling' pathways (all with $p < 0.01$) were upregulated as a common response to all T. cruzi strains. 'Glutathione and one-carbon metabolism' pathway, and 'Cellular nitrogen compound metabolic process' GO term (all with $p < 0.001$) were upregulated exclusively in the cardiomyocytes infected with the TcI/II strains. Upregulation in the oxidative stress-related and hypertrophic responses are shared hallmarks with myocarditis caused by Coxsackie virus, another inflammatory cardiac pathology. Nitrogen metabolism upregulation and Glutathione metabolism

affection may represent the relation of nitrosative stress and poor oxygen radicals scavenging in the pathophysiological events that lead of chagasic cardiomyopathy.

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## GENOME ASSEMBLY OF TRYPANOSOMA CRUZI TULAHUEN STRAIN REVEALS HIGHLY ABUNDANT TRANSPOSABLE ELEMENTS ASSOCIATED WITH VARIABLE SURFACE PROTEINS

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Trypanosoma cruzi is the causative agent of Chagas disease, which causes 10,000 deaths per year. Despite the high mortality caused by the disease, there is very little whole genome data available for the parasite. T. cruzi has a highly repetitive genome with multiple copies of variable surface proteins, as well as large scale structural genome rearrangements, which makes resolving whole genomes incredibly challenging. Even laboratory strains frequently used in biomedical research do not have publicly available genomes. Using long read Nanopore sequencing, we have generated a high-quality, partially phased whole genome assembly of the hybrid Tulahuen strain, a Type VI strain commercially available from ATCC. Using automated tools and manual curation we have annotated transposable elements in our newly assembled genome. We report a genome with 35% simple repeats, 24% surface proteins, and 22% transposable elements. Moreover, we find that regions with transposable elements are significantly enriched for surface proteins, and that on average surface proteins are closer to transposable elements compared to other coding regions. This finding supports an interesting possible mechanism for diversification of surface proteins that involves mobile genetic elements such as transposons.

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## ZOONOTIC HEPATITIS E VIRUS GENOTYPE 3 STRAIN DETECTED IN A CAPYBARA (HYDROCHOERIS HYDROCHAERIS) FECAL SAMPLE, BRAZIL

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Hepatitis E virus (HEV) is an emerging zoonotic pathogen associated to relevant public health issues. HEV was reported infecting wild rats worldwide; however the importance of wild rodents as potential HEV reservoirs or their zoonotic role is still unknown. Capybara (Hydrochoerus hydrochaeris) is the world´s largest rodent species distributed throughout South America and known to carry potentially zoonotic agents. The aim of this study was to investigate the HEV species Paslahepevirus balayani's presence in free-living capybaras inhabiting urban parks in São Paulo state, Brazil. Molecular characterization and phylogenetic analysis of positive samples was also undertaken. A total of 337 fecal samples collected during 2018-2020 were screened for HEV using RT-qPCR and confirmed by conventional nested RT-PCR targeting ORF1 and ORF2 regions. HEV genotype and subtype was determined combining Sanger and next generation sequencing. HEV was detected in one specimen (0.3%) collected in 2019, and assigned as HEV-3f. Phylogenetic analysis of ORF1 and ORF2 regions revealed that Brazilian capybara HEV-3f strain are closely related to European swine, wild boar and human strains (90.7%-93.2% nt), suggesting an interspecies transmission. Molecular epidemiology of HEV is poorly investigated in Brazil and so far, subtype 3f has only been reported in swine, hampering that more robust conclusions could be drawn from the phylogenetic analysis. This is the first detection of HEV in capybaras stool samples. Nevertheless, this data must be treated with caution once identification of HEV in these mammals may not necessarily be associated with natural infection and not indicate that these animals play a role in the HEV transmission. Capybaras are continuously exposed to fecal-borne viruses as these animals display semi-aquatic habit and are living in human-animal interface environments. The implementation of systematic molecular surveillance of HEV in the One Health concept, including human, wildlife, domestic pigs, wild boars and environment samples are vital to elucidate HEV-3 subtype's role and circulation in the country.

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## ASYMPTOMATIC VISCERAL LEISHMANIASIS PREVALENCE IN MILITARY WORKING DOGS COMPARED TO SOLDIERS DEPLOYED TO IRAQ

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Visceral leishmaniasis (VL) is a zoonotic, vector-borne disease that poses a significant health risk to canines and humans. We previously reported 19.5% of American Soldiers deployed to Iraq had asymptomatic visceral leishmaniasis (AVL). Canines are the main reservoir of Leishmania infantum, a cause of VL. Military Working Dogs (MWDs) deploy worldwide and work closely with Soldiers. The aim of this study was to determine the rate of AVL in MWDs that deployed to Iraq to assess their infection and transmission risk. This retrospective study examined records of 104 MWDs that deployed to Iraq from the Remote Online Veterinary Record database and the Federal Records Center between 2005-2022. Banked pre- and post-deployment serum and whole blood samples were obtained from the Department of Defense Food Analysis and Diagnostic Laboratory. Leishmania diagnosis was confirmed using the canine rk39 Rapid test, Indirect Fluorescent Antibody (IFA) Assay and quantitative polymerase chain reaction (PCR). The prevalence of canine VL was 2.9% (n=3/104); 2 were PCR positive, one seroconverted. Sand fly prevention was documented in 65% of the MWDs. Of the 3 MWDs that tested positive, 66% (n=2) did not receive sand fly prevention and 100% were asymptomatic for VL. The odds of a VL positive test were 4.1 times more likely in a MWD without sand fly prevention compared to MWDs with sand fly prevention. Iraq-deployed human American Soldiers who were tested for AVL responded on a survey that 138/200 (69%) rarely or never used insect repellent, 14 (7%) worked or handled MWDs, 18 (9%) cared for local dogs, and 75 (37.5%) reported others had local dogs in their unit. Despite canines being the main host and reservoir for L. infantum, MWDs deployed to Iraq had a considerably lower VL prevalence than their human counterparts. Increasing the use of sand fly prevention in leishmaniasis endemic areas may better protect Soldiers deployed to these regions."The opinions and assertions expressed herein are those of the author(s) and do not reflect the official policy or position of the Uniformed Services University of the Health Sciences or the Department of Defense."

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## DOES CAVE USE POSE A RISK FOR PATHOGEN SPILL? A CASE OF CHEKWOPUTOI CAVE IN MT ELGON EASTERN UGANDA

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Bats are increasingly singled out as a source of disease pathogens, what is not very clear though, is how the pathogens cross over to humans. Using camera traps in caves with known bat colonies, we monitorg the inner cave usage by bats and other vertebrates to establish if there may be a possible connection that could provide an avenue for dispersal of bat borne pathogens to humans or human environments.Our preliminary results suggest continuous bat activity in their cave day roost, which we suspect is a result of presence of some other vertebrate in the cave roost that stirs the bats to fly around.From camera trap data so far our results show: --The continued presence of bats in the cave during the day but not in the night.-A total six other vertebrate species (including humans) present in the cave when the bats were also in the cave.-No direct depredation on bats by the other vertebrates in the cave.We continue to monitor the cave use by the bats and the other vertebrates to understand what the potential risk for pathogen (if ever they are determined to be in the bats) spill could be. Two of the species (genet and civet are carnivores that could potentially eat bats). The porcupine and giant rats could potentially feed on guano while all species are exposed to aerosols in the cave. Two species the Porcupine and the Giant rats are species commonly hunted for meat by some members of the local community.

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## ACTIVITY PATTERNS OF INSECTIVOROUS BATS IN THE MT. ELGON REGION-UGANDA: IMPLICATION FOR DISEASE SURVEILLANCE

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Uganda is home to over 110 bat species, including 77 species of insectivorous bats playing critical roles of economic importance including pest insect consumption, pollination and fertilizer production. However, some bat species are associated with highly pathogenic viruses such as coronaviruses, paramyxoviruses and filoviruses which pose public health concerns. It has always been a suppositional debate on how these viruses spill-over to humans. In Uganda, humans visit caves to collect manure, minerals and use caves as shelter for both humans and their animals. This poses a spill-over risk of potentially infectious agents. We are currently undertaking a five-year project in Kapchorwa district for bio-surveillance of potential zoonotic pathogens in bats. Where we also investigate the ranging patterns of bats in this region using LOTEK GPS tags to understand how far bats move from their cave roosts to forage and how this behaviour can predispose human communities to disease outbreak should a bat be carrying pathogens; this method has proven useful in providing insightful information on bat species' movements. However, the small size of some insectivorous bats makes it impossible to have a GPS tag mounted on their back. Furthermore, the nocturnal and elusive behaviours of bats make it difficult to capture them for tagging. Using SM4+ bat Detectors we passively monitored the presence and activity of the insectivorous bats species to inform our understanding of the bat community composition in the region. Our results so far point to over 14 species recorded in human occupied landscapes; two of which species have previously shown positive tests for infectious pathogens. We continue collecting long term acoustic data to enable our understanding of seasonal bat activities to enable us draw inferences on the potential risk of spill over of pathogens to humans. Overall, our research underscores the importance of continued research into bat ecology and zoonotic disease surveillance, and will also lead to generating a national library of acoustic for insectivorous bats which shall be point of reference for further studies of bats in Uganda.

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## FACTORS INFLUENCING BAT BORNE VIRAL PATHOGENS PREVALENCE AND SPILL OVER IN UGANDA: IMPLICATIONS FOR ONE HEALTH INITIATIVES

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Although bats have been implicated as one of the key reservoirs for various viral pathogens, and also reported to participate in spill over of pathogens; there is still no very clear evidence at what critical threshold of events the viral pathogens spill to humans. And yet, like many other vertebrates, bats have lived with several types viruses for a long time. In recent decades emerging diseases such as Ebola, Murburg, SARS and other viral zoonotic diseases linked to bats, continue to pose critical global public health concerns. This research attempts to contribute to the body of knowledge of how and when bat-borne viral pathogens could spill. Using systematic review, predictive modelling approaches, and active sampling of bat species to detect viral pathogens in different cave roosts of Mt Elgon region, Uganda; we seek to identify the critical threshold levels of spatiotemporal, ecological, physiological and anthropogenic determinants that might trigger bat-borne virus spill over. We suggest that an interplay of several factors at given thresholds might be responsible for triggering likelihood for spillovers. We predict the critical level of interaction of environmental factors, seasonal factors, bat community ecology, bat population size, roost characteristics, roost fidelity by bats, human activity in the caves, and bat physiological stress; that would influence the occurrence and spill over of bat-borne viral pathogens. This in turn is linked to the implications for One health initiatives.

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## PURCHASE, CONSUMPTION, AND OWNERSHIP OF CHICKENS AND CHICKEN PRODUCTS AMONG HOUSEHOLDS IN MAPUTO, MOZAMBIQUE: A CROSS-SECTIONAL STUDY

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Poultry farming provides an important source of income and nutrition in low- and middle-income countries; however, chickens are also the major reservoir for zoonotic enteropathogens, which are responsible for a significant burden of foodborne illness. Informal food systems pose the risk of transmission of enteropathogens from poultry to humans. To gain a better understanding of the potential exposure to enteropathogens of poultry origin, we conducted one of the first cross-sectional, population-based surveys assessing chicken consumption, purchase, and rearing practices using in Maputo, Mozambique. We surveyed 570 households using a structured questionnaire between May and June 2021. We found that about half of 570 households purchased broiler chicken meat (weighted n=250, 44.8%) and eggs (263, 46.5%) in the week leading up to the survey. The most common location where households purchased broiler chicken meat was corner stores (i.e., small stores on streets) (weighted n=141, 56.7%), followed by informal wet markets (43, 17.5%) and directly from farmers (45, 16.8%). Of 570 households, 97 (16.4%) reported that they raised live chickens at the time of the survey. Chicken feces were observed on the floor or ground at 49 (51%) of these 97 households. Of 39 households with children under five that raised chickens, 11 (28%) reported that their children take care of live chickens. Of 570 households, 58 (10.2%)

reported that they applied chicken litter compost to their gardens. Our study highlights the importance of implementing food safety measures, particularly at informal food systems that often do not have access to adequate hygiene facilities, to control foodborne illnesses resulting from poultry products.

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## HIGHLY PATHOGENIC AVIAN INFLUENZA A (H5N1) IN MARINE MAMMALS AND SEABIRDS IN PERU

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Highly pathogenic avian influenza (HPAI) A/H5N1 viruses (lineage 2.3.4.4b) are rapidly invading the Americas, threatening wildlife, poultry, and potentially evolving into the next global pandemic. In November 2022 HPAI arrived in Peru, causing massive pelican and sea lion die-offs. We report complete genomic characterization of HPAI/H5N1 viruses in five species of marine mammals and seabirds (dolphins, sea lions, sanderlings, pelicans and cormorants) sampled since November 2022. All Peruvian viruses belong to H5N1 lineage 2.3.4.4b, but they are 4:4 reassortants where 4 genomic segments (PA, HA, NA and MP) position within the Eurasian lineage that initially entered North America from Eurasia, while the other 4 genomic segments (PB2, PB1, NP and NS) position within the American lineage (clade C) that was already circulating in North America. These viruses are rapidly accruing mutations as they spread south. Peruvian viruses do not contain PB2 mutations E627K, D701N, K702R previously linked to mammalian host adaptation and enhanced transmission, but at least 8 novel polymorphic sites warrant further examination. This report of HPAI A/H5N1 in marine birds and mammals from South America highlights an urgent need for active local surveillance to manage outbreaks and limit spillover into humans of a highly pathogenic avian influenza strain with clear pandemic potential.

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## EXPLORING THE HEALTH SEEKING BEHAVIOR OF SNAKEBITE VICTIMS AND COMMUNITY PERCEPTIONS IN THE VOLTA AND OTI REGIONS OF GHANA

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Snakebite envenoming is a neglected tropical disease with incidence growing globally. In Ghana, an average estimate of 9,600 snakebites occur annually ,thus a serious public health concern . Mortality rates due to snakebites are linked to delays in reporting for treatment in health facilities. This study examined health-seeking behavior of snakebite victims and community members perceptions on treatments for victims of snakebites in the Volta and Oti Regions of Ghana. This was a mixed method study conducted in ten (10) selected communities in both the Volta Region (Ho Municipality, Kpando Municipality) and Oti Region (Nkwanta North District, Jasikan District). Communities were selected using purposive sampling techniques, and data were analyzed using the Statistical Package for the Social Sciences (IBM SPSS) version 26.0. Descriptive statistics was used for sociodemographic characteristics. Bivariate and multivariate binary logistic regression analysis was used to assess the factors associated with health-seeking behavior among participants. A total of 397 participants were included in the study, with a snakebite incidence of 15.9% among community members. The mean age of participants was 39.56 (±SD=14.78) with 42.3% being females and 57.7% males. This study found 43% of participants had a family member who had been bitten by a snake. The multivariate logistic regression analysis showed that those whose family members previously experienced snakebites were more likely (aOR=1.93, 95%CI:1.25-2.99) to seek health care than those who had never experienced snakebites. In Kpando,54% of participants attended the traditional healer as their first treatment for snakebites while Jasikan, Kpassa and Ho showed higher attendance to health facilities with the highest (86%) in Ho. There exists high awareness among community members about seeking healthcare from a health facility however they still believe in using traditional remedies or visiting the traditional healer. Culturally appropriate interventions that seek to improve the health-seeking behavior of snakebite victims within the community will be imperative to reduce the burden.

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## PREVALENCE AND RISK FACTORS FOR HUMAN LEPTOSPIROSIS IN A PASTORALIST COMMUNITY, ENDULEN, TANZANIA

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Although leptospirosis is suspected to be common in rural Tanzania, data are limited. We sought to determine leptospirosis prevalence, identify infecting Leptospira serogroups, and investigate risk factors for leptospirosis in a rural area of Tanzania where pastoralist animal husbandry practices and sustained livestock contact are common. We enrolled patients at Endulen Hospital, Arusha Region, Tanzania, presenting with a history of fever within 72 hours, or a tympanic temperature of ≥38.0°C. We administered structured questionnaires covering recent symptoms, animal-related activities, and livestock health. Serum samples were collected at enrolment and 4-6 weeks later and were tested using microscopic agglutination testing with 20 Leptospira serovars from 17 serogroups. Leptospirosis was defined as a ≥four-fold rise in antibody titer between acute and convalescent serum samples, or a reciprocal titer ≥400 in either sample. Leptospira seropositivity was defined as a single reciprocal antibody titer ≥100 in either sample. The predominant reactive serogroup was that with the highest titer. We explored risk factors for leptospirosis and Leptospira seropositivity using multivariable logistic regression modelling. Of 229 participants, 99 (43.2%) were male and the median (range) age was 27 (0, 78) years. Participation in at least one livestock related activity was reported by 160 (69.9%). We identified 18 (7.9%) cases of leptospirosis, with Djasiman 8 (44.4%) and Australis 7 (38.9%) the most common predominant reactive serogroups. Overall, 69 (31.1%) participants were Leptospira seropositive and the most common predominant reactive serogroups were Icterohaemorrhagiae 21 (30.0%), Djasiman 19 (27.1%), and Australis 17 (24.3%). Milking cattle (OR 6.27, 95% CI 2.24-7.52) was a risk factor for leptospirosis, and milking goats (OR 2.35, 95% CI 1.07-5.16) was a risk factor for Leptospira seropositivity. Leptospirosis caused nearly one in

twelve hospitalizations with febrile illness in this predominantly pastoralist population. Interventions that reduce risks associated with milking livestock may reduce human leptospirosis.

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## CRIMEAN CONGO HEMORRHAGIC FEVER IN TANZANIA: RELEVANCE OF ONE HEALTH APPROACH ON UNDERSTANDING THE EPIDEMIOLOGY OF A PRIORITY ZOONOSIS

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The World Health Organization's Research and Development Blueprint lists Crimean-Congo hemorrhagic fever (CCHF) virus on their priority A list and hemorrhagic fever diseases are on the list of priority pathogens in Tanzania due to high risk to public health. A One Health approach is necessary to study CCHFV, a tick-borne zoonotic virus, that circulates between ticks and several vertebrate hosts without causing overt disease and thus can be present in areas without being noticed. A systematic sampling method is conducted across a gradient of different human disturbance levels such as pristine, pastoral and agro-pastoral areas in northern Tanzania to assess CCHF seroprevalence and prevalence in ticks, small mammals, cattle and people. More than a thousand ticks have been collected and identified thus far for virus detection by polymerase chain reaction (PCR). Five mainly genera of ticks have been collected and identified namely Rhipicephalus, Amblyomma, Hyalomma and Haemaphysalis. This One Health approach to sample people, cattle, small mammal and vector populations simultaneously will strengthen the study's power to identify associations among these populations as well as risk factors for virus exposure and circulation across the gradients of environmental disturbances. By One Health approach from this study we will be able to create research-based data on the epidemiology of CCHF virus in ticks, cattle, small mammals, people and risk factors associated with the virus exposure. Furthermore, with very limited data present on CCHF in Tanzania, findings from the study will be important in improving Tanzania's capacity to manage the risk of CCHF as well as making evidence-based prevention and control recommendations.

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## ARPHILAKE CONSORTIUM COMBATING ANTIBIOTIC RESISTANCE IN PHILIPPINES' LAKES: ONE HEALTH UPSTREAM INTERVENTIONS TO REDUCE THE BURDEN

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Lakes are critical aquatic ecosystems and provide essential natural resources for populations in low-middle income countries like the Philippines. A growing concern has been the contamination of lakes with determinants conferring antimicrobial resistance (AMR) from animal and human sources. In particular, a growing literature supports that beta-lactam resistance is a burgeoning issue in Asian lakes and rivers. However, few interventions have been rigorously evaluated to determine if the impact of environmental contamination from hospitals and agriculture and livestock production can be reduced. Here, we propose specific interventions with pre- and post-evaluations to determine their impact on beta-lactam resistance. Following a One Health the ARPHILAKE consortium composed of partners from Canada, Spain, UK, Ireland, and the Philippines will: i) Conduct environmental monitoring to identify antimicrobials and antimicrobial resistance genes in Laguna Lake and surrounding areas for mitigation at point sources; ii) Enable feasible, economic, and effective treatment method for farm effluents and hospital wastewater based on solar light; iii) Determine the AMR profile of bacteria isolated from backyard farms animals in the Laguna Lake area and exame the driver for antimicrobial use and prescription in the veterinary setting; and iv) Implement point of care rapid testing for key antimicrobial resistant organisms and antimicrobial stewardship in selected hospitals. This is the first project following a One Health approach conducted in the Philippines to reduce the risks of AMR in the lake environment. The outcomes of this project will serve as starting points to replicate the interventions in other lakes in the Philippines and other Southeast Asian countries. The scientific data produced will be used to promote awareness on AMR through community participation and develop government policy interventions. Such knowledge and interventions to reduce antimicrobials and AMR bacteria will help preserve the lake that provides economic services to the lakeshore communities.

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## PARASITOLOGICAL RISK AT THE INTERFACE WILDLIFE-DOMESTIC ANIMALS IN NAZINGA RANCH, BURKINA FASO

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Changes in land use, habitat fragmentation, habitat loss, climate change and human population growth are modulating the relationships between humans, domestic animals and wildlife. These events have led to the emergence of new infectious diseases. While some of these diseases have their origins in wildlife, wild populations are also exposed to pathogens from humans and their animals. This situation impacts wildlife conservation. This study aim to highlight parasites at the wildlife and domestic animals interface in Burkina Faso. Fresh droppings of wild and domestic animals were collected in the Nazinga Ranch and its surroundings following transects line that cover the conservation zone, the buffer zone, the hunting zone and the interface of the park where wild, domestic animals and humans can meet. Coprological analyses were done to isolate parasites. A total of 76 samples belonging to eight different species of mammals were collected. These animals were Antelopes (Hippotragus equinus), Defassa Cobs(Kobus ellipsiprymnus defassa), Buffon's Cobs (Kobus kob), Domestic Goat (Capra hircus), Bushbuck (Tragelaphus scriptus), the Elephant (Loxodonta africana), Warthog (Phacochoerus africanus), and Ox (Bos taurus). The prevalence of parasites was 75.0% for Nematoda, 13.16% for Trematoda, 68.42% for Cestoda and 26.31% for Protozoans. At the interface, the droppings encountered belonged to Loxodonta africana, Capra hircus and Bos taurus with infection rate of 66.6%, 64.5% and 100% respectively. While Capra hircus carried only Tapeworms, Bos taurus and Loxodonta africana had several parasites in common such as genera of Haemonchus, Cooperia, Taenia, Moniezia, Eimeria. A total of 16 species of parasites were encountered during this study. This parasite richness and the modes of dissemination of these parasites show that there is a high risk of transmission between wildlife, domestic animals and humans in this area. The high prevalence of infection in elephants is therefore, another threat to this species, which is already heavily impacted by human-elephant conflicts.

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## CHARACTERISING PSYCHOSOCIAL IMPACT OF TUBERCULOSIS AND THE SOCIAL SUPPORT NEEDS FOR PEOPLE WITH TUBERCULOSIS IN INDONESIA

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Stigma towards people with TB (TB-Stigma) is a crucial challenge to TB elimination and is associated with other TB psychosocial consequences. We evaluated TB-Stigma, depression, quality of life (QoL), and psychosocial support needs among people with TB in Indonesia. In seven provinces of Indonesia, from February to November 2022, we interviewed adults diagnosed with drug-susceptible (DS)-TB (a) receiving treatment at public facilities, (b) receiving treatment at private facilities, (c) lost to follow up (LTFU) to treatment, and (d) receiving TB retreatment. We used our previously-validated Indonesian TB-Stigma Scale to measure TB-Stigma, Patient Health Questionnaire-9 (PHQ-9) to assess depression, EQ-5D-5L to quantify QoL, and additional questions to determine needs for psychosocial support. We applied general linear models and regression analyses to identify factors associated with TB-Stigma and correlations between TB-Stigma, depression, and QoL. Of 612 study participants, 60.6% experienced moderate TB-Stigma. TB-Stigma scores were higher among people receiving treatment at private facilities (adjusted B (aB)=2.48;0.94-4.03), those LTFU (aB=2.86;0.85-4.87), males (aB=1.73;0.59-2.87), losing or changing job due to TB (aB=2.09;0.31-3.88) and living in a rural area. Participants experienced mild-to-moderate (36%) and moderately severe to severe (6%) depression. Experiencing TB-Stigma was associated with moderately severe to severe depression (adjusted odds ratio=1.23;1.15-1.32), and higher combined stigma and depression levels were associated with lower QoL. Participants had a high unmet need of peer support including peer-to-peer emotional support (52%), peer-to-peer education (63%), and peer-led group counselling (78%). In conclusion, there is a sizeable and intersecting burden of TB-Stigma and depression, which is associated with lower QoL. Participants reported a substantial unmet need for psychosocial support from peers. Based on these findings, we are now co-designing a community-based peer-led psychosocial intervention for TB-affected people in Indonesia.

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## DIAGNOSTIC ACCURACY OF THE NOVA TUBERCULOSIS TOTAL ANTIBODY RAPID TEST FOR DETECTION OF PULMONARY TUBERCULOSIS AND INFECTION WITH MYCOBACTERIUM TUBERCULOSIS

**GIDEON NSUBUGA**

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The NOVA Tuberculosis Total Antibody Rapid Test is a commercially available lateral flow serological assay that is intended to be used as an aid in the diagnosis of tuberculosis. We conducted a study to estimate diagnostic accuracy of this assay for diagnosis of active pulmonary tuberculosis disease and for detection of M. tuberculosis infection. This study used existing frozen plasma specimens that had been obtained previously from consenting adults whose tuberculosis status was rigorously characterized. The investigational assay was performed in a single laboratory by laboratory staff specifically trained to conduct the assays according to the manufacturer's procedures. In addition, intensity of the test band was subjectively assessed. Plasma specimens from 150 participants were tested. All testing attempts yielded a determinate result of either positive or negative. For diagnosis of active pulmonary tuberculosis disease, test sensitivity and specificity were 40.0% (20/50, 95% confidence interval [CI] 27.6% to 53.8%) and 85.0% (95% CI 76.7% to 90.7%), respectively. For detection of M. tuberculosis infection, test sensitivity and specificity were 28.0% (95% CI 20.5% to 37.2%) and 86.0% (95% CI 73.8% to 93.0%), respectively. Among the 35 positive tests, no statistically significant band intensity trend was found across participant groups (p=0.17). Study findings do not support a role for the NOVA Tuberculosis Test in current tuberculosis diagnostic algorithms.

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## EXPLORING THE POTENTIAL OF A SALIVA-BASED, RNA-EXTRACTION-FREE PCR TEST FOR THE MULTIPLEXED DETECTION OF KEY RESPIRATORY PATHOGENS

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Efforts to control and monitor transmissible infectious diseases rely on large-scale screening initiatives. The use of saliva as a non-invasive sample type could alleviate bottlenecks encountered in mass testing strategies in community settings. Having extensively demonstrated saliva as sensitive for the detection of SARS-CoV-2, we sought to validate this approach for other common respiratory pathogens. From May-July 2022, de-identified saliva samples were collected from consenting adults ≥18 years of age with respiratory symptoms (New Haven, CT, USA). Saliva samples from SARS-CoV-2-negative individuals were stored at -80ºC until further processing in the research laboratory. We modified our RNA-extraction-free SARS-CoV-2 PCR test for multiplexed detection of four additional key respiratory viruses ("SalivaDirect+"): influenza A, influenza B, RSV and hMPV and for singleplex testing for pneumococcus. Sample stability was tested after storage at +4°C, room temperature (~19°C) and 30°C for 72 hours. We confirmed a limit of assay detection at 4 copies/µl for each virus target. From 804 saliva samples tested with SalivaDirect+, 17 (2.1%) tested positive for one of the viruses targeted, with 7 (0.9%) positive for influenza A, 4 (0.5%) positive for RSV, and 6 (0.7%) samples positive for hMPV. No sample tested positive for influenza B. For influenza A and RSV, detection by SalivaDirect+ was comparable to testing following RNA extraction but detection of hMPV was less sensitive. We confirmed that virus detection remained stable at +4°C, room temperature and 30°C for up to 72 hours. In singleplex testing, 87 (10.8%) samples tested positive for pneumococcus. By testing saliva samples from symptomatic, SARS-CoV-2-negative individuals we detected respiratory viruses which were otherwise missed in testing focused solely on SARS-CoV-2. Being less invasive and less resource-intensive than other sample types, saliva-based testing can lead to more equitable and sustainable testing and surveillance programs. As a result, saliva can bolster the public health response, particularly in low-resource and remote environments.

5645

## PREVALENCE OF NASOPHARYNGEAL CARRIAGE OF MACROLIDE RESISTANCE-ASSOCIATED ERYTHROMYCIN RIBOSOME METHYLASE (ERM) GENES AMONG HEALTHY CHILDREN AND ADULTS IN A PERI-URBAN COMMUNITY IN LIMA, PERU

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Erythromycin ribosome methylase (erm) genes, which confer macrolide resistance, are commonly detected in healthcare settings. Yet, their prevalence among healthy individuals in the community is unknown. Here

we provide an initial assessment of erm nasopharyngeal carriage in healthy children and adults. Nasopharyngeal swabs were systematically obtained at enrollment and weekly thereafter from children and adults enrolled in a household-based prospective cohort study in Lima, Peru. Samples were sequenced using the Illumina Respiratory Pathogen/ID AMR Panel to detect common respiratory bacteria and antimicrobial resistance genes. We defined 'any erm gene' (erm) as the detection of at least one of the specific erm gene classes. We compared the prevalence of erm carriage at enrollment among age groups (ages 0-4, 5-17, 18-44, and 45+ years) using the Fisher's exact test. There were 114 individuals were included in this analysis; 74% were female and median age was 24.2 years (IQR 4.6, 41.8). An erm gene was detected in 51 (44.7%) of individuals, most commonly ermC (15.8%) and ermB (7%). The prevalence of erm gene detection was high and similar among age groups: [0-4 years (19/33, 57.6%), 5-17 years (12/20, 60.0%), 18-44 years (11/34, 32.4%) and 45+ years (9/27, 33.3%) (p=0.056)]. These preliminary results indicate that erm genes were commonly detected in healthy community-dwelling children and young adults in Lima, Peru. Future analysis will assess changes in erm carriage over time, transmission among household members, and its clinical relevance.

5646

## VACCINATION FOLLOWING THE EXPANDED PROGRAM ON IMMUNIZATION SCHEDULE COULD HELP TO REDUCE DEATHS IN CHILDREN UNDER FIVE HOSPITALIZED FOR PNEUMONIA AND SEVERE PNEUMONIA IN A DEVELOPING COUNTRY

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Pneumonia is the leading cause of under-five mortality worldwide. An expanded programme on immunization (EPI) is one kind of evidence-based tool for controlling & even eradicating infectious diseases. This study aimed to explore the impact of EPI vaccination among children of 4-59 months hospitalized for pneumonia & severe pneumonia. Additionally, we evaluated the role of 10 valent pneumococcal conjugate vaccine alone on clinical outcomes in such children. In this retrospective chart review, children 4-59 months of age with the WHO defined pneumonia & severe pneumonia, admitted in the Dhaka Hospital of icddr,b between August`2013 & December`2017 who had the information on immunization as per EPI schedule by 4 months of age, were included in the analysis. Comparison was made between the children who were fully immunized & who were not immunized (consists of partial immunization and no immunization). A total of 4625 children had pneumonia & severe pneumonia during the study period. Among them, 2605 (56.3%) had information on immunization. Between them, 2195 (84.3%) were fully immunized by 4 months of age according to EPI schedule & 410 were not immunized. In log-linear binomial regression analysis, it has been revealed that immunization in children 4-59 months of age was associated with lower risk of diarrhea (p=0.033), severe pneumonia (p=0.001), anemia (p=0.026) & deaths (p=0.035). Importantly, the risk of developing severe pneumonia (1054/1570 [67%] vs. 202/257 [79%], p<0.001) and case-fatality-rate (57/1570 [3.6%] vs.19/257 [7.4%], p=0.005) were still significantly lower among those who were immunized with PCV-10 than those who were not. Under-five children immunized as per EPI schedule were at a lower risk of diarrhea, severe pneumonia, anemia & deaths, compared to unvaccinated children. In addition, PCV-10 was found to be protective against severe pneumonia & deaths in such children. The overall results underscored the importance of the continuation of immunization scrupulously adhering to EPI schedule to reduce the risk of morbidities & mortalities in such children, especially in resource-limited settings.

5647

## RESPIRATORY SYNCYTIAL VIRUS INFECTION IN CHILDREN ADMITTED TO A PEDIATRIC INTENSIVE CARE UNIT IN GHANA AMID COVID-19 PANDEMIC

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Respiratory Syncytial Virus (RSV) infection is a seasonal illness that affects about 97% of children by the age of 2 years. Although RSV infection can be life-threatening during the first year of life and is the leading cause of hospitalization in infants, there are limited data available on the burden of RSV in critically ill children to inform on the prevention and treatment of the disease with specific viral therapeutics. The current study assessed the burden of RSV among children aged less than 2 years in a pediatric intensive care unit (PICU) during the COVID-19 pandemic in Accra, Ghana. Children below the age of 24 months with severe respiratory tract infections who were admitted to the PICU of the Korle Bu Teaching Hospital were recruited with parental consent. Nasal swabs were obtained within 72 hours of admission and tested for RSV and Influenza virus using the ID NOW Point of care test machine at the PICU and then confirmed by real-time quantitative reverse transcription-polymerase chain reaction (RT-qPCR) at the Noguchi Memorial Institute for Medical Research. Samples were also investigated for the presence of other viral agents such as the SARS-Cov 2 virus. Twenty-eight children were enrolled from June to November 2021. RSV was confirmed in 9/28 (32%) of the children who were all below the age of 12 months. Among the RSV-positive group, infants <3 months old had a higher incidence of infection (67%, p<0.01). RSV A predominated 7/9 (78%) cases. One (1) patient each tested positive for Influenza virus and SARS-CoV-2. Two patients (22%) were preterm and 5 (56%) had congenital abnormalities. Besides supportive treatment, children were on antibiotics routinely per the PICU protocol. The average length of stay of patients at the PICU was 19.11 days and one (1) RSV death occurred. RSV remained an important cause of severe respiratory illness during the COVID-19 outbreak in Ghana. The PICU burden of RSV was heavy on infants during the first year of life. And there is a need for targeted interventions such as the introduction of vaccines against severe RSV disease.

5648

## MOLECULAR INVESTIGATION OF THE AETIOLOGY OF TUBERCULOSIS-LIKE CLINICAL SYNDROMES IN ADULTS PRESENTING FOR PRIMARY HEALTH CARE AT LIMBE AND NDIRANDE HEALTH CENTRES

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The suboptimal nature of diagnostics leads to empirical broad-spectrum and tuberculosis treatment for most patients with respiratory illness in low-income settings, where also the aetiology is poorly described especially at the primary care level. Our ACT-TB Trial demonstrated that empirical broad-spectrum antibiotics do not add much diagnostic or clinical benefit for patients presenting to primary care. We hypothesized that the bulk of the respiratory symptoms may have been caused by respiratory viruses and atypical bacteria whose diagnostics are not readily available. To investigate the aetiology and prevalence of respiratory pathogens that are not traditionally investigated, we conducted a cross-sectional study including a random selection of patients from the ACT-TB trial participants (trial included: aged ≥18 years, attending primary care, unwell for at least 14 days, with cough, with no immediate indication for hospitalization). Participants provided nasopharyngeal swab samples and we used real-time-Polymerase Chain Reaction (Siemens® FTD 33kit) to detect pathogens. We included 297 participants (45% male), and the most common pathogens were Bordetella (87%), Klebsiella pneumoniae (77%), salmonella species (70%), HCoVHKU1 (57%), Haemophilus influenzae (56%), Chlamydia pneumoniae (54%), Human rhinovirus (49%), Haemophilus influenzae B (46%) Staphylococcus aureus (45%), and ICV (44%). Our preliminary results demonstrate the high prevalence of respiratory viruses and atypical bacteria, all of which are not covered by any of the antibiotics used for empirical therapy. This mismatch highlights the need for National tuberculosis and antimicrobial stewardship programs to improve diagnostic protocols and limit outpatient prescriptions of antibiotics. Future research and investment should focus on strengthening diagnostics for tuberculosis and other respiratory pathogens. At the conference, we hope to present additional analysis by TB status and clinical improvement status.

**5649**

## PULMONARY-UROGENITAL TUBERCULOSIS: A DELAYED DIAGNOSIS

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Urogenital tuberculosis (TB) is responsible for one-third of cases of extrapulmonary TB and occurs in up to 20% of patients with pulmonary TB. We illustrate a diagnostic challenge in a case of pulmonary-urogenital TB with HIV infection. A 60-year-old woman with HIV infection diagnosed in May 2020 following an initial presentation of Escherichia Coli bacteremia complicated by HIV-related immune thrombocytopenic purpura. Computed tomography (CT)thorax revealed tree in bud in bilateral upper lobes with mild hydronephrosis of the right kidney compared to the left kidney. She underwent bronchoscopy and her TB work-up was negative. She was treated for latent TB with Isoniazid-based regime and commenced on antiretroviral therapy and prednisolone. She presented again in February 2023 following a 3-day history of fever with documented temperatures of 380C but no obvious source of infection. She had thrombocytopenia of 45X109/L but normal white cell count, renal and liver function. Her chest radiograph was normal. She was treated as occult sepsis with intravenous (IV) Ceftriaxone and IV Hydrocortisone with subsequent resolution of fever. She had recurrence of her fever with tenderness at right lumbar region after five days of Ceftriaxone. Her antibiotic was escalated to IV Meropenem. Ultrasound abdomen showed gross right hydronephrosis with pyonephrosis. Contrast-enhanced CT thorax and abdomen revealed multiple tree-in-bud nodules prominent in both upper lobes with multiple lung nodules. There were no cavitating lung lesions and enlarged mediastinal lymphadenopathy. There was right hydronephrosis with dilated pelvic-calyces, stenosed infundibulum and right mid ureteric stricture. She underwent a right nephrostomy with subsequent right ureteral stenting. Direct smear urine for acid fast bacilli was positive and Mycobacterium TB(MTB) GeneXpert was positive with indeterminate Rifampicin-resistant, pending urine MTB culture and sensitivity. Treatment with anti-TB medications was commenced, with steroid replacement. In conclusion, Urogenital TB can be missed in view of asymptomatic manifestation of hydronephrosis

**5650**

## PREVALENCE OF MALARIA-PNEUMONIA OVERLAP IN RURAL GAMBIA: NINE YEARS OF CLINICAL EXPERIENCE IN ENDEMIC AREA

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Globally malaria and pneumonia account for almost 40% of the mortality in under-five children in sub-Saharan Africa. Respiratory distress is a common feature of severe falciparum malaria in both children (40%) and adults (25%). We aimed to assess the prevalence of malaria and radiological pneumonia (RP) overlap and the proportion of children with these overlapping who truly require pneumonia treatment along with malaria management. The surveillance population included in the Upper River Region (URR) all Basse Health and Demographic Surveillance System (BHDSS) residents aged 2-59 months. Nurses screened all outpatients and inpatients at Basse Health Centre (BHC) of a referral health facility during the surveillance period from May 2008 to December 2016. Clinicians then applied criteria for patient investigation and treatment was provided based on national guidelines. Rapid diagnostic tests (RDT) for malaria were done from August to December during malaria transmission season. During the population-based surveillance, 22061 patients aged 2-59 months were registered at BHC. We found 1178 cases (6.65%, 1178/17705) of malaria positive, and 1649 cases (12.32%, 1649/13389) of RP. We identified 1198 cases (97.6%, 1198/1227) of RP patients had malaria negative, and 29 cases (2.4%, 29/1227) of RP had malaria positive. 472 cases (5.3%, 472/8858) of no RP had malaria positive and 8386 cases (94.7%, 8386/8858) of no RP had malaria negative. A total of 59.1% (696/1178) (95% CI 56 to 62) of malaria cases had respiratory findings. Among malaria positive and negative cases, we identified 106 cases of Invasive Bacterial Diseases (IBD), Streptococcus pneumoniae, 8 cases of Salmonella spp, and 8 cases of Staphylococcus aureus. Radiological pneumonia is uncommon with malaria RDT-positive. An empiric antibiotic is not indicated with respiratory symptoms and malaria RDT positive. Point of care test for radiological pneumonia/bacterial pneumonia is needed to target antibiotic therapy in children with respiratory symptoms and malaria RDT positive.

**5651**

## DETERMINANTS OF TUBERCULOSIS OUTCOMES DURING THE COVID-19 PANDEMIC AT A REFERRAL HOSPITAL IN RURAL HAITI

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Tuberculosis (TB) is a major public health threat in Haiti, with an estimated annual incidence of 159/100,000. Little is known about determinants of TB outcomes in Haiti since 2020, when multiple events coincided that were likely to impact TB care: the Covid-19 pandemic, progressively worsening national political and security crises, and an earthquake in 2021. To address this, we conducted a prospective cohort study at the St. Boniface Hospital (SBH), a rural referral center in Southern Haiti. Eligibility criteria included: age greater than 18 years, clinically suspected or microbiologically confirmed (by smear, NAAT, or culture) TB, receiving standard first-line TB treatment. We excluded patients with drug-resistant TB and those who had already received at last 7 days of treatment. We administered a baseline

questionnaire, collected baseline and outcome clinical data, and analyzed laboratory samples. We assessed risk factors for unfavorable outcomes (death, treatment failure, loss to follow up) using multivariable logistic regression models. We enrolled 250 patients (37% female) between May 2020 and January 2023, with a median age of 35 years (IQR 25-45). Only 13% had completed secondary education and 20% reported no formal education. Food insecurity was common (55%), and the median probability of poverty (< $1USD/day) was 45% (IQR 28-77). The median time to reach the hospital was 120 minutes (IQR 90-180), the median duration of symptoms prior to presentation was 12 weeks (IQR 4-20), 40% had undernutrition, and 54% met criteria for depression. Of participants with finalized outcome data, 179 (80%) had a favorable outcome, and 10 (4%) died. Independent risk factors associated with unfavorable outcome were age (Adjusted Odds Ratio [AOR] 1.03 per year, 95% CI 1.01-1.06), travel time (AOR 1.07 per 10 minutes, 95% CI 1.02-1.12) and being a smoker (AOR=2.77, 95% CI 1.21-6.32). Our findings highlight factors that can be deleterious in the course of TB, and can serve as a guide to improve intervention strategies (for example, community outreach for patients living far from health facilities) aimed at minimizing the impact of TB.

**5652**

## SHORT VERSUS LONG DURATION MACROLIDE TREATMENT FOR RESPIRATORY TRACT INFECTIONS: A SYSTEMATIC REVIEW AND META-ANALYSIS OF EFFICACY, SAFETY, AND ADHERENCE OUTCOMES

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Respiratory tract infections are a cause of death and disability worldwide. Management involves a course of antibiotics for a period of 7-14 days, however prolonged exposure to antibiotics may lead to the development of antimicrobial resistance. We compared the efficacy, safety, and adherence of short-course with long-course macrolide treatment for respiratory tract infections. We conducted a systematic review and meta-analysis by searching the Embase, MEDLINE, and Cochrane Central Register of Controlled Trials for randomized controlled trials published from inception to 25 August 2021. Studies that recruited patients with respiratory infections and reported treatment outcomes of at least two macrolide antibiotics given for different durations were eligible. We used Rob 2.0 to assess the quality of studies and a random effects meta-analysis to estimate effects and 95% confidence intervals. 2695 articles were retrieved. 9 randomized controlled trials published between 1984 and 2005 involving 2900 participants from 27 countries in Africa, America, Asia, Europe, and Oceania were eligible for inclusion. The target respiratory infections were tonsillo-pharyngitis, atypical pneumonia, acute exacerbation of chronic bronchitis, community-acquired pneumonia, sinusitis and upper respiratory tracts infection, community-acquired acute maxillary sinusitis and Group A beta-haemolytic streptococcal tonsillitis/pharyngitis. Short duration treatment did not differ from long duration treatment with respect to efficacy assessed using incidence of treatment failure (RR= 0.88, 95% CI= 0.71-1.09). The proportion of treatment non-adherent participants was also similar between short and long duration macrolide groups (RR= 0.85, 95% CI=0.65, 1.11). The risk of adverse events was lower among patients in the shorter duration group compared to the longer duration group (RR =0.88, 95% CI=0.80, 0.96). We found that short duration treatment is as effective and safe as long duration treatment. This result should inform the design of antibiotic dosages to preserve the lifespan amid the dryness of the product development pipeline.

**5653**

## PREVALENCE OF EXTRA-PULMONARY TUBERCULOSIS IN AFRICA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Mycobacterium tuberculosis primarily causes pulmonary disease. Disease in other organs is called extra-pulmonary tuberculosis (EPTB). The burden of EPTB is not well quantified in TB endemic countries such as those in sub-Saharan Africa. This study aimed to quantify the burden of EPTB via a systematic review of the prevalence of EPTB in African countries. Studies were retrieved by searching five databases; 84 studies published between 1990 and 2020 were included. The studies described the prevalence of EPTB among TB patients (53 studies) or patients with conditions other than TB including HIV (12), meningitis (3), renal failure (3) and other comorbidities some of which are cancer (9). The focus of the meta-analysis was on EPTB among TB patients (53 studies). Meta-analysis was performed in the 53 studies (198,781 participants) using a random-effects model to estimate pooled prevalence of EPTB. Meta regression was used to explore possible explanations for the heterogeneity observed, according to regions and time period. The pooled prevalence of EPTB among TB patients was 24% (95% CI 21- 28%). There was substantial heterogeneity in prevalence for the five African regions. The Eastern region had the highest prevalence with 30% (95% CI 26-35%) and the lowest prevalence was in Western Africa, 17% (95% CI 10-27%). There was no significant difference in the prevalence of EPTB when compared between 3 ten-year time periods. This systematic review and meta-analysis provides an estimate of the prevalence of EPTB in TB patients in Africa.

**5654**

## NEUTRALIZING ANTIBODIES TO SARS-COV-2 IN A ONE YEAR CROSS-SECTIONAL STUDY IN KISUMU COUNTY, KENYA

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Neutralizing antibodies (NAbs) to SARS-CoV-2 spike protein are a good predictor of protective immune response to coronavirus disease 2019 (COVID-19). In this cross-sectional study carried out at Kombewa, Kisumu County, Kenya during Alpha, Delta and Omicron waves, total of 1,385 subjects were enrolled, out of which 827 (61.7%) had NAbs. The mean magnitude of NAbs increased over the successive waves (0.90 U/mL at alpha, 1.80 U/mL at Delta and 5.7 U/mL at Omicron), while frequency of SARS-CoV-2 by qPCR progressively declined; 25% at alpha, 3% at Delta and 10% at Omicron. Further analyses are recommended to dichotomize NAbs emanating from SARS-CoV-2 infections and those from vaccinations.

**5655**

## PREDICTION OF DISEASE OUTCOME USING A DEFINITE CUT-OFF VALUE IN CHEST X-RAY SCORING, OBSERVATION FROM A RESOURCE LIMITED COVID-19 TREATMENT FACILITY

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Evaluation of potential outcomes of COVID-19 affected pneumonia patients using radiological imaging chest computed tomography (CT) scans, may not be conceivable in low-resource settings. Thus, this study aimed to evaluate the performance of chest X-ray (CXR) scoring in

predicting the disease severity and outcomes of adults hospitalized with COVID-19. This was a retrospective chart analysis consuming data from COVID-19-positive adults who had CXR availability and were admitted to a temporary COVID-19 unit in Bangladesh from 23rd April 2020 to 15th November 2021. We reviewed baseline CXR, clinical data, and blood test results of all enrolled patients. At least one clinical intensivist and one radiologist combinedly reviewed each admission CXR for the presence of consolidation, ground-glass opacities, reticular opacities, and pleural effusion. CXR scoring varied from 0 to 8, depending on the area of lung involvement with 0 indicating no involvement and 8 indicating ≥75% involvement of both lungs. The receiver operating characteristic curve (ROC) was used to determine the optimum CXR cut-off score for predicting fatal outcomes. Out of 263 COVID-19 affected adults, a total of 218 (82.9%), with a mean age of 53.52 ± 16 years, were included in the study. The ROC demonstrated the optimum cut-off as ≥3 and ≥5 for disease severity and death, respectively. In multivariate logistic regression analysis, a CXR score of ≥3 was found to be independently associated with disease severity (aOR, 8.70; 95% CI, 3.82, 19.58, p <0.001) and a score of ≥5 with death (aOR, 16.53; 95% CI, 4.74, 57.60, p <0.001) after adjusting age, sex, antibiotic usage before admission, history of fever, cough, diabetes mellitus, hypertension, total leukocytes count, and C-reactive protein (CRP). Using CXR scoring derived cut-off values at admission might help to prompt identify the COVID-19 affected adults with different severity of pneumonia and who are at risk of severe disease and mortality. This may help to initiate early and aggressive management of such patients as well as reduce their fatal outcomes.

**5656**

## STREPTOCOCCUS PNEUMONIAE NASOPHARYNGEAL CARRIAGE AND SEROTYPES DISTRIBUTION IN URBAN (KIBERA) AND RURAL (ASEMBO) KENYA AMONG CASES WITH SEVERE ACUTE RESPIRATORY ILLNESS 6-9 YEARS POST INTRODUCTION OF 10-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV10)

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Pneumococcus is a leading cause of pneumonia globally. Pneumococcal conjugate vaccines (PCV) protect against vaccine-serotype disease and nasopharyngeal carriage, leading to reduced transmission and herd protection. Kenya introduced 10-valent Synflorix (PCV10GSK: serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F) in 2011 and switched to Pneumosil (PCV10SII: 1, 5, 6A, 6B, 7F, 9V, 14, 19A, 19F and 23F) in 2022. PCV13 includes PCV10GSK types plus 3, 6A and 19A. We examined pneumococcal carriage prevalence and serotype distribution among predominantly outpatient severe acute respiratory illness (SARI) cases, to understand persistence of PCV10GSK serotypes 6-9 years after introduction and to assess serotype coverage of PCV10SII and PCV13. We leveraged ongoing SARI surveillance at two sites (Kibera, urban informal settlement in Nairobi and Asembo, rural western Kenya) collecting nasopharyngeal swabs for culture from patients of all ages meeting a standardized SARI case definition. Culture-positive isolates were serotyped by conventional multiplex PCR and/or Quellung. From January 2017 to April 2020, 3,067 patients in Asembo and 838 in Kibera had swabs collected and tested. Pneumococcal carriage prevalence was 43.0% (n=1,320) and 58.1% (n=487) in Asembo and Kibera, respectively; among children aged <5 years it was 63.3% (606/958) and 78.3% (144/184), respectively. Among 1,298 (97.7%) serotyped isolates from Asembo, most common serotypes were 3 (n=148), 6A (n=108), 35B (n=90), 19F (n=69) and 19A (n=58); among 482 (96.7%) serotyped isolates from Kibera, most common were 3 (n=50), 35B (n=29), 11A (n=26), 16F (n=22), 13 (n=20) and 19A (n=18). The prevalence of carriage with PCV10GSK, PCV10SII and PCV13 serotypes was 5.2%, 10.4%, and 15.5%, respectively in Asembo, and 8.9%, 11.8%, and 18.7% in Kibera; among children <5 years it was 8.0%, 16.6%, 21.2% in Asembo and 9.2%, 17.4%, and 26.6% in Kibera. Pneumococcal carriage in SARI is not reflective of etiology yet provides insight into circulating serotypes. PCV10GSK serotypes, most notably 19F, persisted up to 9 years after vaccine introduction. PCV10SII will offer broader serotype coverage.

**5657**

## HUMAN IMMUNODEFICIENCY VIRUS INFECTION AND DIABETES MELLITUS IN PEOPLE WITH TUBERCULOSIS IN ODISHA, INDIA

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This study evaluated the burden of human immunodeficiency virus (HIV) infection and diabetes mellitus (DM) in tuberculosis (TB) cases from Odisha, India, during 2019, and its impact on the TB treatment outcome. The study utilized data on TB patients of Odisha during 2019, from the NIKSHAY portal, the health management information system of TB in India. This is a retrospective observational registry-based cohort study, which evaluated a linkage between socio-demographic predictors, clinical diagnostic and treatment predictors, time of treatment predictors, and co-morbidity with TB. Data were retrieved electronically in Microsoft-Excel and analysis was done using STATA 16. Data for 47831 TB cases of Odisha was extracted from the Nikshay application for 2019. The highest prevalence (31.1%, 14863/47831) of TB was observed among young participants aged 15-30 years, whereas the prevalence was least among children <14 years (4.4%, 2124/47831). Of the 47831 TB cases, 7.6% (3659/47831) had DM, along with TB. 1.2% (571/47831) had HIV along with TB, while only 0.08% (37/47831) had both DM and HIV along with TB. 88.2% (3148/3569) of cases with DM and TB had a favorable outcome, compared to 82.3% (449/541) of cases with HIV and TB. People with TB who did not have DM had a significantly higher favorable outcome (OR 1.6, 95% CI 1.5-1.8) compared to those with TB and DM. Similarly, TB cases who did not have HIV infection had a significantly higher favorable outcome (OR 2.4, 95% CI 1.9-3.0) compared to those with TB and HIV. In conclusion, our study showed that presence of DM and/or HIV in TB patients had an impact on the TB treatment outcome.

**5658**

## SCHISTOSOMAL CIRCULATING ANODIC ANTIGEN CLEARANCE IN PRESCHOOL AGED CHILDREN FROM THE PIP (PRAZIQUANTEL IN PRESCHOOLERS) TRIAL

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Circulating Anodic Antigen (CAA) is known to be specific to schistosomiasis and is assessed using the highly sensitive Up-Converting reporter Particle Lateral Flow (UCP-LF) CAA assay. The CAA assay is an important tool in determining worm burden hence is relevant in estimating treatment efficacy and reinfection rates. Limited data on CAA clearance after praziquantel (PZQ) treatment is available in Preschool Aged Children (PSAC). As part of the PIP trial, a phase II trial exploring different PZQ dosing regimens in children aged 12-47 months in Uganda, we present findings assessing clearance by measuring CAA in urine before and four weeks after PZQ treatment. The trial enrolled PSAC infected with Schistosoma mansoni as diagnosed by stool Kato-Katz (KK) living on the shores of Lake Albert,

Uganda. Schistosomal CAA was analyzed at baseline and at four weeks post treatment along with egg count by KK. 348 participants were enrolled to the trial with a median age of 36 months (IQR 28.0 – 42.0) and 51% of the participants were male. KK infection intensities at baseline were categorised as light (56.9%), medium (24.0%) and heavy (19.1%). Median CAA concentration was 154.8pg/ml (IQR 41.3 - 673.8) at baseline and 4pg/ml (IQR 0.2 - 58.0) at four weeks post treatment. Overall, there was a marked reduction in CAA levels at four weeks post treatment with 41% 'antigen cure' (95% CI 35.4 – 46.7). In comparison, parasitological cure based on KK was 91% (95% CI 86.9 – 94.2) for the 300 participants with complete CAA data. Out of those who showed parasitological cure at four weeks, 52.8% (95% CI 46.4 – 59.3) were still CAA positive but with relevantly reduced CAA concentrations. Findings demonstrate expected high egg reduction after PZQ treatment but with poor 'antigen cure', raising concerns of residual untreated infection in this trial setting. The disparity in diagnostic clearance is likely due to the poor sensitivity of KK at low egg burdens.

**5659**

## GAPS IN BEDSIDE PROTOCOLS AND POLICIES FOR MANAGEMENT OF FEMALE GENITAL SCHISTOSOMIASIS IN ENDEMIC SOUTH AFRICA AND NON-ENDEMIC NORWAY

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Female genital schistosomiasis (FGS) is a prevalent gynaecological disease amongst women and girls in schistosomiasis-endemic parts of Africa. However, it is often misdiagnosed as a sexually transmitted disease or cervical cancer. FGS may cause abnormal vaginal discharge, infertility, paediatric genital symptoms, and may increase the susceptibility to HIV. The diagnosis entails cervicovaginal examination. WHO recommends praziquantel 40-60 mg/kg as a single dose. To adequately serve immigrants and travellers, health professionals in non-endemic countries should also be aware of FGS as a differential diagnosis. This literature study sought to explore current grey literature for FGS and gaps in patient management protocols in endemic South Africa and non-endemic Norway. Healthcare professionals in ten institutions provided access to bedside protocols used in their clinical practice. The heads of the cervical cancer screening programmes in both countries provided National Department of Health policies and protocols. Management protocols from private practitioners and hospitals were not included for practical reasons. Ten management and policy documents from each country, such as the national Standard Treatment Guidelines in South Africa and the regional hospital guidelines in Norway, were examined. This included reading through the relevant chapters, word searching and exploring the references. No guidelines mentioned FGS in the diagnosis or management of abnormal vaginal discharge, bloody discharge, infertility, or cancer. In order to change the current under-diagnosis, misdiagnosis and mismanagement of women it is imperative that the recommendations for diagnosis and treatment of FGS are included in healthcare policies and management protocols. Further research and collective action are needed to address the gaps in national guidelines and bedside protocols in both countries.

**5660**

## NEXT STEP TOWARDS POINT-OF-CARE MOLECULAR DIAGNOSIS OF FEMALE GENITAL SCHISTOSOMIASIS: EVALUATION OF AN INSTRUMENT-FREE LAMP PROCEDURE

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Detection of Schistosoma DNA in gynaecological samples by real-time polymerase chain reaction (qPCR) is considered to be the reference laboratory test for the diagnosis of Female Genital Schistosomiasis (FGS). However, qPCR is expensive and needs highly trained technicians. Loop-mediated amplification (LAMP) is a more field-friendly isothermal DNA amplification procedure, but it still requires electrically powered equipment. Here we validated an S. haematobium-specific Sh-LAMP procedure and tested a fully instrument-free isothermal DNA amplification and detection procedure by using a novel low-cost and reusable T-cup device. Specific primers were selected based on published assays, targeting the ribosomal intergenic spacer region of S. haematobium. Technical validation of the Sh-LAMP was performed using 20 negative controls, including DNA extracts of soil transmitted helminths and S. mansoni, and a 10-fold dilution series (100-10-3) of DNA extracted from a single S. haematobium egg (n=4). For clinical validation, the Sh-LAMP was used on 125 DNA samples extracted from swabs of cervicovaginal lesions of a previous FGS study in Madagascar. Results were compared with the cycle threshold value (Ct) of the standard ITS-2 targeting qPCR. The T-cup performance was evaluated in a representative sub-selection (n=10) of Sh-LAMP positive clinical samples. Single S. haematobium egg DNA up to a 10-2 dilution and a Ct<35 were all Sh-LAMP positive. The specificity was found to be excellent (100%). In the clinical samples Sh-LAMP showed comparable results with the qPCR, with 35.2% and 33.6% positives, respectively, and a concordance of 79.2% (99/125). Most false-negatives were seen in the qPCR positive samples with a Ct≥35 (5/7), while Sh-LAMP detected 14 additional cases missed by qPCR. The T-cup was found to be a very user-friendly method, but it missed 1 of the 10 Sh-LAMP positive samples. The Sh-LAMP was found to be a suitable alternative to qPCR for the diagnosis of FGS in gynaecological samples, with high potential for the T-cup as a fully instrument-free DNA amplification device for point-of-care diagnosis in low-resource settings.

**5661**

## POPULATION LEVEL IMPACT OF NOVEL DRUGS TARGETING JUVENILE SCHISTOSOMES ON CONTROL AND ELIMINATION OF SCHISTOSOMIASIS

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Praziquantel (PZQ), the standard drug for schistosomiasis, has minimal activity against juvenile schistosomes (within 6 weeks of infecting humans) and imperfect cure rates. The effect of treatment-resistant juvenile schistosomes and imperfect PZQ clearance on transmission dynamics of Schistosoma is unknown. We model the population level effects of novel drug candidates targeting both juvenile and adult schistosomes on schistosomiasis control programs in various settings, which can guide drug development decisions. We created an individual-based dynamic mechanistic model of S. mansoni transmission that simulates mass drug

administration programs using novel drugs. The model simulates low, medium, and high prevalence settings using data from SCORE trials, and explicitly models juvenile and adult schistosomes for each infected person. We simulated five years of annual mass drug administration with: i) 1-dose PZQ (40 mg/kg; per WHO guidelines); ii) 2-dose PZQ (6 weeks apart); and iii) novel drug with activity against juvenile and adult schistosomes. Treatment parameters for PZQ were calibrated to literature values (84% and 88% egg reduction for 1- and 2-dose PZQ), and novel drug efficacy was varied. We assumed 75% coverage. In a low prevalence setting (baseline 15%), 1-dose PZQ, 2-dose PZQ, and a novel drug (PZQ-equivalent activity against adult worms plus juvenile activity) led to 1.0%, 0.8%, and 0.8% prevalence at year 5, respectively. In a high prevalence setting (baseline 50%), 1-dose PZQ, 2-dose PZQ, and a novel drug led to 9.0%, 8.0%, and 7.4% prevalence at year 5, respectively. Increased efficacy of a novel drug against adult schistosomes compared to PZQ (+25% potency) had larger effects on prevalence reduction than comparable activity against juvenile schistosomes. A novel drug that can kill both adult and juvenile schistosomes with higher efficacy will have measurable public health gains compared to PZQ alone. A novel drug will have larger effects in high prevalence settings and small relative reductions in near elimination settings. Targeting juveniles is generally less important than increasing efficacy against adults.

**5662**

## DEVELOPMENT OF ANTIGEN-BASED MULTIPLEX IMMUNODIAGNOSTICS FOR TWO PREDOMINANT SCHISTOSOMA PARASITES IN SUB-SAHARAN AFRICA

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Schistosomiasis is a disease of poverty that is highly prevalent in Sub-Saharan African countries. Cross-reacting proteins in Schistosoma mansoni (Sm) and S. haematobium (Sh) have been alluded to pose diagnostic challenges in the development of serological diagnostics. Proteins specific to these two parasites can leverage diagnostic challenges associated with cross-reactivity and the development of a multiplex diagnostic tool for the two parasites. The aim of this study is to identify protein markers from S. mansoni and S. haematobium for concurrent diagnosis of the two parasites. High-quality protein extracts were prepared from the eggs [(soluble egg antigen (SEA) and adult worms (Schistosoma worm antigens (SWA)] of S. haematobium and S. mansoni. The diagnostic performance of the proteins was evaluated using a quantitative ELISA method with sera obtained from S. mansoni-infected mice. The immunoreactive proteins in the helminths were identified by conventional Western blot analyses and the specific protein markers for concurrent diagnosis of the two parasites were confirmed by double-binding Western blot analyses. Sm SEA gave the best diagnostic performance (Sensitivity (SS) = 1.00, Specificity (SP) = 0.96, AUC = 0.96). Sh SEA slightly performed better in diagnosing S. mansoni infection (SS = 0.96, SP = 0.87, AUC = 0.95) compared to Sm SWA (SS = 0.91, SP = 0.95, AUC = 0.93). Diagnostic protein markers of size 100 kDa in Sh SWA, > 250 kDa (Sh SEA), 25-37 kDa (Sm SWA), and 50-150 kDa (Sm SEA) were specific for the concurrent diagnosis of S. mansoni and S. haematobium. This study showed the plausibility of developing multiplex immunodiagnostic tools for S. mansoni and S. haematobium. Ongoing studies with 2D Western blots and LC-MS seek to identify novel biomarkers for operational diagnosis of these two Schistosoma species simultaneously.

**5663**

## BASELINE SEROPREVALENCE OF SCHISTOSOMA IN ZAMBIAN WOMEN ENROLLED IN A COHORT STUDY (THE ZIPIME WEKA SCHISTA STUDY)

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Schistosomiasis, a disease caused by the waterborne parasite Schistosoma (S.) spp., continues to present a significant public health problem across sub-Saharan Africa. Schistosoma seroprevalence surveys provide an opportunity for the assessment of the burden of schistosomiasis in low-prevalence areas. This study aimed to assess Schistosoma seroprevalence in women as part of an ongoing cohort study and identify potential risk factors across two communities (Livingstone and Kafue) in Zambia using a field-applicable assay. Community health workers recruited women into the Zipime Weka Schista study through home visits and obtained genital self-swabs, urine samples, rapid tests for HIV and Trichomonas vaginalis (Tv), and administered a questionnaire. Venous blood was obtained at a follow up clinic visit. Serological testing was performed for the detection of antibodies to soluble egg antigen (SEA) using the IVD SEA-ELISA. Urine microscopy was conducted for the detection of S. haematobium (Sh) eggs in urine. Testing for High-Risk Human Papillomavirus (HR-HPV) was performed with Gene Xpert. Descriptive statistics were used to analyse sociodemographic characteristics and logistic regression was conducted to explore associations with other infection parameters. A total of 601 serum samples were analysed. Overall prevalence of egg-patent Sh was 5.7% (34/592). Approximately one third of participants (35.8%, 216/601) were found to be Schistosoma seropositive. Seroprevalence was higher in Livingstone than Kafue (40.1% vs. 32.0%, p=0.053). Seropositive participants were more likely to report daily water contact during childhood (p=0.006). Most seropositive participants had no Sh eggs observed in urine (90.0%, 188/209) suggesting chronic exposure. No crude or adjusted associations between Schistosoma seropositivity and any sexually transmitted infection were found including HR-HPV, HIV and Tv. Further visual and molecular genital testing for female genital schistosomiasis is ongoing and will refine our estimates.

**5664**

## DEVELOPMENT OF AN ELISA TO DETECT ANTIBODY TO SCHISTOSOMA JAPONICUM INFECTION USING A BACTERIAL EXPRESSED RECOMBINANT ANTIGEN SJ10.3

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Schistosoma japonicum is a trematode parasite endemic in China, the Philippines, and parts of Sulawesi, Indonesia. Traditional diagnosis is based on identifying S. japonicum eggs in fecal samples using microscopy; however, diagnosis by microscopy requires trained personnel to make and read slides and has low sensitivity in light infections. Antibody detection for serological diagnosis can be useful to indicate schistosome infections in individuals who have traveled to schistosomiasis endemic areas, for mapping in control programs, and when attempting to assess interruption of transmission or perform surveillance. We selected protein Sj10.3 based on its homology to an S. mansoni antigen (Sm10.3) and S. haematobium antigen (Sh-SAP-1) that were identified by epitope library selection and peptide array screening. Using GenBank to obtain the sequence, we synthesized the Sj10.3 gene and expressed it as a GST fusion protein using a bacterial expression vector. The protein was purified using

glutathione magnetic beads and confirmed as reactive with sera from persons with S. japonicum infection using immunoblot. After optimization of the ELISA, we evaluated the assay performance using an S. japonicum serum panel obtained from WHO that consisted of 242 negative and 340 positive samples. These sera were confirmed positive by S. japonicum adult microsomal antigen immunoblot, the current CDC serological reference assay. Based on this serum panel, the assay's sensitivity and specificity are 91% and 99%, respectively. Planned validation studies include assay evaluation against a panel of potential cross-reactive sera such as other trematode infections, including paragonimiasis, fascioliasis, clonorchiasis, and opisthorchiasis. Availability of a recombinant antigen specific for schistosome infections improves the ability of control programs to map endemic areas, evaluate interruption of transmission, and perform surveillance for schistosomiasis in several Asian countries.

**5665**

## URINARY HPV ANALYSIS AS A COMPLEMENTARY DIAGNOSTIC TEST AMONG WOMEN AT RISK FOR CERVICAL CANCER AND FEMALE GENITAL SCHISTOSOMIASIS

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The triad of HIV, cervical cancer and Female Genital Schistosomiasis (FGS) is found mainly in Africa. FGS is a neglected disease that is associated with HIV and is often misdiagnosed as cervical atypia. Most at risk are young women, aged 15 and above, constituting almost 3 million of the South African (SA) population. They may have limited access to healthcare and gynaecological investigations, may be reluctant to undergo a gynaecological examination, and are high at risk for this triad of diseases. The present study sought to compare HPV DNA in urine and cervico-vaginal lavage samples and explore their association with cytology results (Pap smears) and urinary Schistosoma haematobium microscopy results. The study was conducted among 220 young women aged 16-23 years of age from rural high schools in KwaZulu-Natal, South Africa. HPV DNA analysis was done in urine and vaginal lavage. Cytology samples were analysed for squamous cell atypia and urine microscopy was used for the identification of Schistosoma ova. The participants reported to have been sexually active from 17 years of age (SD=1.39). Schistosoma ova were found in 46 (20.9%) of the participants and the HIV prevalence was 43 (19.5%). Cervical cell atypia was detected in 120 (54.5%) of these young women. HPV DNA was detected among in 133 (60.5%) of the urine samples and in 168 (76.8%) of the cervico-vaginal lavage samples ($p < 0.001$). Using cervico-vaginal lavage as a gold standard, HPV PCR DNA urine analysis had a sensitivity of 75.15% and a specificity of 88.24%. There was no association between the HPV DNA in urine and cervico-vaginal lavage and cytology or urinary schistosomiasis results, respectively. It was also found that the young women who were HIV positive were 2,5 times more at risk for HPV in urine/vaginal lavage than those who were HIV negative. Urine has the potential of being optimized as a less invasive, alternative test for HPV among this young adolescent population at risk. In light of the diagnostic challenges urinary HPV testing might be a sustainable solution for targeted intervention among this neglected group.

**5666**

## POPULATION PHARMACOKINETICS OF PRAZIQUANTEL IN PRE-SCHOOL AGE CHILDREN PARTICIPANTS IN THE PRAZIQUANTEL IN PRESCHOOLERS (PIP) TRIAL

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Praziquantel (PZQ) remains the drug of choice for treatment of schistosomiasis, a major neglected tropical disease with over 250 million people infected globally; children bear more than 50% of the disease burden. However, limited pharmacokinetic (PK) data is available to guide dosing of PZQ in children. The objective of this study was to establish and describe the population PK of PZQ in pre-school age children. Children aged 1 to 4 years old with Schistosoma mansoni infection were enrolled into a clinical trial and randomised to receive either 40mg/kg or 80mg/kg dose of PZQ during the study. The doses were split into two and administered 3 hours apart (i.e. 40mg/kg and placebo; 40mg/kg and 40mg/kg). PK samples were collected at four time points; 1.5, 3, 4, and 6 hrs post dose. Plasma concentrations of PZQ enantiomers (R- and S-PZQ) were quantified using a liquid chromatography – tandem mass spectrometry (LC-MS/MS) assay. A hundred and ninety children (54.2% males) were enrolled with a mean age of 33 (± 9.23) months. There was significant variability in the plasma concentration profile of both R and S enantiomers. The plasma concentrations of both enantiomers were quantified up to 6 hours post dose. Praziquantel dosing at 80mg/kg resulted in higher drug exposure than at 40mg/kg. Further PK/PD modelling is ongoing pending trial unblinding.

**5667**

## PHARMACOLOGIC MONITORING OF PLASMA CONCENTRATION OF PRAZIQUANTEL ON THE INTENSITY OF SCHISTOSOMA INFECTION IN A THERAPEUTIC EFFICACY MONITORING STUDY IN PERSONS TREATED FOR SCHISTOSMIASIS IN ABUJA, FEDERAL CAPITAL TERRITORY, NIGERIA

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Treatment and control of schistosomiasis depend heavily on praziquantel (PZQ) which is administered mainly as a single dose of 40mg/kg body weight based on WHO recommendation. PZQ is the backbone of Mass Drug Administration (MDA), a strategy for the control and elimination of schistosomiasis. PZQ is well tolerated by patients and has few side effects, but there have been concerns of tolerance/resistance/treatment failure. Continuous therapeutic monitoring is critical for successful mass drug administration programme. We monitored therapeutic efficacy of praziquantel in a cohort of 65 participants from whom urine, plasma and stool samples were collected and processed by microscopy for Schistosoma haematobium (Sh) eggs detection by urine filtration technique, while Kato-Katz method was used for stool examination for the detection and quantification of of S. mansoni (Sm) using standard protocol. Of the 65 persons, 5years and above, a single dose of PZQ, using a praziquantel, given as DOT. After about 2weeks of follow up the blood samples of the

cohort were taken again. 51 of them [32males (62.7%) and 19 females (37.3%] with mean age of 14.5years were selected based on intensity of infection for detection and quantification of plasma levels of PZQ using High Profile Liquid Chromatography (HPLC). Plasma PZQ level was recorded in micrograms per milliliter (µg/ml). Of the 51, 44 were positive for eggs (infection group): 15 low infection (1-50 eggs), 22 moderate infections (50-400 eggs), and 6 heavy infection (>400 eggs). 7 had zero egg count (non-infection or control group). Mean plasma PZQ concentration for the non-infection group was 12.84 µg/ml, low infection 4.22 µg/ml, and 2.71ug/ml for moderate infection. Heavy infection (>400 parasites); no PZQ detected. In addition, mean plasma PZQ concentration was higher for S. haematobium infection (3.20 µg/ml), so was ERR as compared with S. mansoni. This study shows differential effect of PZQ for Sh and Sm, thus indicating an association between intensity of infection of schistosomiasis and plasma levels of PZQ and efficacy. It also shows that PZQ is rapidly taken up by schistosomes following its administration.

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## THE PREVAILING INFECTION OF SCHISTOSOMA JAPONICUM AND OTHERZOONOTIC PARASITES IN BUBALINE RESERVOIR HOSTS IN THE RICEFIELD OF LAKE ECOSYSTEM: A CASE IN LAKE MAINIT THE PHILIPPINES

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Bovines are important reservoir hosts of schistosomiasis, placing humans and animals in rice fields areas at risk of infection. This study reported the prevailing infection of zoonotic parasites from bovine feces in the rice fields adjacent to Lake Mainit lake scape in the Philippines. Formalin Ethyl Acetate Sedimentation (FEASD) was performed on 124 bovine fecal samples from rice fields and documented eggs and cysts from eight parasites: Schistosoma japonicum, Fasciola sp., Balantidium coli, coccidian oocyst, Ascaris sp., Strongyloides sp., and hookworm species. Among these parasites, Fasciola sp. harbored the highest infection with a 100% prevalence rate, followed by hookworms (50.94%), B. coli (32.22%), and S. japonicum (13.33%) respectively, while Strongyloides sp. (2.83%) reported lowest. The intensity of infection of Schistosoma eggs per gram (MPEG=4.19) among bovines is categorized as “light.” Bovine contamination index (BCI) calculations revealed that, on average, infected bovines in rice fields excrete 104,750 S. japonicum eggs daily. However, across all ricefield stations, bovines were heavily infested with fascioliasis with BCI at 162,700 Fasciola eggs per day. The study reports that apart from the persistent cases of schistosomiasis in the area, bovines in these rice fields are also heavily infested with fascioliasis. The study confirms the critical role of bovines as a reservoir host for continued infection of schistosomiasis, fascioliasis, and other diseases in the rice fields of Lake Mainit and requires immediate intervention to manage the spread of these diseases.

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## POOLED PEAKS PIPELINE (P3): AN R-BASED PROGRAM FOR POPULATION GENETIC ANALYSES IN POOLED SAMPLES

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Over 1.5 billion people globally are infected with soil-transmitted helminths, and 230 million are infected with schistosomiasis. Although the primary diagnostic method is the microscopic examination of stool or urine, essential transmission dynamics can be identified using population genetics. Pooled egg samples facilitate the analysis of large parasite and infrapopulation numbers. While using microsatellite markers on pooled samples can measure genetic diversity and differentiation, few methods of batch analysis are currently available, and even fewer are available offline and for free. DNA sequencers and genetic analyzers store fragment analysis data as .fsa files that are read into licensed software requiring internet access, such as Peak Scanner. The Pooled Peak Pipeline (P3) is written in R and builds off the previously published Fragman package. P3 provides added quality control, produces reviewable tracings, formats the output to perform duplicate sample comparison, converts raw peak heights to allele frequencies, and computes population-level measures, including Nei’s GST, Jost D, principal coordinate analysis plot, and phylogenetic tree. P3 was tested using fsa files from Schistosoma haematobium lab strain samples and Schistosoma mansoni samples from infected Brazilians. The P3 results of the S. mansoni samples were compared to a previous analysis using traditional tools and therefore serve as an excellent control for the accuracy of P3. In conclusion, this analysis pipeline will provide free and offline access to genetic analysis following initial download, enhancing the capability to use pooled samples, egg or otherwise, for genetic analysis in low-resource settings. Although microsatellite markers are not a diagnostic tool, they are used in various genetic studies of infrapopulations, including studies of host-parasite coevolution, transmission dynamics, and drug resistance. A link to P3’s beta version will be provided.

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## RISK FACTORS FOR HIGHER-INTENSITY SCHISTOSOMA MANSONI INFECTION IN LAKE ALBERT COMMUNITIES, UGANDA: A CROSS-SECTIONAL STUDY

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Schistosomiasis, a chronic trematode disease, is persistent in Lake Albert, western Uganda, the site of the Praziquantel in Preschoolers (PIP) trial. This cross-sectional study aimed to describe the infection and morbidity burden in household members of trial participants and identify risk factors to explain the persistent infection burden. Recruitment was by convenience sampling from four villages and data collection involved a survey, single Kato-Katz (KK) stool examination, hepatic ultrasound and household GPS measurement. Multilevel logistic regression assessed risk factors for moderate-to-heavy infection. Of 243 participants from 66 households, 66% were female and the median age was 22 years (IQR 12 - 33). Participants lived a median of 242m (IQR 178 - 384) from the shore, most (82%) visited the lake at least twice per day and 77% had unimproved sanitation. Despite local campaigns, only 37% reported taking praziquantel in the past year. Most participants (71%) were positive for Schistosoma mansoni on KK and 50% had moderate-to-heavy infection. Twenty-five (11%) participants had periportal fibrosis (image pattern C - F). In multivariable analysis, age and village were associated with moderate-to-heavy infection: adjusted odds ratio (aOR) 5.53 (95% confidence interval [CI] 1.72 - 17.76) for those aged 10 - 19 years vs. <10 years and aOR 0.11 (95% CI 0.03 - 0.46) for the lowest- vs. highest-burden village. There was weak evidence for a negative association with recent treatment and no association with distance to the lake or water contact. There was evidence of household clustering of moderate-to-heavy infection (intraclass correlation coefficient = .13). Schistosome infection and disease is pervasive in lakeshore communities with poor sanitation and low treatment coverage. We found clustering in infection risk which may be targetable by control programmes. Lake proximity was unimportant at the scales studied. High-intensity infections despite recent treatment suggest rapid reinfection or treatment failure. There is an urgent need for more intensive preventive chemotherapy and improved water and sanitation in endemic areas.

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## SNAIL-SCHISTOSOME DYNAMICS IN COMPLEX ECOLOGICAL COMMUNITIES

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Schistosomes are transmitted between human and snail hosts via free-living stages in freshwater. Historically, biocontrol of host snail species using predators and competitors has been attempted with mixed success. In this study we investigated the interactions between the ecological community and snail-schistosome dynamics in two settings. We first conducted a mesocosm experiment to investigate the impact of competition by non-host genera Melanoides and Physa on the population dynamics and schistosome transmission in Biomphalaria, host of Schistosoma mansoni. We tracked snail abundance, body size, reproduction, resources, and schistosome production for 16 weeks. Melanoides decreased the abundance of Biomphalaria and algae, suggesting strong resource competition. Physa also reduced Biomphalaria abundance. However, they increased algae and Biomphalaria body size, cercarial production, and reproduction – effects inconsistent with resource competition, but consistent with intraguild predation. Lab experiments confirmed that Physa is a voracious predator of Biomphalaria eggs. Given these strong effects of competition and predation, we surveyed non-host snail competitors and predators of in 57 waterbodies surrounding Mwanza, Tanzania, at 3 time points over the schistosome transmission season. We found significant variation in predator community composition and that community composition impacted host snail size and infection status: waterbodies with a greater abundance of notonectids and Corixidae had larger snails and a greater infection prevalence. We speculate that these smaller predators are limited to consuming small snails, thereby contributing to larger snail size and infection prevalence. Collectively, our work demonstrates that non-host species in the ecological community of host snails may have a great impact on schistosome transmission dynamics. Synthesizing these community-level processes and understanding which traits of predators and competitors matter can yield important insights into schistosome biocontrol.

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## A COMPLEX INTERPLAY BETWEEN FOOD, HEALTH AND LIVELIHOODS - LIVE FLUKE (OPISTHORCHIS VIVERRINI) IN NORTHEAST THAILAND

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Transmission of the human-infective and carcinogenic trematode Opisthorchis viverrini (OV), a parasite acquired from eating raw or undercooked fish that causes cancer of the bile duct, is ongoing in Southeast Asia. The resulting cancer mainly afflicts individuals aged 40+ and once progressed is rapidly fatal with limited treatment options. Despite decades of control, this cholangiocarcinoma caused by OV remains one of the leading causes of death in Thailand and Laos and incidence rates in Northeast Thailand remain the highest in the world. Determining which interventions will be most effective requires an understanding of fundamental epidemiological processes, many of which are poorly understood. The communities most heavily affected by the parasite are lower-income rural households around the Mekong River and its tributaries where the main sources of employment are fishing, rice farming and fish farming. There is therefore a close connection between people's livelihoods and infection. Persistent public health campaigns have stigmatised the practise of eating raw fish, which has contributed to a disconnect between reported behaviour and actual eating habits. To better understand this disconnect and the complex interplay between diet, health and livelihoods from the community perspective, a food festival at a well-established food market was conducted in the highly endemic region of Maha Sarakham. Questionnaires, discussion groups and interactive activities, all conducted in the Thai language by staff and students from Khon Kaen University, formed the basis of the comprehensive qualitative and qualitative data collection from this event. This event engaged with previously overlooked community members – such as fisherman and non-risk-group individuals. Novel evidence and metrics were gathered from this festival that will be used to inform intervention methods, surveillance, treatment, and health policy. This project will also be used as a template for community engagement and epidemiological data collection and has informed research and public health policy for the region.

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## THE SNAIL-TREMATODE-MICROBIOME TRIPARTITE INTERACTION: FROM LAB MANIPULATIONS TO THE FIELD

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Snail-borne diseases affect over 250 million people worldwide and pose a substantial burden on the livestock industry. A fundamental understanding of the drivers of the epidemiology of these diseases is crucial for the development of sustainable control measures. The microbiome is increasingly being recognized as an important player in the interaction between parasitic flatworms and snail intermediate hosts. In order to better understand this interaction, field and lab-based studies, including microbiome transplant experiments whereby the microbiome is transferred between a donor and a recipient host, are needed. We conducted a transplant and an infection experiment in the lab on Biomphalaria glabrata and collected field data in the Senegal River Basin (SRB) focused on Bulinus spp. First, a multiplex PCR was used to detect flatworm infections in snails. Next, an amplicon sequencing workflow was used to genotype up to 25 infected snails per species per site and their infecting flatworms. Finally, the microbiome of the selected snail specimens was profiled through 16S metabarcoding. We conducted a successful snail microbiome transplant and show that the phylogenetic relatedness between the recipient and donor snail affects the recipient's survival probability. Furthermore, the microbiome changes throughout flatworm infection development in B. glabrata. Moreover, sympatric host-parasite combinations might affect the microbiome differently compared to allopatric ones. Finally, the SRB field dataset showed marked variation in the microbiome between species and across regions but not between infected and uninfected samples. Combined with information on the co-infection status of our samples, these findings could provide further insights into the relationship between infection status and microbiome. Transplant experiments, complemented by field-based studies, could facilitate future research endeavors to investigate the role of specific bacteria or bacterial communities in parasitic flatworm resistance of freshwater snails and might ultimately pave the way for microbiome-mediated control of snail-borne diseases.

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## USING MATHEMATICAL MODELS TO UNDERSTAND SCHISTOSOMIASIS TRANSMISSION IN A UGANDAN HOTSPOT

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Schistosomiasis is a neglected tropical disease of profound medical importance, infecting approximately 240 million people, 90% living in Sub-Saharan Africa. Severe schistosomiasis is associated with periportal fibrosis and portal hypertension which can cause death without appropriate disease management. A cornerstone of international efforts to eliminate schistosomiasis as a public health problem is mass drug administration

(MDA) with praziquantel. However, despite nearly two decades of MDA, the prevalence of infection and the incidence of periportal fibrosis remains high in communities along the shore of Lake Albert in Uganda, representing a conspicuous failure of the current intervention strategy. The FibroScHot Consortium is addressing this urgent public health need by conducting a randomised controlled trial to evaluate the effectiveness of delivering MDA at frequencies of up to four times per year. Reporting on research conducted as part of the Consortium, this talk presents progress from mathematical model-based analyses of the historical impact of MDA on the infection and morbidity dynamics of Schistosoma mansoni along the shore of Late Albert and discusses how severe schistosomiasis may be tackled in transmission hotspots.

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## MALE GENITAL SCHISTOSOMIASIS AMONG LOCAL FISHERMEN ALONG SOUTH SHORELINE OF LAKE MALAWI IN MANGOCHI DISTRICT

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Male genital schistosomiasis (MGS) is an ignored consequence of urogenital schistosomiasis (UGS) with schistosome eggs and pathologies in genitalia. First reported by Madden in 1911, its epidemiology, diagnostic testing and management are not well described owing to limited research and diminishing focus. Furthermore, expansion of Human immunodeficiency virus (HIV) epidemic across sub-Saharan Africa has renewed interest in MGS owing to their plausible but under-explored interactions. A longitudinal cohort study was set up among fishermen along southern Lake Malawi shoreline in Mangochi district to investigate prevalence and morbidity of MGS. Fishermen aged 18+ years were recruited and administered questionnaires, assessing knowledge, attitudes and practices associated with MGS. They submitted urine and semen for parasitological and molecular diagnostic tests: dipstick, point-of-care circulating cathodic antigen (POC-CCA), filtration, microscopy and real-time PCR. Abdominopelvic and scrotal ultrasonography were conducted for abnormalities in prostate, seminal vesicles, epididymis, testes and other organs. Standard Praziquantel (PZQ) treatment (40mg/kg) was offered to all and follow-up was done at 1, 3, 6 and 12 months. 376 fishermen (median age: 30 years, range: 18-70) were recruited and interviewed. Below 10% reported experiencing MGS symptoms, like genital or coital pain. Baseline MGS prevalence by semen microscopy was 10.4% (n=114, median: 5.0/ml) while real-time PCR was 26.6% (n=64). UGS prevalence was 17.1% (n=210, median: 2.3/10 ml). None of MGS positive participants experienced MGS symptoms. 130 fishermen had ultrasonography and 9 (6.9%) had abnormalities, 1 with prostatic nodule and another a testicular nodule. Subsequent analyses on follow-up indicated variable detection dynamics, with fewer abnormalities observed. MGS is prevalent among local fishermen along southern Lake Malawi, which calls for improved availability and accessibility to advanced diagnostics, PZQ treatment and control interventions to reduce prevalence and better manage MGS.

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## PREVALENCE OF SCHISTOSOMIASIS AND IMPLEMENTATION OF SCHISTOSOMA PREVENTION PROJECT IN GEZIRA STATE, SUDAN 2022-23

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In several developing countries, including Sudan, schistosomiasis remains a public health problem particularly in Gezira state. The needs of clear prevalence and testing an effective environmental and social impact methods were essential to eradicate schistosomiasis .This study is composed of two major sections first to measure the prevalence of schistosomiasis in Gezira state -the second part is to implement environmental-social based interventional projects to cover an area of the gap in knowledge and attitude in one of the most prevalent villages after the primary survey result and the total study period was 18 months. The first part of study was done by Descriptive Prevalence Study design [DPS]using a case-counting survey in major health centers in Gezira state .the second part composed of three phases :-pre intervention knowledge and attitude survey . -intervention through health education session .-Post-intervention evaluation survey.The primary survey reveals S.haematobium was found in 32%, while S.mansoni infection was found in 43% of the Gezira state population, and 13% of the total number of cases in Gezira state from Alhafayer village out of hundreds of villages, that's why we choose Alhafayer village for the interventional project, the outcome of 14 months of interventions: The morbidity rate in alhafayer village has dropped from 82% to 24%. Raising awareness among the village population from 13% to 78% after assessing attitudes and practice of schistosoma-preventing methods. Opening spared section in Alhafayer heath center for diagnosis, provide treatment , and scheduling regular health education sessions for all social classes .The need for clean water sources was discussed with the Gezira government and charities .however the clean water project will start soon to cover Alhafayer and two neighboring villages .Collaboration across sectors for water supply, environmental management, and health education an intersectoral approach needed to eradicate Schistosoma. Also an Operational research is essential to develop intervention methods, diagnostic procedures, and human behavior facilitation.

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## EVALUATION OF THE BURDEN AND RISK FACTORS ASSOCIATED WITH FEMALE GENITAL SCHISTOSOMIASIS IN TWO ENDEMIC AREAS IN MALAWI AS PART OF THE MORBIDITY OPERATIONAL RESEARCH FOR BILHARZIASIS IMPLEMENTATION DECISIONS (MORBID) STUDY

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Female genital schistosomiasis (FGS), caused by Schistosoma haematobium (Sh) is prevalent in Sub-Saharan Africa (SSA). FGS is associated with sexual and reproductive life morbidity, and increased HIV and cervical precancer prevalence. There are no control programmes for FGS screening and diagnosis in endemic countries, hindering precise disease burden estimation. This study evaluated the burden of FGS by visual and molecular diagnostic methods in two districts in Southern Malawi. Women aged 15-65 years, sexually active, not currently menstruating, or pregnant, were enrolled from the larger MORBID study. A midwife completed a symptoms questionnaire, obtained a cervicovaginal swab and cervicovaginal lavage (CVL), and assessed FGS-associated

genital lesions using hand-held colposcopy. Visual-FGS was defined as the presence of sandy patches, rubbery papules, or abnormal blood vessels. Molecular-FGS was defined as Sh DNA detected by real-time PCR from a cervicovaginal swab. Egg-patent Sh infection was detected by urine microscopy. 950 women completed the questionnaire (median age 27, [IQR] 20-38). Visual and molecular FGS prevalence was 26.9% (260/967) and 8.2% (78/942), respectively. Of the 584 women with available genital and urinary samples, 6.5% (38/584) had egg-patent Sh infection. Multivariable logistic regression showed a positive significant association between molecular and visual FGS (OR=2.9, p<0.01). Self-reported urinary, genital, sexual and reproductive health symptoms were not predictive of FGS (p>0.05). Molecular-FGS was associated with egg-patent Sh infection (OR=7.5, p<0.01). There was no significant correlation between visual-FGS and Sh infection status (p = 0.5). Some villages had high prevalence of molecular-FGS, even with <10% prevalence of urinary Sh in school-aged children (SAC), a common treatment threshold. No significant association between visual-FGS and village Sh prevalence was found (p=0.2). This study highlights the significant burden of FGS in Southern Malawi and importance of using field-deployable screening methods for FGS diagnosis in SSA countries and determining treatment need.

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## PRESENCE OF SARS-COV-2 RNA IN DIFFERENT SOURCES OF WATER OF NEPAL

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Wastewater-based epidemiology (WBE) has been one of the most promising surveillance tools for monitoring the status, trend of infection, possible future outbreaks of coronavirus disease-2019 (COVID-19), to gather the information on disease surveillance, epidemiological models, genetic diversity, and geographic distribution. This study reports the presence and reduction of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) at two wastewater treatment plants (WWTPs) of Nepal, along with river water, hospital wastewater (HWW), and wastewater from sewer lines collected between July 2020 and February 2021. SARS-CoV-2 RNA was detected in 50%, 54%, 100%, and 100% of water samples from WWTPs, river hospitals, and sewer lines, respectively, by at least one of four quantitative PCR assays tested (CDC-N1, CDC-N2, NIID_2019-nCOV_N, and N_Sarbeco). The CDC-N2 assay detected SARS-CoV-2 RNA in the highest number of raw influent samples of both WWTPs. The highest concentration was observed for an influent sample of WWTP A (5.5 ± 1.0 log10 genome copies/L) by the N_Sarbeco assay. SARS-CoV-2 was detected in 47% (16/34) of the total treated effluents of WWTPs, indicating that biological treatments installed at the tested WWTPs are not enough to eliminate SARS-CoV-2 RNA. One influent sample was positive for N501Y mutation using the mutation-specific qPCR, highlighting a need for further typing of water samples to detect Variants of Concern. Furthermore, crAssphage-normalized SARS-CoV-2 RNA concentrations in raw wastewater did not show any significant association with the number of new coronavirus disease 2019 (COVID-19) cases in the whole district where the WWTPs were located, suggesting a need for further studies focusing on suitability of viral as well as biochemical markers as a population normalizing factor. Detection of SARS-CoV-2 RNA before, after, and during the peaking in number of COVID-19 cases suggests that WBE is a useful tool for COVID-19 case estimation in developing countries.

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## POOR OUTDOOR BATHROOMS DRAINAGE SYSTEMS OF CHING'AMBO RESIDENTS IN MZUZU CITY AS A SAFE HAVEN AND TOOL FOR INCREASED EXPOSURE TO TROPICAL PARASITES

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Bathroom drainage wastewater is a major source of water borne diseases and injuries especially in densely populated communities with a higher water table. However, water sanitation remains one of the neglected areas of research and health promotion in the fight against diseases of poverty. For this project, we aimed at investigating the implications of poor drainage systems of traditional bathrooms among residence of Ching'ambo, in Mzuzu city. We conducted an exploratory survey using quantitative and qualitative data collection methods which included photography of out-door bathrooms drainage systems, observation checklists, and in-depth interview questionnaires. A total of 60 households with traditional bathrooms were identified through random sampling technique and calculated using n=N/1+N (e)2 with 95% confidence interval participated in the study. SPSS version 16 statistical software was used for the generation of descriptive analysis. The results demonstrated that inhabitants were highly exposed to vectors or parasites that transmit neglected tropical diseases of poverty such as mosquitoes, worms, ticks, snails and other insects of medical entomology. In the study, 60% of participants reported to be victims of snakebite and physical injury while 70% perceived themselves as having experienced zoonotic diseases due to close contact with stray dogs that habited these bathrooms as safe havens. The research showed that poor and inadequate spacing for construction of out-door bathrooms wastewater pathways were important contributors for poor drainage systems which were in agreement with the reports of 68% of households respondents. Consequently, the findings of this study provide an impetus for further wastewater epidemiology and related research. Additionally, there is need for intense interventions tailored towards promotion of proper wastewater disposal leadership among residents of Ching'ambo and surrounding semi-urban communities in Mzuzu city.

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## SOCIOCULTURAL INFLUENCES ON ACCEPTANCE AND HEALTH RISK OF WATER RESOURCES IN REMOTE COMMUNITIES IN GHANA

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Access to clean water has an important role to play in ensuring good public health and to reduce health risks, so there are clear guidelines and pollution limits to safeguard the health of populations. Water should be free of microbial inputs, chemical inputs (heavy metals), with water quality indices values between 70 and 100. With these requirements met, consumers can accept and utilize water; however, water may be utilized for other reasons. The relationship between rural people and their environment, vis à vis water resources is deeply rooted in socio-cultural values and norms; this can influence utilization and acceptance regardless of vital health risks, but this gap has not been researched particularly in rural, remote communities. Additionally, water quality assessments usually end at comparing concentrations with WHO guideline values, however, this study assessed health risk. Key Informant Interviews were conducted among twelve purposively sampled community leaders, using an interview guide and results analyzed using Interpretative phenomenological analysis method and health risk of heavy metals were also assessed using USEPA models. Cultural norms and History influenced acceptance, utilization and protection of the water resource. Management of water resources is shrouded in Taboos and Beliefs, with little scientific underpinning; meanwhile water quality is compromised by run-off from riparian agricultural activities and some heavy metals potentially having long term effects despite recording

low concentrations. Therefore, water and policy managers need to conduct proper community engagement, to reduce any sociocultural barriers and assess health risks, in water protection interventions.

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## MOLECULAR DETECTION OF PATHOGENIC LEPTOSPIRA AND HELICOBACTER PYLORI IN ENVIRONMENTAL SPECIMENS COLLECTED FROM THE OPISTHORCHIASIS ENDEMIC AREAS AT KHON KAEN PROVINCE, THAILAND

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Opisthorchiasis is a foodborne trematodiasis common in Thailand. Interestingly, opisthorchiasis endemic areas were found to have substantial incidences of leptospirosis as well as Helicobacter pylori infection. The parasite causing opisthorchiasis (Opisthorchis viverrini) has a complex life cycle involving different intermediate hosts before maturing into the infective metacercariae stage and infecting humans through ingestion of mainly raw fish. O. viverrini infection is the main risk factor for cholangiocarcinoma in humans. Since O. viverrini, Leptospira spp. and H. pylori are present in various aquatic environments, we attempted to elucidate the relationships of these pathogens at the opisthorchiasis endemic areas around the Lawa Lake region at Khon Kaen Province, Thailand. Specimens were collected from ten study sites around the Lawa Lake region at Khon Kaen Province. Mud, water, snail and fish mucus were collected from the study sites. One metacercariae specimen was also included in the study. DNA extracted from the specimens were subjected to PCR assays targeting the LipL32 (pathogenic Leptospira) and cagA (H. pylori) genes. PCR positive products were sequenced and compared using the BLAST database for identification. Sequencing of the PCR positive products revealed that the mud, fish mucus and metacercariae specimens were positive for the presence of H. pylori. Remarkably, the same mud specimens that were positive for H. pylori were also positive for the presence of pathogenic Leptospira, suggesting co-localization of these two pathogens at the same site. Our findings suggest that H. pylori and pathogenic Leptospira are prevalent in opisthorchiasis endemic areas at Khon Kaen Province, Thailand.

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## ASSESSMENT OF THE MICROBIAL CONTAMINATION OF DELIVERY BOXES OF ONLINE FOOD DELIVERY SERVICES PROVIDERS IN ACCRA

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The sanitary conditions under which the food is transported is vital for safety. This study aimed at enumerating and identifying microbial contaminants of delivery boxes of food delivery service providers in Accra as an indicator of microbial quality and hygiene. Swab samples were randomly obtained from forty (40) food delivery operators at the beginning and end of week. The Total Aerobic, Total Coliform Enterobacteriaceae, Fecal Coliform, yeast and mold counts, were determined as an index of microbial quality and safety. Pathogens from the delivery boxes were isolated, characterized and identified. There was significant level of bacterial and fungal contamination in the delivery boxes. The maximum Total Aerobic count, coliforms, E. coli yeast and mold in the delivery box were 1.03×106cfu/cm2, 1.7×105cfu/cm2, 4.92×103cfu/cm2, 6.57×102 and 5.60×102 respectively at the beginning of the working week. These increased to 9.56×106cfu/cm2, 2.18×106cfu/cm2, 3.51×104 cfu/cm2, 7.61×103 cfu/cm2 and 8.31×102 cfu/cm2, respectively at the close of the week. Bacillus sp., Staphylococcus sp., Acinetobacter and Pseudomonas stutzeri. Saccharomyces cerevisiae, Rhodotorula rubra, Aspergillus niger and Penicillium sp. were isolated from the delivery boxes. The high contamination levels recorded in this study indicates the poor hygienic state and practices of the food delivery service operators in Accra. Foods and other items being transported for delivery in these boxes have a potential and risk of contamination from these pathogens and contagions, posing a public health threat to recipients and unsuspecting customers who patronize food delivery services in the highly urbanized population of Accra. Hygiene training of food delivery service providers is an urgent necessity.

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## INCREASING THE ACCESSIBILITY AND HANDWASHING PRACTICES THROUGH TIPPY TAPS IN CABO DELGADO PROVINCE, MOZAMBIQUE

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Diarrheal diseases including cholera are responsible for a high mortality and morbidity in humanitarian emergencies due to overcrowding, malnutrition, lack of healthcare access, water and sanitation facilities as well as handwashing stations. The province of Cabo Delgado, Mozambique, has been immersed in a severe humanitarian crisis due to conflict and insecurity since 2017. Hygiene promotion campaigns have been implemented since August 2022 including construction of a simple and low-demanding resource handwashing station, named tippy tap, in Internal Displaced Persons (IDPs) sites and host communities. The aim of this study was to describe the accessibility, use and community ownership of tippy taps. A cross-sectional survey was conducted in 5 districts after 6 months of a hygiene promotion campaign implementation. A total of 409 people participated in the survey. Overall, 63% of participants were females, the largest age group were people between 26 and 35 years old (26%) and 64% of households had at least a person with the primary education level. Thirty-one per cent of households reported to have a tippy tap within 500 meters and 22% of participants reported to use a tippy tap within the last 24 hours. A hundred and fifty-one households (37%) reported at least one case of diarrhea or stomachal pain within the previous month. The use of tippy taps was strongly associated with their accessibility ($P<0.001$) and knowledge of causes of diarrhea ($P=0.006$). Moderately evidence was found between use of tippy taps and self-reported diarrheal cases within the household during the previous month ($P=0.04$). Further construction of 1550 tippy taps in program targeted areas without programmatic support showed the strong uptake of communities demonstrating the sustainability and ownership of tippy taps as a tool to improve hygiene practices. The provision of functional and feasible handwashing stations in humanitarian contexts is crucial to increase handwashing practices for cholera and diarrheal disease prevention.

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## WORK RELATED INJURIES: WHAT FACTORS DETERMINE ITS SEVERITY IN A LOW RESOURCE SETTING?

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It is estimated that more than 2.3 million people (both males and females) die from work-related injury or disease; this links to over 6000 deaths daily globally. This study assessed factors influencing the severity of workplace injuries reported to Teaching Hospitals in Ghana. fr  The study employed a cross-sectional prevalence survey with a quantitative approach. A simple random sampling method was used to select the study respondents. Data were captured using REDCAP and was exported from REDCap directly into Stata 16 statistical software for analysis. The total number of respondents

involved in this study was 523. Statistical significance for all testing was set as 0.05.ResultsA total of 523 workplace injuries data were extracted at the various teaching hospitals, less than half the number of injured patients (38.43%) were admitted at KATH. The respondents who resided in rural areas were 2.60 more likely to experience severe workplace injuries (AOR=2.60, 95% CI=1.19-5.69). Exposure to burns, blunt and penetrating injuries increased the likelihood of experiencing severe workplace injuries (AOR=20.46; 95% CI=2.25-186.06), (AOR=19.39; 95% CI=2.14-175-27) and (AOR=9.33; 95% CI=1.53-56.99) respectively. ConclusionThe sentinel hospital site, type of settlement, and mechanism of injury were established to be influencing the severity of workplace injuries. The authors recommend the prioritization of stakeholder involvement such a as industry management team to work towards practical steps to address this burden education

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## ASSOCIATION OF PRENATAL ENVIRONMENT FACTORS WITH UNDER FIVE NUTRITIONAL GROWTH OUTCOMES IN UGANDA

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Children with nutritional growth failure are more susceptible to infections and may experience cognitive, physical, and metabolic developmental impairments. Many countries in Africa depend on rain-fed agriculture for food security which is reflected in meteorological environment factors. The objective of the current study is to assess meteorological factors associated with village-level rates of child nutritional growth outcomes in Uganda. This was a cross-sectional study of 5219 children ages 0 to 59 months with anthropometric measures using the 2016 Ugandan Demographic and Health Survey. Geostatistical models parameterized by meteorological, land topography and sociodemographic factors were developed to determine their association with height-for age z-scores (HAZ), weight-for-age z-scores (WAZ), and weight-for-height z-scores (WHZ). Of the 5219 children ages 0 to 59 months included in the analysis, 50% were male, and mean age was 33 (SD:16) months. Large disparities in the burden of child growth failure exist within Uganda at smaller and larger spatial scales; villages in the northeastern and southeastern areas of the country bear the highest prevalence of all forms of child undernutrition. Higher SPEI 3 months pre-birth was positively associated with child nutritional growth outcomes. Additionally, higher location mean rainfall 11 months pre-birth was positively associated with HAZ consistent with SPEI findings. Aridity index association with WAZ and WHZ were consistent with findings of SPEI. Slope angle, land surface mean temperature, travel time to the nearest city, and nighttime light emissions were not consistent with nutritional growth outcomes. In conclusion, pre-birth availability of agricultural water was associated with nutritional child growth outcomes.

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## BARRIERS OF EFFECTIVE AND SUSTAINABLE WATER, SANITATION AND HYGIENE (WASH) SERVICES AT SCHOOLS IN BANGLADESH

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Considering the potential effect of a poor environment in schools on children, every school requires appropriate WASH initiatives that include provision of WASH facilities and sustainable operation and maintenance (O&M) of those facilities. In Bangladesh, all government schools are mandated to have a School Management Committee (SMC) who are responsible for ensuring WASH O&M. However, in majority of the schools SMCs didn't perform better in sustainably operating and maintaining their WASH infrastructure. We investigated the barriers for successful O&M of WASH services in schools to help inform strategies. We conducted qualitative interviews with 36 SMC members from purposively selected 18 primary schools in three cities in Bangladesh between Feb- Apr 2020. All interviews were audio recorded, transcribed verbatim and we performed thematic content analysis. The major problem identified was economic. Due to low funding from government, lack of private contributions and lack of financial mechanism, SMCs were unable to make investment decision to ensure supplies as well as recruiting a janitor. Inadequate supplies of cleaning products, handwashing agents left toilets unclean and allow students to wash their hands with water only. Moreover, if maintenance is required when facilities were broken or blocked and need the septic tanks emptied, or replacement of tube wells, SMCs were unable to do it as they did not have their own fund. Children were not aware of how to use the facilities properly, and did not have good hygiene practices. Community contribution was poor in terms of proving good hygiene practices to children and in improving WASH facilities at schools. Communication and coordination gaps between SMCs and other stakeholders were also an important barrier. Moreover, there was an institutional lack of planning and adoption of WASH policies or they lack the capacity, time, and resources for appropriate decision making and planning of WASH services for their schools. Providing information and train SMC for capacity building, establishing a strong financial mechanism may be useful for successful O&M of school WASH in a sustainable way.

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## EVALUATION OF A MULTI-LEVEL, PARTICIPATORY INTERVENTION TO REDUCE ARSENIC EXPOSURE IN AMERICAN INDIAN COMMUNITIES: A CLUSTER RANDOMIZED CONTROLLED TRIAL OF THE COMMUNITY-LED STRONG HEART WATER STUDY PROGRAM

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Chronic arsenic exposure has been associated with an increased risk of cardiovascular disease, diabetes, cancers of the lung, pancreas and prostate, and all-cause mortality in American Indian communities in the Strong Heart Study. The Strong Heart Water Study (SHWS) designed and evaluated a multi-level, participatory community-led intervention to reduce arsenic exposure among private well users in partnership with Northern Great Plains American Indian Nations. A cluster randomized controlled trial (cRCT) was conducted to evaluate the effectiveness of the SHWS program over a two-year period on: (1) urinary arsenic; and (2) reported use of arsenic safe water for drinking and cooking. The cRCT compared the installation of a point-of-use arsenic filter and a mobile health (mHealth) program (3 phone calls; SHWS mHealth & filter arm) to a more intensive program, which included this same program plus 3 home visits (3 phone calls and 3 home visits; SHWS intensive arm). A 48% significant reduction in urinary arsenic (geometric mean 13.2 to 7.0 µg/g creatinine) was observed from baseline to the final 2 year follow-up when both study arms were combined (Geometric mean ratio (GMR): 0.52 (95% Confidence Interval: 0.39, 0.69)). By treatment arm, the reduction in urinary arsenic from baseline to the final follow-up visit was 57% in the mHealth & filter arm and 29% in the intensive arm. There was no significant difference in urinary arsenic levels by treatment arm at the final follow-up visit (GMR comparing the intensive vs. mHealth & filter arms: 1.21 (95% CI: 0.77, 1.90). In both

arms combined, exclusive use of arsenic-safe water from baseline to the final follow-up visit significantly increased for water used for cooking (17% to 50%) and drinking (12% to 43%). Delivery of the community-led SHWS program, including the installation of a point-of-use arsenic filter and an mHealth program on the use of arsenic-safe water without home visits, resulted in a significant reduction in urinary arsenic and significant increases in reported use of arsenic-safe water for drinking and cooking during the two-year study period.

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## A CLUSTER RANDOMIZED CONTROLLED NON-INFERIORITY TRIAL TO COMPARE THE PROTECTIVE EFFECTIVENESS OF SULFADOXINE PYRIMETHAMINE AND AMODIAQUINE AND DIHYDROARTEMISININ PIPERAQUINE FOR SEASONAL MALARIA CHEMOPREVENTION AMONG CHILDREN 3 TO 59 MONTHS, IN THE CONTEXT OF HIGH PARASITE RESISTANCE, KARAMOJA REGION, UGANDA

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In areas with highly seasonal malaria transmission, seasonal malaria chemoprevention (SMC) with sulfadoxine-pyrimethamine and amodiaquine (SPAQ) is recommended for age groups at high risk of severe malaria. However, due to widespread prevalence of resistance markers associated with parasite resistance to SP and AQ in east and southern regions of Africa, there was need to explore an effective alternative regimen for SMC that could be used if SPAQ starts to lose efficacy. We assessed the effectiveness of SMC for prevention of malaria among 3,853 children aged 3-59 months using a three-arm open-label prospective cluster-randomized controlled trial (cRCT) in Karamoja sub-region in Uganda. A total of 3,749 children were randomized to receive SMC with either SPAQ (1,698) or dihydroartemisinin-piperaquine (DHAPQ) (1,667) while 384 acted as control and relied on standard malaria care over the five-month high transmission period. In total, 554,155 person-days (251,414 in the SPAQ arm, 249,069 in the DHAPQ arm and 53,672 in the control arm) were generated for analysis. There were 464 events of clinical malaria (76 in the SPAQ arm, 66 in the DHAPQ arm and 322 in the control arm). These represent an incidence rate of 8 confirmed malaria events per 10,000 person-days in the entire study population (3 per 10,000 person-days in the SPAQ arm, 3 per 10,000 person-days in the DHAPQ arm and 60 per 10,000 person-days in the control arm). 750 samples were corrected one month before and after implementation of SMC and assessed for markers of resistance for SP, AQ and DHAPQ. Compared with children in the control arm, those in the SPAQ arm had a 94% lower risk of having an RDT-confirmed malaria episode; Hazard Ratio (HR): 0.06 (95% confidence interval [CI], 0.04 - 0.08, p<0.001); while those in the DHAPQ arm had a 96% lower risk; HR: 0.04 (95% CI, 0.03 - 0.06, p<0.001). The hazard ratio for the protective effectiveness of SPAQ was non-inferior to that of DHAPQ. In our setting, DHAPQ was not superior to SPAQ in terms of prevention of clinically significant malaria in SMC-eligible children.

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## MATAMAL: A CLUSTER - RANDOMIZED PLACEBO-CONTROLLED TRIAL TO EVALUATE THE ADDITIVE IMPACT OF IVERMECTIN TO DIHYDROARTEMISININ-PIPERAQUINE SEASONAL MASS DRUG ADMINISTRATION FOR MALARIA CONTROL ON THE BIJAGOS ARCHIPELAGO OF GUINEA-BISSAU

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Ivermectin (IVM) is an anthelminthic drug that effectively kills anopheles mosquitoes which blood-feed on treated individuals. Small phase 2 and 3 clinical trials have demonstrated its potential to reduce malaria incidence, and modelling studies suggest that it could be an effective tool to reduce malaria transmission, particularly if given as Mass Drug Administration (MDA) in combination with Dihydroartemisinin-Piperaquine (DP) MDA. The safety and efficacy of IVM and DP in combination has been demonstrated previously. MATAMAL is a cluster-randomised placebo-controlled parallel assignment clinical trial conducted on the Bijagos Archipelago of Guinea-Bissau, testing whether the addition of IVM (300mcg/kg/day for 3 days) to DP MDA each month for three months during the rainy season significantly reduces malaria prevalence compared to giving DP MDA with placebo ((IVM-P), 300mcg/kg/day for 3 days). MDA was given in the context of standard malaria control measures, including insecticide-treated bed nets. MATAMAL is the largest trial to evaluate the additive impact of IVM MDA, and the only trial to evaluate combined IVM/DP MDA versus a placebo control. The trial arms consisted of 24 clusters, with 12 allocated to each of DP+IVM and DP+IVM-P, distributed monthly in July, August and September of 2021 and 2022. The primary outcome is the difference between arms in malaria prevalence, measured by estimating the cluster-adjusted prevalence of Plasmodium falciparum by varATS qPCR in a random sample of individuals of all ages during the endpoint survey, conducted during the peak transmission season. Secondary outcomes include safety and tolerability, clinical malaria incidence, entomological outcomes and the wider impact of IVM on co-endemic IVM-sensitive infectious diseases. Approximately 24,000 individuals received MDA for 2 years. The MDA was well-tolerated with relatively few adverse events that were predominantly mild in severity. 4,305 individuals were sampled in the primary outcome survey. The primary outcome and select secondary outcome data will be presented.

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## MONITORING SUSTAINED IMPACT ONE YEAR AFTER MASS DRUG ADMINISTRATION IN A LOW-MODERATE MALARIA TRANSMISSION SETTING OF SENEGAL WITH OPTIMIZED CONTROL INTERVENTIONS

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Mass drug administration (MDA) is not recommended for malaria transmission reduction in moderate transmission settings due to a lack of evidence of sustained impact. Short-term results of an open-label, cluster-randomized controlled trial in a low-moderate malaria transmission setting demonstrated MDA targeting individuals ≥3 months of age was associated with a 53% [95% CI: 29%, 69%] greater reduction in malaria incidence in all ages from baseline compared to standard-of-care which included seasonal malaria chemoprevention (SMC) given to children 3-120 months of age. Here we assessed if the impact was sustained one year after MDA. The study occurred in Tambacounda, Senegal, where district-level annual incidence was 50-200/1000 population in 2016-2019. Prior to the trial, optimized control interventions were introduced (i.e., proactive community case management and mass distribution of pyrethroid-piperonyl butoxide nets) in all 60 study villages. Villages were randomized 1:1 to receive 3 rounds of MDA with dihydroartemisinin-piperaquine+single low-dose primaquine (DP+SLD-PQ) or standard-of-care which included 3 cycles of SMC with sulfadoxine-pyrimethamine+amodiaquine; coverage was 80-84% and 93-94% of the eligible population, respectively. In 2022, the year after MDA rounds were completed, SMC was resumed in all villages. The primary outcome was malaria incidence confirmed by rapid diagnostic test by village health workers and at health facilities during the high transmission season of the post-intervention year, with subgroup analyses by MDA coverage, age (≥ vs. <10 years), and baseline malaria endemicity. Incidence rates in the MDA arm were 188 cases/1000 in 2020, 93/1000 in 2021, and 130/1000 in 2022, compared to 211, 179 and 152 cases/1000 in the control arm. The adjusted rate ratio (MDA:control) in the post-intervention year was 0.81 [95% CI: 0.51, 1.29]. While a substantial reduction in incidence was seen in the intervention year, this was not sustained in the year after MDA. Higher population coverage and/or more rounds and/or years of MDA may be needed to achieve a sustained impact. Subgroup analyses will be presented.

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## TREATMENT OF UNCOMPLICATED MALARIA USING ARTEMISININ-BASED COMBINATION THERAPY IN THE FIRST TRIMESTER OF PREGNANCY: EXPERIENCE FROM TANZANIA

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Artemether-lumefantrine (AL) has been the recommended first-line treatment for uncomplicated malaria and has been widely available in retail markets in Tanzania since 2006. The World Health Organization recommended AL for the treatment of uncomplicated malaria in pregnancy (MiP) in the first trimester of pregnancy in November 2022. This decision came two years after Tanzania revised its malaria treatment guidelines in 2020 recommending AL for the treatment of MiP in the first trimester. We describe the Tanzanian experience following this policy change. We reviewed data from public and private health facilities from the Health Management Information Systems (HMIS) in all 26 regions of Tanzania between January 1, 2016 and December 31, 2022. The updated treatment guidelines were disseminated throughout Tanzania to all facilities (>9,000) by December 2021. In Tanzania, all pregnant women are tested for malaria at their first antenatal care (ANC) visit. However, as midwives may not prescribe antimalarials, all women who test positive at ANC are referred to the outpatient department for treatment. The HMIS does not disaggregate malaria treatment by pregnancy status; therefore, the proportion of pregnant women who tested positive during ANC was used as a proxy indicator for MiP treatment. The proportion of pregnant women tested for malaria during ANC increased from 75% 2016 to 98% in 2022. The malaria test positivity rate ranged between 6-7% between 2016-2022. All pregnant women who tested positive regardless of trimester received AL following implementation of the 2020 treatment policy. It was noted that the HMIS did not capture whether cases were uncomplicated or severe, nor was there a way to directly report adverse drug reactions. AL stock remained >95% in both public and private facilities. Our review suggests a high level of adherence in Tanzania to the updated policy recommending AL for treatment of MiP in the first trimester.

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## ANTI-GAMETOCYTE ACTIVITY AND POST-TREATMENT PROTECTIVE EFFICACY OF ARTEMETHER-LUMEFANTRINE VS. DIHYDROARTEMISININ-PIPERAQUINE FOR UNCOMPLICATED MALARIA: PRELIMINARY RESULTS OF A MULTI-DOSE PHARMACOKINETIC/PHARMACODYNAMIC TRIAL

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Mass drug administration (MDA) for P. falciparum malaria elimination remains a controversial practice. Currently, the favored agent is dihydroartemisinin-piperaquine (DP) due to its longer half-life and its designation in Africa as second-line to artemether-lumefantrine (AL). We conducted a randomized trial of the comparative anti-gametocyte and secondary chemopreventive effects of AL vs. DP to elicit information relevant to their deployment in different contexts. Zambian children <5 years old (n=182) with uncomplicated malaria were randomized to AL or DP, admitted 72 hours for parasite and drug kinetics, and followed for 9 weeks. 173 participants (95%) contributed 1,424 person-weeks of observation time. Participants were similar at baseline across all covariates, including parasite and gametocyte density. From thin smears, asexual clearance did not differ between the groups but gametocyte clearance was more rapid and durable in the AL group. In those with baseline gametocytemia, 100% (8/8) in the AL group were clear by 1 week compared to 62% (5/8) in the DP group (p=0.04). In those with gametocytes at any time, gametocyte density was significantly lower in the AL group (AUC 38 gam.*h/mL (95% CI 13-107) vs. 136 (95% CI 34-550) in the DP group, p<0.001), the proportion with gametocytemia was significantly lower in the AL group at all weeks, and those treated with DP were significantly more likely to have emergent gametocytemia. The overall incidence of recurrent asexual parasitemia was higher in the AL vs. DP group (hazard ratio 1.9, 95% CI 1.3-2.7, p=0.001) but by 9 weeks there was no significant difference in the cumulative prevalence of recurrent parasitemia (AL 58% vs. DP 49%, p=0.31) or clinical failure (AL 19% vs. 18%, p=0.89). Molecular and chemical assays to generate high-resolution gametocyte dynamics and drug pharmacokinetics are underway, but these preliminary results support a differential impact on gametocytes that could have relevance for how these agents are deployed in different malaria contexts, pointing to a need for pairing primaquine with DP to eradicate gametocytes or prioritizing an alternative combination for MDA.

5693

## PHARMACOMETRIC ASSESSMENT AND DOSE-OPTIMIZATION OF PRIMAQUINE IN THE RADICAL CURE OF PLASMODIUM VIVAX MALARIA IN CHILDREN: AN INDIVIDUAL PATIENT DATA META-ANALYSIS

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Primaquine is one of only two registered antimalarials for the radical cure of P. vivax malaria. The main burden of malaria is in young children and yet there is no suitable pediatric formulation. After decades of use, primaquine dosing recommendations in the pediatric population are still ambiguous. Weight-based and age-based dosing have yet to be optimized, resulting in increased variability in drug exposure and under-dosing in young children. Pharmacokinetic studies were identified through a systematic literature review of articles published in PubMed, Google Scholar, Embase, ClinicalTrials.gov, or conference proceedings between 1960 and 2021, following PRISMA guidelines. Investigators were invited to contribute individual-level patient data to the WWARN repository as part of the study group. Primaquine concentration-time data were analyzed using nonlinear mixed-effects modelling, with a particular focus on the impact of body weight and age. A total of 24 studies (15,862 measured primaquine and 10,693 carboxyprimaquine concentrations), including 2,399 adults and 226 children <5 years, were identified and included in this pooled meta-analysis. Primaquine pharmacokinetics were best described by a one-compartment distribution model, with transit absorption. Nonlinear relationships between total drug exposure and body weight and age were observed, highlighting the need for a pharmacometric approach to evaluate and design evidence-based optimal dosing in young children. The final pharmacokinetic model was used to explore different dosing scenarios with the available dose strengths of 5, 7.5, and 15 mg tablets, which demonstrated unacceptably high exposures in young children. Our findings support the development of novel pediatric formulations that can be used more accurately in this specific population. With the current tablet strengths, a tablet needs to be broken or dissolved in water to administer an appropriate dose in young children. An optimized weight-based dosing was successfully derived with current dosing strengths for patients ≥12 kg body weight, comprising five weight-bands.

5694

## FDA-APPROVED KINASE INHIBITORS AS POTENTIAL ADJUNCTIVE THERAPY CANDIDATES FOR ENDOTHELIAL DYSFUNCTION IN CEREBRAL MALARIA

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Cerebral malaria is associated with cerebral coagulopathy, breakdown of the blood-brain barrier (BBB), and severe brain swelling in children, but currently there are no adjunctive drug treatments. To identify new therapeutics for inflammatory injury to blood vessels, we first screened in vitro 31 FDA-approved kinase inhibitors (KI) drugs in primary human microvascular brain endothelial cells monolayers challenged with the procoagulant protein thrombin or with Plasmodium falciparum lysates and down-selected promising candidates to evaluate in the experimental cerebral malaria model. From the in vitro screen, the BCR-ABL family of drugs showed diverse endothelial barrier phenotypes ranging from protective, neutral, to barrier disruptive. In addition, BCR-ABL drugs had anti-malaria activity with Nilotinib and Bosutinib preventing Plasmodium falciparum ring-stage parasite progression to trophozoite stage and Imatinib targeting late-stage parasites. From in vivo analysis in the ECM model, Bosutinib and Imatinib had modest protection in an early-stage treatment regimen (days 4 to 7). By comparison, Nilotinib, showed substantial protection against neuropathologic injury and lung injury measured by a rapid murine coma and behavior score, associated with BBB protection and parasitemia control. Moreover, when given as a late-stage treatment, Nilotinib on its own improved animal survival by 40% and reduced vascular leakage at the brain, lungs, and kidneys, and further increased animal survival and accelerated parasite clearance when given as an adjunctive drug with artesunate. Our work demonstrates that kinase inhibitors are an attractive drug-repurposing option to treat vascular leakage at vital organs during malaria infection and to pursue for cerebral malaria adjunctive therapy.

5695

## MULTI-OMIC PROFILING OF CUTANEOUS LEISHMANIASIS INFECTIONS REVEALS MICROBIOTA-DRIVEN MECHANISMS UNDERLYING DISEASE SEVERITY

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Leishmania braziliensis infection results in inflammation and skin injury, with highly variable and unpredictable clinical outcomes. Here, we investigated the potential impact of microbiota on infection-induced inflammatory responses and disease resolution by conducting an integrated analysis of the skin microbiome and host transcriptome on a cohort of 62 L. braziliensis-infected patients. We found that overall bacterial burden and microbiome configurations dominated with Staphylococcus spp. were associated with delayed healing and enhanced inflammatory responses, especially by IL-1 family members. Dual RNA-seq of human lesions revealed that high lesional S. aureus transcript abundance was associated with delayed healing and increased expression of IL-1β. This cytokine was critical for modulating disease outcome in L. braziliensis-infected mice colonized with S. aureus, as its neutralization reduced pathology and inflammation. These results implicate the microbiome in cutaneous leishmaniasis disease outcomes in humans and suggest host-directed therapies to mitigate the inflammatory consequences.

5696

## REPROGRAMMING EIF4A-DEPENDENT MRNA TRANSLATION TO CONTROL LEISHMANIA INFECTION

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Protozoan parasites of the genus Leishmania are causative agents of leishmaniasis, a spectrum of tropical neglected diseases. The lack of efficient vaccines and failure to control emerging parasite resistance reflect the need to identify novel targets for therapeutic intervention. More specifically, to counteract parasite resistance, a host-directed therapy, boosting and/or modulating host responses to control the infection is desired. Our laboratory demonstrated that one third of protein-coding mRNAs in macrophages are differentially translated upon infection by Leishmania donovani. In silico analysis indicated activated translation

dependent on the mRNA helicase eIF4A upon infection. Notably, pharmacological inhibition of mammalian eIF4A reduced L. donovani and L. amazonensis survival within macrophages. Selective inhibitors of eIF4A, named rocaglates, have immunomodulatory and antimicrobial properties associated with their ability to fine-tune macrophage functions. Hence, we postulate that pharmacological inhibition of eIF4A-dependent translational programs contributes to control Leishmania infection. To begin testing this hypothesis, we assessed the leishmanicidal activity of a panel of 20 synthetic rocaglates with high affinity for mammalian eIF4A1 (i.e., strong eIF4A1 clampers). As negative controls, we included another subset of 20 rocaglates that promote either low or no clamping of eIF4A1 (i.e., weak/dead eIF4A clampers). Our screening identified four strong eIF4A1 clampers (i.e., C26, C29, C37 and C38) that reduce the infection index in bone marrow-derived macrophages (BMDMs) by more than 50% when compared to DMSO-treated control cells. Surprisingly, five eIF4A1 weak clampers (i.e., C3, C7, C11, C18 and C20) were also able to control intramacrophage Leishmania replication. Additionally, selected rocaglates showed an effect in reducing the infection index of infected THP-1 derived macrophages, validating our findings in a human model. In line with these data, the leishmanicidal activity of rocaglates with strong affinity for eIF4A1 was either partially or completely abrogated in BMDMs derived from Elf4A1+/- mice. In contrast, the anti-parasitic action of eIF4A weak clampers was resistant to reduced eIF4A1 levels in BMDMs. As expected, polysome tracing experiments revealed that only strong eIF4A1 clampers inhibit macrophage mRNA translation rates. To further elucidate the mechanism through which rocaglates act in infected macrophages, we performed a time course of infection in treated cells and identified what appears to be stalling of the parasite replication, instead of parasite killing. These stalled parasites, however, can reinfect and replicate in new, untreated BMDMs, hinting at a host-directed effect, with macrophages being the main target of rocaglate treatment. Taken together, our data allow us to conclude that strong clamper rocaglates seem to inhibit the replication of L. amazonensis in a host eIF4A-dependent manner, both in mouse and human macrophages. Weak clampers, on the other hand, are not dependent on host eIF4A1 for their anti-leishmanial effect and do not seem to be affecting the translation rate of the host cells, suggesting a yet-to-be identified translation-independent host or parasite target that is responsible for the observed effect. Currently, we are implementing a host- and parasite-based multi-omics approach to identify changes in the translatome and the proteome of intramacrophage parasites and their host cells associated with the leishmanicidal activity of strong and weak eIF4A1 clampers identified in our screening. Our long-term goal is to provide insight on the mechanistic basis and therapeutic potential of modulating eIF4A-dependent and -independent translational programs to reduce morbidity and mortality associated with visceral and cutaneous leishmaniasis.

**5697**

## BORRELIA BURGDORFERI CO-EXPOSURE ENHANCES IN VITRO HOST CELL SUSCEPTIBILITY TO LEISHMANIA INFANTUM AND INDUCES TH17-LIKE CELL RESPONSES IN L. INFANTUM-SEROPOSITIVE DOGS

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Canine leishmaniasis (CanL), a zoonotic disease caused by Leishmania infantum, is classically transmitted via sand flies from reservoir dogs to nearby people. Dogs with CanL are often coinfected with tick-borne bacteria, such as Borrelia burgdorferi. These co-infections were causally associated with disease progression and mortality, but specific mechanisms affecting microbicidal responses against L. infantum are unknown. We hypothesize B. burgdorferi co-infection impact host and T cell responses, prompting L. infantum replication and survival. Exposure to B. burgdorferi significantly increased L. infantum parasite burden in DH82 canine macrophage cells. Late apoptosis was significantly decreased in co-infected DH82 cells compared to uninfected and L. infantum-single infected cells. Co-infected DH82 cells showed an enhanced inflammatory response, with upregulation of TNFA, IL6, and IL1B, and concomitant release confirmed via ELISA. Ex-vivo stimulation of PBMCs from L. infantum-seropositive subclinical dogs with spirochetes and/or TLA had limited induction of IFN-γ. In L. infantum-seropositive dogs, co-exposure significantly induced expression of pro-inflammatory cytokines known to induce Th17 differentiation and effector functions, such as IL23p19, IL17A, and IL22, compared to unstimulated and TLA-single stimulated cells. Co-stimulation significantly increased the production of IL-17A and chemokines associated with neutrophilic (IL-8 and CXCL1) and monocytic (CCL2) recruitment compared to TLA-single stimulated PBMCs. L. infantum and B. burgdorferi co-infection promoted robust alterations in the host cell immune response and death, contributing to enhancing L. infantum survival and replication in DH82 cells. B. burgdorferi exposure induced pro-inflammatory and Th17-like responses in PBMCs from L. infantum-seropositive dogs. Excessive inflammation has been shown to contribute to the development of visceral leishmaniasis. Preventing tick-borne disease through already available preventatives will make a significant difference in CanL control and prevent spread of L. infantum in dogs and humans.

**5698**

## STAT6-DEPENDENT/IL-5-MEDIATED EOSINOPHILIA PRIMED BY PRE-EXPOSURE TO UNINFECTED SANDFLY VECTOR BITES ENHANCE SUBSEQUENT LEISHMANIA INFECTION

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The immunological environment primed by pre-exposure to uninfected sandfly bites is thought to have a significant impact on infection by the sand fly vector transmitted parasitic pathogen Leishmania. However, the nature of the immune response to uninfected vector bites and how this response changes over the life history of a mammalian host is poorly understood. We investigated the immune response elicited by exposure to the bites of uninfected Lutzomyia longipalpis sandflies. As previously shown, short-term exposure to sandfly bites activated salivary antigen-specific interferon (IFN)-gamma-producing dermal-derived CD4+ Th1 cells. However, upon repeated exposure, the immune response underwent diversification at the population level to include multiple salivary antigen specific CD4+ subsets (Th1, Th2, Th17, and TREG) and enhanced IL-5 production. Analysis of delayed-type hypersensitivity (DTH) at the bite site during ongoing chronic exposure revealed four phases of DTH, the last of which correlated with a high degree of immunoregulation, a local inflammatory cell infiltrate comprised primarily of eosinophils, an alteration in the maturation of inflammatory monocytes, and enhanced disease upon subsequent challenge with Leishmania plus salivary gland homogenate (SGH). Employing mice genetically deficient in T cells (TCR-/-), Th2 immunity (STAT6-/-), or Eosinophils (GATA-1-/-) revealed that long-term exposure to uninfected bites primes a T cell dependant Th2 response characterized by eosinophil infiltration. GATA1-/- and STAT6-/- deficiency resulted in decreased inflammation, decreased generation of M2-like monocytes-derived macrophages, fewer eosinophils, and enhanced IFN-g response. Disease exacerbation mediated by long-term exposure was found to be dependent on IL-5, as anti-IL-5-mediated reduction of eosinophils resulted in improved control of Leishmania infection. These results show that IL-5-induced eosinophilia mediated by T cells/STAT6 following exposure to uninfected bites influences the virulence of a vector transmitted disease.

5699

## DISSECTING PROTECTIVE NK CELL RESPONSES TO TRYPANOSOMA CRUZI INFECTION IN THE HUMAN SKIN

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Chagas disease is caused by the protozoan parasite Trypanosoma cruzi. It is mainly prevalent in Latin America and is classified as one of the most neglected tropical diseases. Since T. cruzi parasites are mainly transmitted by blood-sucking triatomine bugs, the skin is the main entry route and the first affected organ. Although homeostasis and immunosurveillance of this important anatomic site are crucial to limit parasite burden, the initiation of the local immune response in the skin, as well as the systemic consequences of these early events, remain elusive. Due to the obligatory intracellular life cycle of T. cruzi, natural killer (NK) cells substantially contribute to the control of parasite spread. Therefore, our study aimed to dissect the response of human NK cells to T. cruzi infected non-immune cells resident in the skin, mainly fibroblast and keratinocytes. For this purpose, we established in-vitro cultures of primary human cells, characterized T.cruzi infections, and performed NK cell co-cultures. Our findings describe for the first time and in a comprehensive manner how NK cells sense T.cruzi infection in the human skin. We found remarkable differences in the expression of NK ligands between infected keratinocytes and fibroblasts. Furthermore, we demonstrate that T. cruzi infected primary human skin cells promote NK cell degranulation and show how NK cells orchestrate the following immune response by differential cytokine release. Finally, we performed transcriptome analysis of infected fibroblasts and identified pathways leading to contact-independent activation of NK cells. Taken together, we established reliable in-vitro models to study mechanisms of the NK cell response to T. cruzi infected human skin cells and identified crucial receptors for NK cell activation, which are presumably responsible for the containment of parasite spread in the skin during acute infection. Understanding the contribution of NK cells to parasite control and the mechanisms affecting NK cell function can contribute to target natural killer cells as enhancers of vaccine responses and to develop new therapeutic options.

5700

## INHIBITION OF SRC SIGNALING INDUCES AUTOPHAGIC KILLING OF TOXOPLASMA GONDII INDEPENDENT OF EGF RECEPTOR

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Toxoplasma gondii is an intracellular parasite that can cause severe disease in both immunocompromised and immunocompetent patients, affecting organs such as the brain and eye. T. gondii avoids degradation by the autophagolysosome by manipulating signaling pathways that promote autophagy. Previous studies have shown that the parasite activates EGFR to prevent targeting by autophagy. It is unclear if T. gondii manipulates alternative mechanisms independent of EGFR to avoid autophagic targeting, an important question since various cells in the brain and retina have little to no expression of EGFR. Our study found that T. gondii activates Src and Akt in cells that lack EGFR expression. Genetic or pharmacological inhibition of Src with Saracatinib impaired T. gondii-induced phosphorylation of Src and Akt. Low-dose Saracatinib and knockdown of Src resulted in the accumulation of autophagosomal and lysosomal markers (LC3 and LAMP1, respectively) and promoted killing of T. gondii dependent on the autophagy protein ULK1 and lysosomal enzymes. T. gondii-dependent activation of Src deactivated PTEN promoting PI3K/Akt signaling that prevented autophagic targeting of the parasite. Inhibition of Src disrupts this pathway and allows for autophagic killing of T. gondii. Saracatinib administration to mice with pre-established ocular and cerebral toxoplasmosis reduced histopathology and parasite loads in the eye and brain without altering cell-mediated or humoral immunity. In conclusion, T. gondii can activate Src - Akt signaling to prevent autophagic eradication independently of EGFR. Inhibition of Src may have therapeutic applications against ocular and cerebral toxoplasmosis.

5701

## LOSS OF SIGLEC-7 CORRELATES WITH ENHANCED NATURAL KILLER CELL FUNCTION AND PROTECTION FROM MALARIA SYMPTOMS

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An effective malaria vaccine is urgently needed, but progress towards this goal has been hampered by our limited understanding of the factors responsible for effective malaria immunity. Malaria is a parasitic disease caused by the parasite Plasmodium. Natural killer (NK) cells inhibit the growth of Plasmodium in vitro through antibody dependent cellular cytotoxicity (ADCC). We went on to show that a subset of NK cells in malaria endemic subjects, known as adaptive NK cells, lack the Fc receptor γ chain (FcRγneg) have enhanced ADCC function. However, it was unclear if the lack of Fc γ chain was the reason for increased functionality or if it only served as a marker for adaptive NK cells. Our goal was to know why adaptive NK cells have enhanced cytotoxic function in malaria. Therefore, we developed a CRISPR/Cas9 protocol to ablate genes associated with ADCC in primary NK cells. We then evaluated the effect of those ablations on NK cell function and found that the absence of FcRγ does not enhance NK cell ADCC. We then searched for other alterations in adaptive NK cells in malaria subjects that correlated with enhanced ADCC function. We found that a decrease in the expression of the inhibitory receptor Siglec-7 (Sialic Acid Binding Ig-like lectin 7) correlated with increased NK ADCC function in individuals with a history of malaria infection. Importantly, Sigelc-7 negative NK cells correlated with FcRγneg NK cells in malaria. Because Siglec-7 is an inhibitory receptor, we hypothesized that when the NK cells lack this receptor, this could be cause for adaptive NK cell increased function. We then ablated Siglec-7 using CRISPR/Cas9 and found enhanced ADCC functionality and enhanced killing of Plasmodium-infected red blood cells. Therefore, we predict Siglec-7 is an important protein of interest to study for the protective effects of NK cells in malaria. This is further supported as unaffected individuals without a history of malaria infection did not have a decrease in Siglec-7 expression. The ultimate goal is to use this data to leverage insights from NK cell protective mechanisms to create better therapeutics and vaccines for malaria.

5702

## GUT MICROBIOTA-DERIVED METABOLITES ALTER HUMAN-DERIVED MACROPHAGE STIMULATION AND MAY INCREASE IMMUNE RESPONSES TO ORAL CHOLERA VACCINE

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Immune responses to oral cholera vaccines (OCV) vary among individuals; studies of host factors have only partially explained these differences. We have observed correlations between gut microbes and responses to OCV

in persons receiving two doses of OCV (Shanchol) in Dhaka, Bangladesh, a cholera-endemic area. We previously found that OCV recipients with specific gut microbial profiles were predictive of Vibrio cholerae O-specific polysaccharide memory B cell responses (OSP MBC), a response known to be correlated with protection. Interestingly, the microbes most correlated with OSP MBC produce sphingolipids (SL). To test the association of SL and OCV responses, we isolated microbes from the stool of vaccine recipients that produce SLs including Bacteroides species. Lipids extracted from cultures of Bacteroides xylanisolvens were then applied to human-derived macrophages, a model of mucosal innate immune responses that may contribute to the development of OSP MBC after OCV. We found lower production of interleukin-6 (IL-6) and other inflammatory cytokines after microbe-derived SLs were applied to the model, compared to lipid layers in which SL synthesis was inhibited ($P<0.0001$, t test). This indicates a lower level of baseline innate immune activation in the model when SLs are present. We hypothesize that this low level of baseline stimulation at the mucosal surface (i.e. when microbe-derived SL are present) may increase responsiveness to vaccine antigen. We tested this by preconditioning our model with microbe-derived SLs and then applying lipopolysaccharide (LPS) for 24 hours. Macrophages preconditioned with microbe-derived SL had greater IL-6 production ($P=0.0003$, t test) after stimulation with LPS compared to cell preconditioned with bacterial lipids lacking SL, indicating that the innate immune response to LPS was greater in the presence of microbe-derived SL. Here, we have identified SL-producing microbes isolated from vaccine recipients that correlate with OSP MBC responses in OCV recipients and found that lipids from these microbes impact innate immune responses to vaccine antigens in vitro.

**5703**

## TITLE: ASCERTAINING TRUE CHOLERA BURDEN AND SUBNATIONAL CHOLERA RISK WITH A NOVEL CONTINUOUS DISEASE ENDEMICITY INDEX

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Despite efforts to control and eliminate cholera, it continues to impose large public health impacts in low and middle-income countries. Implementing effective and efficient intervention programs to control cholera in affected regions relies on knowing the actual cholera burden. The current WHO cholera endemicity definition is defined as having reported cholera within three of the past five years, which is subject to bias because of discrepancies in national-level reporting to the WHO. We introduce a novel endemicity index using 20 years of cholera data extracted from publications, WHO, and national ministries of health reports. The index is the sum of the product of weighted reported years and annual incidence rates at national and subnational levels where the years' weights are calculated using a truncated Gaussian distribution. We used Gradient Boosted regression incorporating environmental and socio-economic covariates, potentially associated with cholera occurrence, and a spatial random effect for countries to estimate the endemicity index for subnational regions without adequate cholera data. The endemicity index predictions were largely consistent with previous estimates of endemicity in both endemic and non-endemic areas. Within Africa, Eastern and Central Africa are estimated to have persistent cholera, while western Africa is at a lower risk of cholera transmission except for portions of Liberia and Nigeria. Southern China, the northern provinces of India, Bangladesh, and the Philippines are regions that are at a higher risk of cholera in South East Asia. The endemicity index indicated 29 out of 186 countries are highly endemic (have at least one subnational region with an endemicity index greater than zero), and in 8 of these 29 countries, this held true for 90% or more of their subnational regions. In addition, 11 out of 186 countries showed moderate risk of endemic transmission (an endemicity index between (-5,0)). Our results will help public health agencies to identify cholera-endemic regions, estimate the true cholera burden in these regions, and direct intervention programs to these areas more effectively.

**5704**

## ENHANCED CHOLERA SURVEILLANCE AS A TOOL FOR IMPROVING VACCINATION CAMPAIGN EFFICIENCY

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Oral cholera vaccines (OCVs) are effective in reducing risk of cholera caused by Vibrio cholerae O1 and deployed increasingly for preventive vaccination campaigns, although global demand has exceeded global supply in recent years. Systematic testing for V. cholerae O1 is rare, even in high burden cholera surveillance systems, which means that the limited supply of vaccines may not be delivered to areas with the highest true cholera burden. We explored the effect of increasing V. cholerae testing capacity on OCV campaign impact using a spatial modeling framework that simulates detection, vaccine targeting, susceptibility and burden for projected cholera in 35 African countries from 2022-2035. We compared public health impact, vaccination campaign efficiency (number of true cholera cases averted per 1,000 fully vaccinated persons), and cost-effectiveness across 18 vaccination scenarios with different assumptions about bacteriological confirmation capacity and OCV targeting strategy. When districts with an observed cholera incidence rate exceeding 10 per 10,000 population were targeted with OCV, in scenarios with and without systematic V. cholerae testing, 29 million (95%PI: 21-37 million) and 71 million (95%PI: 65-78 million) individuals were fully vaccinated and 19% (95%PI: 15-22%) and 25% (95%PI: 22-27%) of true cases were averted to yield 10.3 (95%CI: 8.3-13.0) and 5.6 (95%CI: 4.6-6.7) averted cases per 1,000 fully vaccinated persons, respectively; increasing testing cost by $37 (95%PI: 29-52) per averted case reduced OCV campaign costs by $376 (95%PI: 275-556). Compared to status quo, which targets OCV to areas with high clinical cholera incidence rates, expanding V. cholerae testing capacity and targeting OCV campaigns to areas with the highest incidence rate of test-positive V. cholerae has the potential to greatly improve the efficiency and cost-effectiveness of OCV campaigns, while averting nearly as many true cholera cases and using far fewer vaccines. Integration of systematic testing into cholera surveillance, including use of rapid diagnostic tests, could improve efficiency and reach of current global supply of OCV.

**5705**

## RE-EMERGENCE OF CHOLERA IN HAITI LINKED TO ENVIRONMENTAL VIBRIO CHOLERAE O1 OGAWA STRAINS

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Cholera was introduced in Haiti in October 2010. Between February 2019 and August 2022, no further cholera cases were registered. On September 25th, 2022, a new outbreak occurred in the Ouest Department of Haiti, where our group previously demonstrated the establishment of an environmental reservoir of toxigenic V. cholerae O1 in the aquatic ecosystem and its active role in fuelling the epidemic during lull periods. We investigated the origin of the new outbreak by analyzing the full genome sequence of 41 Vibrio cholerae O1 toxigenic Ogawa strains isolated between October 3rd November 21st, 2022 collected by GHEISKIO. We performed maximum likelihood phylogenetic analysis of 2,129 toxigenic V. cholerae O1 sampled worldwide, from 1937 to 2022, and in-depth

Bayesian phylogenetic and molecular clock analysis of 31 new strains obtained from Haiti in 2018, at the nadir of the previous outbreak, as well as 294 Haitian strains sampled between 2010 and 2017, and 16 sequences from the 2022 outbreak publicly available. Our phylogenetic analysis firmly shows the new strains clustering within the Haitian monophyletic clade that emerged at the time of the 2010 outbreak. Our Bayesian phylogenetic also demonstrated that the new strains of V. cholerae cluster shared a most recent common ancestor with a 2018 Haitian Ogawa strain isolated from the aquatic ecosystem and cluster with the previous Ogawa clade that was circulating in 2015-2016. Our case data points out to the original epicenter of the outbreak in Port-Au-Prince and the exponential spread in different directions. Our phylogeography analyses based on the plateau phase of the epidemic, shows further spread within Port-Au-Prince and towards outside. In conclusion, our results show that the new outbreak strains originated from strains that have been circulating undetected at sub-epidemic levels in the aquatic environment. Our data strongly indicates that re-emergence of cholera in Haiti is the likely result of a spill-over event at the aquatic-human interface related to persistence of V. cholerae O1 in the environment.

**5706**

## EFFECTIVENESS OF THE EUVICHOL® ORAL CHOLERA VACCINE AT 2 YEARS: A CASE-CONTROL AND BIAS-INDICATOR STUDY IN HAITI

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The World Health Organization (WHO) recommends use of oral cholera vaccine (OCV) in cholera control efforts. Studies of Shanchol® - a bivalent killed whole-cell OCV - have established its effectiveness in preventing cholera. Euvichol®, a similar product, was pre-qualified by WHO in 2016 and is now the leading component of the Global OCV stockpile, but data on its field effectiveness are limited. Between November - December, 2017, a Euvichol® campaign was implemented in Haiti. We conducted a case-control study to evaluate the effectiveness of this OCV, and a bias-indicator study to evaluate likelihood of study bias. Residents of Mirebalais, Haiti who were eligible for vaccination were enrolled beginning 10 months after the vaccination campaign. Cases were individuals presenting to a cholera treatment unit with acute watery diarrhea (AWD). Stool samples were tested by culture and RT-PCR of the V. cholerae O1 cholera toxin gene. For each case, four control individuals without diarrhea were matched by location of residence, enrolment time, age and sex. Participants were interviewed for sociodemographics, cholera risk factors, and self-reported vaccination. Cholera cases were analyzed in the vaccine effectiveness (VE) study. AWD cases negative for cholera by culture and RT-PCR were analyzed in the bias-indicator study. Data were analyzed by conditional logistic regression, adjusting for matching factors. From 9/12/2018 - 3/12/2020, we enrolled 100 AWD cases. 18 had cholera and were matched to 72 controls. 82 cases without cholera were matched to 325 controls. In the VE case-control, 10 (55.6%) cases reported vaccination compared to 54 (75%) controls. Adjusted VE for 2 doses of OCV was 62% (-67 - 91%). VE for 2 doses of OCV in the bias-indicator study was 40% (-12 - 68%). Between 10 - 27 months after vaccination, Euvichol® was effective at preventing cholera. VE estimates and bias-indicator estimates for Euvichol® in Haiti are comparable to prior estimates of Shanchol® in Haiti, which demonstrated robust evidence of VE up to 2 years post-vaccination. Further research to evaluate the effectiveness of OCV in children and over longer periods are needed.

**5707**

## THE EFFECTIVENESS OF ONE DOSE OF ORAL CHOLERA VACCINE: MATCHED CASE-CONTROL STUDIES FROM UVIRA, DEMOCRATIC REPUBLIC OF CONGO

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Amid a global surge in cholera outbreaks and shortage in oral cholera vaccines (OCV), a single-dose regimen, rather than the standard two-dose regimen, is being used in outbreaks. However, single-dose protection data are limited, especially for children and durations of longer than a few months. We conducted two case-control studies in Uvira, Democratic Republic of Congo, to examine the vaccine effectiveness (VE) of a single-dose of the Euvichol-plus vaccine up to 29 months after vaccination. We recruited matched community controls for confirmed cholera cases admitted at health facilities 12-17 months after vaccination, from October 2021 to March 2022 (Study 1). We then prospectively enrolled community controls for cases admitted 24-29 months after vaccination, from October 2022 to March 2023 (Study 2). Eligible cases included culture and/or PCR-confirmed cholera cases who were eligible for the vaccine during the vaccination campaigns. Four controls with no history of cholera in the past 3 years were matched to each case by age, sex, and neighborhood, as well as by household size and presence of a child under-5 in Study 2. We used conditional logistic regression to estimate VE. We enrolled 1202 participants (268 cases, 934 controls) in Study 1 and 855 participants (167 cases, 688 controls) in study 2 with 18% of the cases being <5 years old in Study 1 and 30% in Study 2. The adjusted single-dose effectiveness for all ages was 50.6% (95% CI 30.1-65.2%) 12-17 months after vaccination and 37.7% (5.5-58.9%) 24-29 months after. VE point estimates were similar across age groups in both studies. Estimates for children under 5 years old were 45.1% (-39-78%) in Study 1 and 43% (66-80%) in Study 2, compared to estimates for older participants of 49% (25-65%) in Study 1 and 41% (3-65%) in Study 2. We demonstrate significant clinical protection from a single dose of Euvichol-plus both one and two years after vaccination. While uncertainty remains around the protection conferred to children under 5, these provide important new data to help with the planning of future vaccination campaigns.

**5708**

## SINGLE DOSE ORAL VAXCHORA VACCINE (CVD103-HGR) FOR THE PREVENTION OF CHOLERA IN TRAVELERS

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Cholera remains an ongoing threat for travelers to areas with endemic/epidemic Vibrio cholerae infections. A single dose vaccine which provides rapid protection, especially for those leaving on short notice, is desirable. To assess the safety, immunogenicity and efficacy of Vaxchora® (Cholera Vaccine, Live, Oral) for protection against cholera diarrhea in travelers, 5 prospective, randomized, double-blind, placebo-controlled trials were performed in 4357 subjects 2-64 years of age in

the US. Endpoints included safety, immunogenicity, shedding, protective efficacy, lot consistency, and immunologic bridging following a single dose. The phase 1 study documented serum vibriocidal antibody (SVA) seroconversion in 88.9% of subjects at day 14, and stool shedding of vaccine organisms in 11% through day 7. In a phase 3 study, protective efficacy against moderate-to-severe diarrhea following challenge with virulent V. cholerae was 90.3% and 79.5% at days 11 and 91, respectively. SVA seroconversion, which occurred in 79.8% and 89.4% of subjects at days 8 and 11, respectively, was a strong correlate of protection. In a phase 3 lot consistency study, SVA seroconversion occurred in 93.5% of adults 18-45 years of age at day 11, while a phase 3 immunogenicity study in older adults 46-64 years demonstrated a non-inferior 90.4% seroconversion rate. A phase 4 study in children 2-17 years of age demonstrated a 98.5% SVA seroconversion rate, which was non-inferior to that seen in the lot consistency study. An adolescent sub-study of the pediatric trial documented persistence of SVA seroconversion at 2 years in 64.5% of vaccine recipients. In a post-hoc analysis of the pediatric trial of those subjects who consumed less than the full dose of vaccine, SVA seroconversion was documented in 100% of those who took 50-80% of the dose and in 69.2% of those who took some but <50%. Vaccine was well tolerated in all studies. A single oral dose of Vaxchora vaccine provides safe and rapid protection in adults and children traveling to areas with cholera. SVA seroconversion, the correlate of protection in the cholera challenge trial, occurs in most individuals in as little as 7 days.

**5709**

## LONG TERM MUSCULOSKELETAL MANIFESTATIONS ARE ASSOCIATED WITH A DYSREGULATED IMMUNE RESPONSE IN POST-EBOLA SYNDROME (PES)

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Long-term post-acute sequelae following viral infections have become recognized as highly prevalent in recent years, including in the contexts of Ebolavirus disease (EVD) and SARS-CoV2. As these diseases continue to emerge—Sudan ebolavirus (SUDV) in Uganda; Marburg virus (MARV) in Equatorial Guinea—the long-term health consequences following acute infection are garnering attention. In the context of EVD, a large proportion of survivors develop a range of long-term sequelae with complaints including ophthalmologic, auditory, musculoskeletal (MSK), neurocognitive, and psychiatric. Our group recently defined these sequelae phenotypically into three groups: symptomatic survivors with or without MSK involvement, and asymptomatic. The mechanisms defining long-term sequelae, including MSK, are currently unknown making targeted treatment challenging. Due to the association of inflammatory biomarkers with MSK sequelae in other viral contexts (ie: HIV/AIDS and Chikungunya virus), we developed a 20-plex Luminex panel to assess the relationship between inflammatory markers and MSK sequelae in an ongoing cohort of EVD survivors from Eastern Sierra Leone. Our custom Luminex panel included pro- and anti-inflammatory cytokines, metalloproteinases, and markers of mucosal integrity associated with inflammation. We found that contrary to our hypothesis of increased inflammation in survivors suffering from long-term MSK, that in fact survivors with MSK had lower concentration of serum biomarkers compared to asymptomatic EVD survivors. In collaboration with colleagues at WSU, we tested antibody driven Fc-mediated innate effector function and observed significantly lower levels of monocyte/macrophage phagocytosis and decreased complement deposition in survivors in the MSK group. We are currently investigating alternative hypotheses including the presence of autoantibodies, a phenomenon that has been observed in the context of SARS-CoV2 following recovery from acute disease. Our preliminary data suggest an immune dysregulation and possible immunosenescence associated with long-term MSK sequelae in EVD survivors.

**5710**

## EXPLORING BAT INNATE IMMUNE CELL RESPONSES TO FILOVIRUSES

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Various species of bats are reservoirs of diverse zoonotic viruses, including multiple filoviruses such as Marburg virus (MARV, naturally transmitted by Egyptian rousette bats; Rousettus aegyptiacus) and Bombali ebolavirus (BOMV, recently isolated from Angolan free-tailed bats; Mops condylurus). The ability of bats to host viruses that are highly-pathogenic to humans likely correlates with co-evolved functional anti-viral immune response mechanisms. How bats achieve efficient anti-viral innate or adaptive immune responses at the molecular or cellular level, without developing overt signs of disease themselves, remains to be addressed. In this study, we profile the phenotypes, functionality and anti-viral immune responses of dendritic cells (DC), key primary host cellular targets of filoviruses. We infected bone marrow-derived DCs (bmDCs) from R. aegyptiacus with recombinant MARV or Sudan ebolavirus (SUDV) expressing fluorescent proteins, allowing us to directly compare how DCs respond to infection by a filovirus that these bats harbour in nature (MARV), to a filovirus efficiently cleared by rousette bats in vivo (SUDV). Despite similar rates of progeny virus production, bmDCs supported increased intracellular MARV-ZsG replication out to 3 days post-infection, while ZsG median fluorescence intensity (MFI) in SUDV-infected bmDCs was decreased, indicating differential control of infection of these two viruses. Conversely, MARV- and SUDV-infected bmDCs both upregulated CD40 and HLA-DR surface expression, indicative of functional cell activation. Similarly, bmDCs from M. condylurus sustained steady rates of replication with fluorescently-tagged Zaire ebolavirus (EBOV-GFP) and displayed a notable upregulation of CD40 at 1 day post-infection. Our findings highlight that despite being susceptible to infection, bat DCs display unimpaired activation and antigen presentation capacities following disparate filovirus infections, in contrast to the described impaired maturation and functionality observed in filovirus-infected human DCs.

**5711**

## COMPUTATIONAL DESIGN OF STABILIZED RBD ANTIGENS ENABLES POTENTLY NEUTRALIZING SARS-COV-2 VACCINES

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Waning immunity and emerging variants necessitate continued vaccination against SARS-CoV-2. Improvements in vaccine efficacy, safety, tolerability, and ease of manufacturing would benefit these efforts. Receptor-binding domain (RBD)-based vaccines are effective vaccines used in several

countries, in part due to their ease of manufacturing. Here, we report a novel computational design strategy (SPEEDesign) that improves RBD vaccines. SPEEDesign was used to create RBD immunogens with amino acid changes that enhance neutralizing antibody titers, focus the immune response to neutralizing epitopes, increase production yields, and improve antigen stability. In one application of SPEEDesign, immunogens with 9 amino acid changes elicited neutralizing antibody titers approximately 10-fold greater than the native RBD, and comparable to a benchmark stabilized spike antigen. Crystal structures of these immunogens revealed the structural basis for these improvements. A second application of SPEEDesign produced a stabilized, non-glycosylated RBD that resolved issues hindering the efficient nanoparticle display of the native RBD. This non-glycosylated RBD can be genetically fused to diverse single-component nanoparticle platforms maximizing manufacturing ease and flexibility. All engineered RBD-nanoparticles elicited potently neutralizing antibodies in mice that far exceeded monomeric RBD. A 60-copy particle (noNAG-RBD-E2p) also elicited potently neutralizing antibodies in non-human primates. The neutralizing antibody titers elicited by noNAG-RBD-E2p were comparable to a benchmark stabilized spike antigen and reached levels against omicron BA.5 that suggest it would provide protection against emerging variants. The stabilizing mutations we have identified in both applications of SPEEDesign are adaptable to all vaccine platforms and they are distinct from the changes in emerging variants, making them compatible with updated vaccines. Finally, the SPEEDesign pipeline is a generalizable method that can be used to improve vaccine antigens from diverse pathogens.

5712

## EPSTEIN BARR VIRUS SYNERGIZES WITH PLASMODIUM FALCIPARUM MALARIA TO INDUCE ABERRANT EXPRESSION OF ACTIVATION INDUCED CYTIDINE DEAMINASE

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Early age at EBV infection and repeated episodes of Plasmodium falciparum (Pf) malaria are known risk factors for the development of endemic Burkitt's lymphoma(eBL), the most common pediatric cancer in equatorial Africa. To date, the extent and by what means these two factors interact to drive eBL pathogenesis is yet to be fully understood. Both EBV and P.f have been considered as potential drivers of increased Activation Induced Cytidine Deaminase (AID) activity, the major hallmark of eBL. AID activity is typically restricted to B cells in the germinal center reaction. However, recently low levels of AID expression in immature transitional B cells in peripheral circulation have been reported. Additionally, AID expression has been reported in B cells in peripheral circulation in children and adults from malaria endemic areas. Studies in vitro and in mice have also demonstrated either P.f or EBV can induce expression of AID in peripheral blood. In this study, we hypothesized that EBV and P.f malaria synergistically induce dysregulated expression of AID. We analyzed the expression of AID on B cells from peripheral blood of children experiencing an episode of acute P.f malaria and healthy controls by intracellular flow cytometry. Furthermore, we determined the in vitro induction of AID expression on primary human B cells following infection with EBV with or without CpG (a TLR9 ligand used to mimic P.f DNA). We report a significantly higher frequency and MFI of CD19+ AID+ B cells in children presenting with acute malaria than in healthy controls. In vitro analysis confirmed the synergy between EBV and P.f malaria in the amplification of AID expression since we observed >50% increase in the frequency of CD19+AID+ cells following infection with EBV and CpG. Interestingly, co-stimulation with BAFF, a B-cell cytokine that is elevated during acute malaria infection, further amplified the expression of AID in B cell in vitro. Taken together, our study confirms the synergistic linkage between these two pathogens in the etiology of eBL through the induction of aberrant expression of the highly mutagenic AID that is likely to exacerbates the pathogenesis of eBL.

5713

## ANTIBODY FC CORRELATES OF PROTECTION AGAINST SEVERE DENGUE DISEASE

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Four dengue virus serotypes (DENV1-4) are associated with ~50 million cases annually worldwide, with disease presenting as mild (dengue fever, DF) or severe (dengue hemorrhagic fever/shock syndrome, DHF/DSS). Pre-existing cross-reactive antibodies have been associated with both protection and risk for developing severe dengue upon secondary heterotypic infection, but antibody characteristics that provide protection from severe disease remain poorly understood. We performed systems serology on plasma/serum samples collected from 66 individuals (33 DF and 33 DHF/DSS) before secondary DENV2 and DENV3 infections resulting in either DF or DHF/DSS from a longstanding pediatric cohort study in Nicaragua. We measured binding antibodies by Luminex using beads conjugated to recombinant envelope (E), E domain III, and nonstructural protein 1 (NS1) of DENV1-4 and the related Zika virus (ZIKV). For Fc effector function assays, we used beads conjugated to DENV2, DENV3, and ZIKV antigens. Our study revealed that higher levels of total IgG, IgG2, IgG3, IgG4, and both antibody-dependent complement deposition (ADCD) and antibody-dependent cellular phagocytosis (ADCP) were associated with protection against DHF/DSS. In contrast, neutralizing antibodies to mature DENV2 and DENV3 virions, measured by focus reduction neutralization test on Vero cells, were not significantly different. We also observed a stronger association of ADCD activity with protection when assays were conducted with ZIKV antigens. We validated these findings with a complement-mediated virolysis assay using DENV, ZIKV or yellow fever virus virions. We found that virolysis of ZIKV virions mediated by samples from DENV-exposed, ZIKV-naïve individuals was most strongly associated with protection, suggesting that 1) anti-DENV antibodies that cross-react with ZIKV and 2) antibodies that target virion-specific epitopes are correlated with protection from severe dengue disease. In sum, our study provides important insights into the biophysical features and effector functions of cross-reactive antibodies that may inform the development of effective dengue vaccines.

5714

## ANTIBODY CORRELATES OF SEVERE DISEASE IN SECONDARY DENGUE VIRUS INFECTION AFTER A PRIMARY ZIKA VIRUS INFECTION: A POSSIBLE ROLE FOR IGA

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Sequential infections of dengue virus serotypes 1-4 (DENV1-4) and Zika (ZIKV) can lead to protection or severe disease. We observed in our

long-standing pediatric cohort in Nicaragua that ZIKV infection increases risk of dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) during secondary DENV2 infection, potentially due to antibody-dependent enhancement. However, immune correlates of disease severity are not completely understood. Here, we analyzed antibody characteristics in samples taken after primary ZIKV infection that were associated with DHF/DSS vs. dengue fever (DF) during subsequent secondary DENV2 infection in 24 children (n=12/group) from our Nicaraguan cohort study. We characterized anti-DENV and anti-ZIKV antibodies (IgG1-4, IgA, IgM) in pre-secondary infection samples using a multiplex Luminex assay against recombinant E protein (recE), E domain III, and nonstructural protein 1 (NS1) of DENV1-4 and ZIKV, as well as the Fc effector functions antibody-dependent complement deposition (ADCD) and antibody-dependent cellular phagocytosis. After modelling a dose-response curve, the effective dilution at which mean fluorescence intensity was reduced by 50% (ED50) was significantly associated with DHF/DSS for IgA but not for other isotypes. A bivariate logistic binomial regression showed that a 0.1Log10 increase in the ED50 of anti-NS1 DENV2 IgA and anti-NS1 ZIKV IgA, among others, increased odds of DHF/DSS by 3.07 (95% Confidence Interval [95%CI] 1.62-9.77) and 2.02 (1.31-4.34), respectively. A LASSO multivariate regression selected ED50of anti-NS1 DENV2 IgA as the most relevant feature associated with DHF/DSS. Finally, a Bayesian network analysis revealed that ED50 of anti-NS1 DENV2 IgA and anti-NS1 DENV4 IgA increased the probability of DHF/DSS. We are currently analyzing the amount of IgA1/IgA2 and dimeric vs. monomeric IgA, avidity of IgA against DENV/ZIKV antigens, and markers of neutrophil function in pre-secondary infection and acute-phase samples from these individuals. We hypothesize that IgA may be involved in a pathogenic pathway associated with DHF/DSS that deserves further study.

**5715**

## IN-DEPTH ANALYSIS OF THE IMMUNOGENICITY OF A SINGLE DOSE OF DENGVAXIA IN BASELINE DENGUE-NAIVE CHILDREN IN CEBU, PHILIPPINES

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The first licensed dengue virus (DENV) vaccine, Dengvaxia® sensitizes dengue seronegative (SN) children to experience more severe breakthrough infections compared to unvaccinated children. In clinical trials of this live attenuated tetravalent chimeric yellow fever/DENV vaccine, neutralizing antibodies (NAbs) to the 4 DENV serotypes were detected in most SN vaccinated individuals demonstrating that not all the antibodies that neutralize in vitro are predictive of durable protection in humans. When Dengvaxia® was introduced as a pediatric vaccine in the Philippines in 2016, investigators at the Univ. of Philippines in Manila established a cohort of 2,960 children (60% vaccinated) to study vaccine immunogenicity, efficacy and safety by baseline (BL) DENV serostatus over a 5-year follow-up period. Here we report the results of a sub-study to investigate vaccine immunogenicity in BL SN children 1 Year after receiving a single dose. We analyzed paired blood samples collected at BL and Yr1 from 136 vaccinated and 87 unvaccinated children using a) a multiplex-Luminex assay designed to measure antibodies to 8 DENV antigens (EDIII and NS1 proteins from the four serotypes), b) an ELISA for detecting Abs to yellow fever virus NS1 antigen expressed by the vaccine only, c) a DENV neutralization assay designed to measure NAb responses to contemporary circulating strains of mature DENV1-4. Among the vaccinated children we did not observe any vaccine-induced antibody responses in 34% (44/136) after one dose. Among the children who responded to the vaccine, the frequency of NAbs to each serotype was 66% (DENV4), 21% (DENV1), 15% (DENV2) and 10% (DENV3) supporting a DENV4 biased vaccine. We relied on the presence of DENV NS1 Abs as surrogate to capture all DENV infections in Yr1 blood samples. We observed a high attack rate of DENV infection (53%) between BL and Y1 in the unvaccinated children. Among the vaccinated children, 22% experienced DENV infections demonstrating modest efficacy against infection. Clinical vaccine efficacy and safety in years 1-5 after vaccination and immune correlates analyses are ongoing.

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## ACUTE PUBLIC HEALTH THREATS GLOBALLY: A 10-YEAR WORLD HEALTH ORGANIZATION ANALYSIS

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One of the World Health Organization's (WHO) key activities is the detection and response to acute public health threats to prevent morbidity and save lives. Therefore, WHO adopted a unique all-hazards approach to the global detection and verification of acute public health events of potential international public health concern, under the mandate of the International Health Regulations (2005). Here, we analysed 10-year trends of acute public health events globally. Data on substantiated acute public health events reported between 2013 and 2022 was extracted from an internal WHO platform, the Event Management System (EMS), which is used for tracking health threats globally. Substantiated events are those for which the presence of a hazard was confirmed or the number of human cases exceeded normal thresholds. These events were assessed, by WHO Region and over time, using descriptive statistics in R. Between 2013 and 2022, 3214 acute public health events were recorded globally in EMS, of which six were declared public health emergencies of international concern. For each year of the 10-year period, infectious diseases were the main cause of events, ranging from 68% - 90% per year. Globally the five most common infectious diseases, excluding COVID-19, were cholera, measles, dengue, zika, and avian or human influenza. Natural disasters (9.5%, N=305) were the second most common cause globally. In all WHO Regions infectious diseases were the main cause of public health events, ranging from 47%-84% between Regions. In addition, natural disasters were a common driver of events and the second cause, ranging from 9%-36%, in four of six Regions. Moreover, the proportion of events due to natural disaster has increased in recent years in these Regions. In conclusion, infectious diseases are the main cause of events globally reported to WHO. However, health emergencies by natural disasters are on the rise and marked differences, in health threats, between Regions exist. To prepare for future health threats an understanding of previous acute public health events and changing trends is key.

5717

## DETECTION OF HUMAN CASES OF CRIMEAN-CONGO HEMORRHAGIC FEVER DURING AN ONGOING MULTIDISTRICT OUTBREAK OF EBOLA VIRUS DISEASE IN UGANDA, 2022-23

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In September 2022, Uganda experienced its 5th outbreak of Ebola virus disease (EVD) due to Sudan ebolavirus (SUDV) that spread to multiple districts across the country. During the subsequent national-wide enhanced surveillance activities, several cases suspected of viral hemorrhagic fever (VHF) were found to be caused by other viral etiologies, including yellow fever, Crimean-Congo Hemorrhagic fever (CCHF) and Rift Valley fever. We report the epidemiologic and laboratory findings of human CCHF cases that were concomitantly detected during the EVD response activities. A VHF suspected case was any person presenting with acute onset of fever (≥38.0°C), with no alternative diagnosis, and with other signs and symptoms such as intense fatigue, chills, general body pains, headache, anorexia, vomiting, diarrhea, jaundice, and unexplained bleeding from any site. Between September 2022 and February 2023, a total of 1,107 samples were submitted for VHF testing at Uganda Virus Research Institute, Entebbe, Uganda. Overall, 13 CCHF cases (including 7 deaths; CFR = 54%), aged 4 to 60 years, were identified by PCR from 10 districts, including the same districts as the SUDV outbreak. Of these, most cases were males who also engaged in livestock farming and exposure to wildlife (n = 8; 62%). Four cases were identified in the EVD treatment unit, thus complicating diagnosis and management of CCHF cases during an active SUDV outbreak. Among confirmed cases, the most common clinical symptoms were hemorrhage (n = 12; 92%), followed by fever (n = 11; 85%), anorexia (n = 10; 77%), fatigue (n = 9; 69%), abdominal pain (n = 9; 69%) and vomiting (n = 9; 69%). Further investigations to characterize these viral infections as well as alternate etiologies is ongoing and will be presented. These findings highlight the need for broad diagnostics for VHFs and other viral and high-consequence pathogens, even during confirmed VHF outbreaks, to properly identify all possible causes of acute febrile illnesses detected during periods of heightened surveillance. This approach is especially critical in countries with a broad range of endemic high-consequence zoonotic viral pathogens.

5718

## A COMPREHENSIVE REVIEW OF CLINICAL PRESENTATIONS OF NIPAH VIRUS INFECTION: EVIDENCE GENERATED FROM NIPAH VIRUS OUTBREAKS OF 2023, BANGLADESH

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Nipah virus is endemic to Bangladesh, and human infections have been reported almost every year since 2001. Till March 2023, Bangladesh experienced ten outbreaks of Nipah virus infection. Team of experts led by the Institue of Epidemiology, Disease Control and Research (IEDCR) conducted the outbreak investigations with support from icddr,b, and EcoHealth Alliance to detect more cases linked to the outbreaks and to prevent further spread in the community and healthcare setting. The teams identified the source of infection and obtained comprehensive information of the patients since their exposure to the virus. From January 4 to March 2, 2023, three clusters and seven sporadic Nipah outbreaks were identified from seven districts of Bangladesh. A total of 14 Nipah cases were reported, which included 86% (12/14) laboratory-confirmed and 14% (2/14) probable cases. There were 11 cases of primary infection, all with the history of raw date palm sap consumption prior to symptom onset. During the investigations, 675 contacts were identified; among them, three individuals were infected with the Nipah virus, indicating person-to-person transmission. On average, Nipah infection was confirmed 8 (range, 4 to 14) days after symptom onset and 3 (range, 1 to 9) days after hospitalization. The median incubation period was 11 days, ranging from 1 to 18 days. Most cases were males (57%) with a median age of 20 (15 days to 70 years) years. Ten (71%) cases died during the infection; the rest survived with significant neurological sequelae. In most cases, symptoms started with fever (100%) followed by headache (64%) and vomiting (64%). Subsequently, all patients developed signs of neurological manifestations ranging from incoherent behavior, confusion, increased salivation, neck stiffness, altered level of consciousness to coma. This year's outbreaks exceeded the past eight-year record, and several instances of human-to-human spread indicate the potential to cause larger epidemics in the future. The findings of these outbreak investigations highlight the urgent need for increased efforts in developing vaccines and treatments for Nipah virus infection.

5719

## ENVIRONMENTAL SURVEILLANCE TO DETERMINE COVID-19 PREVALENCE IN DISTRICTS IN NORTHERN GHANA WITH NO REPORTED COVID-19 CASES: EVIDENCE TO INFORM PUBLIC HEALTH INTERVENTIONS

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Environmental surveillance (ES) is a convenient, sensitive, low-cost method to estimate COVID-19 prevalence. Most ES in high-resource countries is implemented in sewered systems. Non-sewered sanitation is common in low-resource settings, and few studies have deployed ES in such environments. We applied ES to detect COVID-19 in two districts with no reported cases of COVID 19 since the beginning of the pandemic to inform public health policy and practice. Mion (pop. 94,930) and Nanumba North (188,680) are rural areas in the Northern region of Ghana. Both localities are sparsely populated. Neither district has a sewerage system, and >70% of the population rely on shared public toilets. Stakeholder analysis was deployed to identify population centers in the community that should be targeted for wastewater or fecal sludge sample collection. Schools (primary, secondary, and college), healthcare facilities, markets, and streams were identified and mapped. From September to November 2022, grab and Moore swab samples were collected daily from septic tanks, pit latrines and surface water. Samples were stored and transported weekly to a central

lab in Accra. Liquid samples were concentrated using Nanotrap particles, RNA extraction, and analyzed by multiplex RT-qPCR. 46 sampling sites were identified, and 194 samples (128 solids samples, 3 Moore swabs, and 63 liquid samples) were collected. 13%, 33.3% and 20% of the liquid, Moore swab, and solids samples tested positive for SARS-COV-2 RNA, respectively. 8.9% of the 78 samples from Mion were positive for SARS-COV-2. Out of the 116 samples from Nanumba North, 24.3% tested positive for SARS-COV-2. Positive samples were from healthcare facilities (6.6%), public latrines (17.7%) and schools (22.5%). No COVID-19 cases were reported from either district during the study period. The Ghana Health Service used the results to develop public health response action plans -including health education and targeted mass vaccination in schools and communities. These findings demonstrate the value of ES to guide the public health response in remote low-resources settings even when clinical cases are not detected.

5720

## MEASLES ANTIBODY RESPONSE AND DURATION IN INFANTS WITH HIGH EARLY-LIFE MALARIA EXPOSURE COMPARED WITH LOW MALARIA EXPOSURE

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Measles continues to cause significant morbidity and mortality globally despite a highly effective vaccine. Immunomodulation caused by high malaria exposure during early-life may impact the response to measles vaccine and the duration of this response. In the present study, we assessed measles antibody levels in a 2-year longitudinal cohort of 166 infants from two sites in Kenya with different levels of malaria transmission. After excluding infants with likely measles exposure prior to vaccination, we categorized measles antibody levels at 10-12 months of age into high/medium/low tertiles and compared the proportion of infants in each tertile between study sites. Using the categories defined by these tertiles at 10-12, 16-18, and 22-24 months of age, we used repeated ordinal logistic regression to compare change between categories over the study duration between study sites. Prior to measles vaccination at nine months of age, 30.6% of infants had measles antibody levels indicative of exposure to the virus. Among infants without pre-vaccine measles exposure, more infants at the low-malaria study site were in the high category of antibody response at 10-12 months compared with infants at the high-malaria study site (42.1% vs. 24.0%, p=0.1). At both sites, more infants moved to the high antibody category throughout the duration of the study, but this change in categories did not differ between study sites. In sum, infants living in the low-malaria community had better vaccine-elicited immunity, on average, than infants in the high-malaria community. The substantial number of infants with likely measles exposure prior to vaccination combined with the increasing antibody levels throughout the duration of the study suggest measles may have been circulating in these populations during the study period. In endemic settings, immunization schedules should take into account gaps in seroprotection prior to vaccination. Further research is needed to understand how to best address potentially lower vaccine response in areas of high malaria transmission.

5721

## MACROLIDE RESISTANCE 36 MONTHS AFTER MASS AZITHROMYCIN ADMINISTRATION IN A CLUSTER-RANDOMIZED TRIAL IN NIGER

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Mass drug administration (MDA) of azithromycin decreases child mortality but has been shown to select for macrolide and nonmacrolide resistance 24 months after initiation. However, resistance prevalence over time is not well studied. Herein, we evaluate resistance 36 months after initial azithromycin treatment and compare mean prevalence between treatment and control arms. In the MORDOR cluster-randomized trial of mass azithromycin distribution, children ages 1-59 months received either azithromycin (15 villages) or placebo (15 villages) every six months. In total, 423 children were randomly selected to provide nasal swab samples after receiving their 36-month administration of treatment or placebo. Streptococcus pneumoniae isolates from cultured swabs were tested for macrolide and nonmacrolide resistance. The primary outcome was prevalence of macrolide and nonmacrolide resistance for treatment versus placebo groups at 36 months. Of the 423 swabs obtained, 322 grew S. pneumoniae (146/202 in the treatment group and 176/221 in the control group). Macrolide resistance prevalence was 14.6% in treatment villages and 8.9% in control villages (OR=2.0, 95% CI: 0.99-3.97) in a mixed effects regression model. Resistance prevalence of several nonmacrolides did not differ between treatment and controls (p>0.18 for all). Our results suggest that increased exposure to resistance determinants or spillover from treatment villages increased prevalence in control villages. Furthermore, although macrolide resistance prevalence increased significantly from MDA initiation to 24 months, overall resistance at 36 months was only marginally greater than at 24 months, indicating that resistance after twice-yearly azithromycin administration to young children flattens before exponential growth occurs. This longitudinal flattening may not hold for MDA regimens that confer high antibiotic pressure by targeting larger proportions of the population with more frequent dosages, like mass azithromycin for endemic trachoma.

5722

## RISK FACTORS FOR COLONIZATION WITH EXTENDED-SPECTRUM CEPHALOSPORIN RESISTANT AND CARBAPENEM RESISTANT ENTEROBACTERALES AMONG HOSPITALIZED PATIENTS IN BANGLADESH: ANTIBIOTIC RESISTANCE IN COMMUNITIES AND HOSPITALS -ARCH-STUDY

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Understanding risk factors for colonization with antimicrobial resistant (AMR) organisms is critical for implementing effective prevention strategies to reduce transmission and risk for developing invasive infections. During Apr-Oct 2019, we conducted a period prevalence study in three hospitals in Dhaka city during which we continuously enrolled patients using simple random sampling. We collected stool samples from hospitalized adult patients to detect extended-spectrum cephalosporin resistant (ESCrE) and carbapenem resistant (CRE) Enterobacterales. We recorded information on patient characteristics, admission wards, antibiotic use, duration of hospitalization at enrollment, and healthcare exposures. Based on a conceptual framework, we used multivariate logistic regression models adjusting for potential confounders to identify the risk factors for colonization. Of 743 enrolled patients, the median age was 40 (IQR:30-55) years, 54% were male, and 44% had underlying chronic illnesses. Median time between hospital admission and patient enrollment was 3 days (IQR:

2 - 6 days) with stool typically collected the following day. Among enrolled patients, 592 (82%) were colonized with ESCrE and 267 (37%) with CRE. Risk factors for ESCrE colonization were stay in a surgical ward compared with a medicine ward (aOR 2.1, CI: 1.3-3.2) and hospitalization for 3-7 days (aOR 1.9, 1.2-3.0). Risk factors for CRE colonization included admission from another healthcare facility (aOR 3.1, 1.5-6.4); stay in a surgical ward (aOR 1.7, 1.1-2.5); hospitalization for 3-7 days (aOR 1.7, 1.2-2.4) and >=7 days (aOR 2.9, 1.8-4.6); invasive procedures (aOR 2.4, 1.3-4.4); urinary catheter use (aOR 2.1, 1.1-4.2); and antibiotic administration in the last 14 days (aOR 2.9, 2.0-4.0). In conclusion, longer hospital stays and more invasive procedures were associated with increased risk for colonization with AMR organisms. These findings emphasize the need for enhanced infection prevention and control efforts to mitigate the transmission of AMR organisms in Bangladeshi hospitals.

**5723**

## MOLECULAR EPIDEMIOLOGY OF ASYMPTOMATIC CRYPTOSPORIDIUM, GIARDIA, AND ENTAMOEBA INFECTIONS: THREATS TO THE HEALTH OF NIGERIAN CHILDREN?

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Intestinal parasitic infections have significant impacts on the health of children. Apart from causing diarrheal diseases, Cryptosporidium and Giardia infections have negative impacts on children's growth and cognitive development and may result in death in immunocompromised individuals. At the national level, there is a scarcity of information on the molecular epidemiology of Cryptosporidium, Giardia and Entamoeba infections in children in Nigeria. As a result, we present for the first time in Nigeria a nationwide assessment of the prevalence, associated risk factors, and genetic profiles of Cryptosporidium, Giardia and Entamoeba species in children aged 10 and below. In total, 985 stool samples were collected from ten states, along with an epidemiological questionnaire. We used real-time PCR and DNA sequencing to detect and genotype the three enteric parasites in stool samples. A variety of statistical and bioinformatic tools were used to analyze the data. The most common parasite found in this study was Giardia sp. (77.4%), followed by Cryptosporidium (19.5%) and Entamoeba (12.3%). Coinfections with two or all three parasites were found in 10% of the children in this study, with Cryptosporidium + Entamoeba coinfection being the least common. Several socioeconomic factors, including toilet type, drinking water source, livestock rearing, and hygiene habits, were identified as risk factors for infection with one or more of the parasites.The main circulating species were identified through genetic analysis as C. parvum, Giardia Assemblage A2 and B, Entamoeba histolytica and Entamoeba dispar. Regardless of the presence or absence of symptoms, our study found a high burden of intestinal parasitic infection in children in Nigeria which was linked to poor hygiene and poverty-related factors.

**5724**

## A CONTINENTAL PICTURE OF SLEEPING SICKNESS: USING MODELS FROM THE DRC TO ESTIMATE GLOBAL GAMBIENSE HUMAN AFRICAN TRYPANOSOMIASIS BURDEN AND PROJECTED RESOURCE USE AND COST UNDER VARIOUS CONTROL STRATEGIES

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Gambiense Human African Trypanosomiasis (gHAT, sleeping sickness) affects several countries across central and western Africa, spanning a diverse range of geographies and epidemiology. In 2019-2020, 70% of all reported gHAT cases were recorded in the Democratic Republic of the Congo (DRC). We have previously fitted a dynamic transmission model to historical data in 168 endemic or formerly endemic health zones of the DRC, across a range of geographies and levels of case reporting from no cases in the last decade to thousands. In this study, we produce a picture of the resources required for gHAT control across the whole continent. We classified the health zones of the DRC for which we have enough data to fit the transmission model by their risk level according to the WHO definitions and then combine them to produce aggregated fits for each risk level. We then used these aggregated fits in combination with WHO estimates of the population living at each level of risk to produce projections across the whole continent. A set of possible interventions were then considered based on estimates of local capacity for screening and geographical suitability for vector control. We made projections under multiple strategies and predicted the disease burden and probability of elimination over time as well as estimating resource use and cost. These projections were used to produce a global health economic analysis under a modified, previously published, version of the net monetary benefit framework. Each strategy's cost-effectiveness is considered with respect to disease burden and its effect on probability of elimination. We also forecast the number of drug doses, diagnostic tests, and tiny targets needed under each strategy and in each country to reach elimination. These projections are made available in a graphical user interface to allow them to be explored and visualised easily.

**5725**

## CHAGATYPER: DEVELOPMENT OF A RAPID RESPONSE, SEMI-AUTOMATED, HIGH-RESOLUTION GENOTYPING PLATFORM FOR CHAGAS DISEASE

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Approximately 30% of Trypanosoma cruzi seropositive individuals develop end-organ cardiovascular and/or gastrointestinal pathology, so-called chronic determinate Chagas disease. The determinants of progression in Chagas disease are not clear. Parasite genetics - at the DTU or sub-DTU level - has long been thought to play a role. However, the difficulty in isolating and genotyping the parasite directly from patients has frustrated efforts to define the link with pathology. Through a collaboration between the University of Glasgow's School of Biodiversity, One Health and Veterinary Medicine (SBOHVM), the Diagnostic Parasitology Laboratory at the London School of Hygiene & Tropical Medicine and the UK Chagas Hub, we aim to establish a prototype clinical genotyping platform for Chagas disease. The UK Chagas Hub has detected and linked into care 100 new cases of Chagas disease in the last year, with screening and active case-finding initiatives ongoing. Over a quarter (27%) of these patients confirmed to be seropositive were also T. cruzi PCR positive. A genome-wide AmpSeq approach to provide high-resolution

genotypes directly from biological samples has been developed. This AmpSeq approach has been successfully piloted with two peripheral blood samples from newly-diagnosed (chronic indeterminate) cases of Chagas disease detected through UK Chagas Hub screening. We are currently generating an AmpSeq reference strain database, with 100 reference strains covering different major genetic lineages (DTUs) and geographic localities. The reference database will support a user-friendly, interactive, online visualisation tool through which PCR amplicons from clinical samples can be compared. The platform will be tested by 100 further UK Chagas Hub clinical samples (data from PCR amplicons sequenced by a third-party added to the online genotyping platform). A semi-automated, high-resolution genotyping platform for Chagas disease will allow the combination of genotype and clinical phenotype information from large cohorts of patients, which can inform our understanding of the determinants of Chagas disease progression.

**5726**

## MOLECULAR-BASED EVIDENCE OF TRANSMISSION OF ATYPICAL TRYPANOSOMIASIS (A-HAT) IN HUMANS IN SELECTED COMMUNITIES IN THE SUHUM MUNICIPALITY OF GHANA

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Human African Trypanosomiasis is transmitted by only two subspecies of Trypanosoma (Trypanosoma brucei gambiense and T. brucei rhodesiense) in sub-Saharan Africa. However, case reports of some Trypanosomes (T. congolense, T. evansi, T. brucei brucei, and Trypanosoma vivax) thought to be natural parasites only to animals have been reported to cause “atypical Human African Trypanosomiasis” (a-HAT). The study therefore aimed at providing molecular-based evidence of the transmission of atypical trypanosomiasis in humans in selected communities in the Suhum Municipality of Ghana. A cross-sectional community-based study design was employed to sample venous blood from 240 human participants. Demographics and risk assessment data from participants and polymerase chain reaction (PCR) assays were performed, using trypanosome internal transcribed spacer 1 (ITS1) generic primers from extracted DNA. PCR products were purified and subcloned into pJET1.2/blunt plasmid. Single clones were checked by ITS1 inner primers (colony PCR). Positive clones were cultured overnight at 37°C at 220rpm in 5mL LB medium with 100µg/mL Ampicillin. The cultures were purified and sent to MicroSynth Labs. Sequences were analyzed and aligned with Geneious Pro 5.5.9. The overall prevalence of trypanosome infection was 15.8% (39/240). Age categories 11-20yr and 41-50yr recorded high prevalence of 21.6% and 22.2% respectively. Infection distribution among males and females was 18.0% and 13.6% (X2=0.9001 p>0.05). The highest prevalence of 19.2% a-HAT was recorded at Zorrh followed by Nkantekwan (17.9%) while no evidence of infection at Santramor No.1 was observed. Trypanosome parasites found in this study area were T. evansi, T. congolense, T. vivax and T. simiae. No association was established between tsetse fly bite and Trypanosomiasis (X2 =1.344 p> 0.05). There is a high prevalence of atypical human trypanosomiasis(a-HAT) in the study area. This underscores the need to investigate the impact of animal parasites on human health in order to institute measures that can help prevent the spread of these parasites

**5727**

## LEISHMANIA INFANTUM VERTICAL TRANSMISSION IN NATURALLY INFECTED DOGS FROM AN ENDEMIC REGION OF BRAZIL

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Visceral leishmaniasis (VL) due to Leishmania infantum is a parasitic illness reported in Europe and Latin America. Dogs are considered the main parasitic reservoir in periurban areas of Brazil. Leishmania transmission in these regions has been traditionally considered to be vector-borne. Although vertical transmission of Leishmania has been shown to have a major impact in maintaining a population of infected dogs, it is not considered yet in control strategies for Brazil. The aim for this study was to evaluate whether and how frequently vertical transmission of Leishmania occurs in a highly endemic setting of Natal, Brazil. We hypothesized that transplacental transmission of Leishmania parasites in Natal may occur at a similar frequency to that observed in dogs in non-endemic regions. To address this, a total of 7 naturally Leishmania-infected pregnant females and their resultant pups (n=48) were recruited and divided into two groups. The first was a cross sectional study to assess presence of parasites in tissues of (aborted) puppies (n=30) from euthanized female dogs (n=4) and the second a prospective evaluation of 3 liters (n= 18), born at a sandfly free facility, to evaluate parasitemia and anti-Leishmania serology for 12 months. In the first study, we found disseminated Leishmania infection in feti, by RT-qPCR, from four pregnant dogs via L. infantum kDNA (89.28%) and detected splenic parasite antigen via IHC in 85.7% of feti. All dogs (100%) from the prospective cohort study were Leishmania positive by both soluble parasite antigen ELISA and parasitemia. Positive correlation between parasitism in umbilical cord blood and in the pup over time supported not only mechanistic evidence of transmission of parasites through umbilical vessels, but also suggests the use of this tissue as a clinical tool to predict Leishmania vertical transmission. These findings support the hypothesis that the incidence of vertical transmission of Leishmania infantum parasites in dogs in endemic areas of Brazil is high and demonstrates the need for novel strategies to control canine VL, including regions where human VL is also endemic.

**5728**

## CLINICAL AND METAGENOMIC CHARACTERIZATION OF CEREBRAL TOXOPLASMOSIS IN THE PERUVIAN AMAZON.

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Neurological opportunistic infections are a significant cause of morbidity and mortality in people living with HIV (PLHIV). We conducted a study of 140 PLHIV with acute neurological symptoms from Iquitos, Peru. Participants were evaluated for cerebral toxoplasmosis. Poisson regression with robust variance was used to assess differences between patient with and without cerebral toxoplasmosis. A subset of samples were evaluated by metagenomic next-generation sequencing (mNGS) of cerebrospinal fluid (CSF) for comparison with standard diagnostic techniques and identification

of additional diagnoses. 27 participants were diagnosed with cerebral toxoplasmosis by CSF qPCR. Compared to participants without cerebral toxoplasmosis, abnormal Glasgow coma score (p=0.05), unilateral focal motor signs (p=0.01), positive Babinski (p=0.01), and multiple intracranial lesions on head computed tomography (CT) (p=0.002) were associated with cerebral toxoplasmosis. mNGS identified 7 cases of cerebral toxoplasmosis, 7 cases of cryptococcal meningitis, and other possible cases of TB(n=5), hepatitis B (n=1), and pegivirus (n=1). CSF mNGS had a positive percent agreement of 71% and a negative percent agreement of 91% with qPCR for T. gondii. An infection was definitively diagnosed by any method for only 35% of participants, demonstrating the challenges of diagnosing neurological opportunistic infections in this population and highlighting the need for broader, more sensitive diagnostic tests for CNS infections.

**5729**

## RISK FACTORS FOR MOTHER-TO-CHILD TRANSMISSION OF TRYPANOSOMA CRUZI AND HEPATITIS B IN THE CROSS-BORDER AREA OF ARGENTINA AND PARAGUAY

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Mother-to-child transmission (MTCT) of Trypanosoma cruzi and hepatitis B virus (HBV) contribute to increased morbidity and disability in Latin America and the Caribbean region. The framework for eliminating MTCT of HIV, syphilis, hepatitis B, and Chagas (EMTCT Plus) has been implemented in the region since 2018 by Mundo Sano in collaboration with several private-public institutions. This study aims to: (i) identify the risk factors for MTCT of T. cruzi and HBV; and (ii) evaluate the effectiveness of the implementation of the framework in the cross-border area of Argentina and Paraguay. Data on T. cruzi and HBV infection among pregnant women and their infants was collected from the antenatal care registries during June 2018 to December 2022. MTCT rates and access to the screening were examined. Bivariate and multivariate analyses were used to explore associations between the infections and socio-demographic factors. Additionally, we conducted 34 Key Informant Interviews (KIIs) for both implementers and service recipients to identify barriers to and promoters for access to screening and treatment of these infections. Finally, we integrated the quantitative and qualitative data using the Consolidated Framework for Implementation Research (CFIR) to evaluate the effectiveness of the intervention. Our preliminary results show approximately 1,493 mothers accessed antenatal care screening for these two infections. The prevalence of T. cruzi among mothers and newborns was estimated at 6.36% (CI95% 5.12-7.60) and 15.9% (CI95%: 1.90-45.44), respectively. However, the screening coverage for newborns who born to the T. cruzi seropositive mothers was only 57.0%. Treatment coverage for newborns was 100%, but post-partum treatment coverage among mothers was only 35.8%. Potential identified barriers were geographical distance to health care services, cultural beliefs, and lack of knowledge about the diseases in the communities. Our findings will be shared with high-level policymakers in the two countries and may be used to improve the implementation of T.cruzi and HBV programs in the region.

**5730**

## ETIOLOGY, GAPS, AND CHALLENGES IN THE DIAGNOSIS AND MANAGEMENT OF SEPSIS AMONG UNDER-FIVES ENROLLED IN THE KENYA CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE NETWORK (CHAMPS) PROGRAM

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Sepsis remains the third leading cause of death in children under five years globally, despite reduced mortality through improved treatment. Diagnosis and clinical management continue to pose a challenge in developing countries. Child Health and Mortality Prevention Surveillance (CHAMPS) is a multi-country surveillance program that systematically identifies causes of under-five mortality from defined catchment areas in seven countries in sub-Saharan Africa and South Asia. Here, we describe the etiology, gaps and challenges in diagnosis and treatment of sepsis in children under 5 years of age enrolled in CHAMPS-Kenya. Causes of death (COD) were determined by a panel of experts using data from post-mortem investigations conducted using minimally invasive tissue specimen testing, clinical records, and verbal autopsy. Between May 2017 and Dec 2022, 697 children below 5 years had their COD determined. Of these, (132) had sepsis in the causal chain leading to death. Of those, 53 (40.2%) were community-based deaths and 48(36.4%) were neonates, 50 (37.9%) were infants, 32 (24.2%) were children above one year of age and 2(1.5%) were stillbirths. The main causative agents of sepsis were Klebsiella pneumonae (42, 34.1%), Escherichia coli (22, 17.9%) and Streptococcus pneumonae (17, 13.8%) respectively while 32 (24.2%) cases had multi pathogen sepsis. One third of hospitalized cases died within the first 24 hours admission; admission diagnoses included shock, severe dehydration, and severe gastroenteritis in 40% of cases. Ninety-five percent of hospitalized cases were not diagnosed with sepsis before death. Blood chemistries were performed in 30% of all hospitalized cases, but none of the cases had peripheral blood cultures or blood gases performed. Sepsis is common among CHAMPS cases in Kenya, with significant antemortem disparities in diagnosis and clinical management of identified cases. Given the complexity of sepsis diagnosis, especially in under-five, maternal health education on danger signs, a high index of clinical suspicion, and training on optimal clinical management could reduce sepsis-related deaths.

**5731**

## GENOMIC CHARACTERIZATION OF EXTRAINTESTINAL PATHOGENIC ESCHERICHIA COLI ISOLATED FROM STILLBIRTHS AND EARLY NEONATAL DEATHS: AN OBSERVATION FROM CHAMPS BANGLADESH

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Extraintestinal pathogenic Escherichia coli (ExPEC) are non-diarrheagenic E. coli (EC) known to cause urinary tract infection, sepsis and neonatal meningitis. Presence of specific virulence genes aids to this characterization. Here we aim to use whole genome sequencing (WGS) to characterize ExPEC isolated from postmortem blood collected from stillbirths and neonates enrolled in the Child Health and Mortality Prevention Surveillance (CHAMPS) project in Bangladesh. CHAMPS uses laboratory diagnosis, clinical, demographic and epidemiological data to determine the causes of stillbirths and under-5 deaths. From Aug 2017 to Dec 2021, blood culture data from 357 post-mortem stillbirths (n = 173) and neonates (n = 184) were analyzed. WGS was performed and analyzed for sequence typing, virulence and antimicrobial resistance genes. EC was detected from

3.9% (14/357) cases including macerated stillbirths (n = 10) and neonates (n = 4). EC was not attributed to be in the causal chain after review by a panel of experts. However, WGS identified ExPEC associated virulence genes from half (7/14) of the EC isolates (5 stillbirths and 2 neonates). All ExPEC isolates belong to different sequence type and serotype. Six (6/7) ExPECs were closely related to uropathogenic EC and harbored virulence genes such as P fimbriae (papA, papC), S fimbriae (sfaD, sfaS), K1 capsule (kpsMII, neuC), cytotoxic necrotizing factor (cnf-1), as well as different siderophore systems (fyuA, iroN) and toxins (clbB, tcpC) which help ExPECs to colonize and infect organs other than the gastrointestinal tract of the fetus. Genomic investigation identified 5 of these ExPECs as multi drug resistant and also extended-spectrum β-lactamase (ESBL)-producers (carrying blaCTX-M-15). However, these were sensitive to carbapenem antibiotics. Presence of virulence features and close relatedness to uropathogenic EC indicated the potential that these organisms were transmitted from mother to the fetus, resulting in negative outcomes. Diagnosis of symptomatic or asymptomatic urinary tract infection in pregnant women may reduce the chance of mother to child transmission of ExPECs.

**5732**

## URECA-LAMP: RAPID, CHEAP AND EFFECTIVE POINT-OF-CARE SCREENING OF CEPHALOSPORIN AND CARBAPENEM RESISTANCE FOR LOW MIDDLE-INCOME COUNTRIES

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Gram-negative bacteria are common causative agents of urinary tract infections (UTIs) worlwide. These bacteria frequently carry genes encoding extended-spectrum beta-lactamases (ESBL) and/or carbapenemases. UTI-screening for these genes with point-of-care testing (POCT) can aid in guiding empirical treatment and reduce antimicrobial resistance (AMR). The novel Urine ESBL and Carbapenemase (URECA) panel is a 7-tube strip. Each well contains primers to detect ESBLs CTX-M-1-group and CTX-M-9-group and carbapenemases OXA-23, OXA-48-like, OXA-50, NDM and VIM. The White Lotus is a novel LAMP-POCT device manufactured by our group. It is portable, battery-operated, low cost and smart phone operable. The aim of this study was to validate the URECA-LAMP panel for rapid screening of ESBL- and carbapenemase-producing bacteria. To this end, a sample set of 70 bacterial strains (10 positive controls per target) was obtained from the well-defined SMART and INFORM bacterial collections. Previous WGS characterization of the strains was used as gold-standard for comparison against URECA-LAMP results. DNA template was prepared by boiling a single bacterial colony from each sample in 0.5 mL of nuclease-free water for 10 minutes. 20 uL of boiled sample was added to each of the wells in URECA panel. 2.5 uL of hydroxy naphthol blue was added to each well for visual detection. Positive results were defined as fluorescence-emitting tubes under blue-led light. After screening the complete sample set, comparison between URECA-LAMP and previous WGS results showed 100% agreement of positive and negative results for each gene. We conclude URECA-LAMP is suitable for detection of ESBLs/carbapenemases in bacteria from UTIs. Our results indicate its feasibility for POC screening of human urine specimens. The lower production cost of the White Lotus instrument makes URECA-LAMP attractive for implementation in low middle-income countries.

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## DEVELOPMENT OF A KLEBSIELLA PNEUMONIAE NEONATAL SEPSIS MOUSE MODEL TO EVALUATE VACCINES

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Klebsiella pneumoniae is a major cause of neonatal sepsis in low-to-middle income countries. With the proportion of multidrug-resistant K. pneumoniae strains increasing globally and the lack of novel antibiotics in the pipeline, vaccination to prevent these infections is an attractive strategy. Several K. pneumoniae vaccines are currently in development, however, there are no animal models of K. pneumoniae-related neonatal sepsis that could be used to evaluate vaccine efficacy. Our goal is to identify a model which produces a 50% lethal dose (LD50) of ~106 CFU of 2- to 3-day-old animals and which shows an age-dependent susceptibility to infection. We have previously evaluated species (mice vs. rats), 3 mouse strains, 3 routes of infection, and 5 K. pneumoniae strains. We found that peroral (PO) infection of 2- to 3-day-old C57BL/6 mice with K. pneumoniae B5055 produced mortality. Here we describe characterization of this model in terms of the LD50 and age-dependent susceptibility to infection. We determined the PO LD50 of K. pneumoniae B5055 to be 8.5 x 106 CFU in 2-day-old C57BL/6 mice. We also tested PO infection with 108 CFU and 109 CFU, demonstrating these inocula could reliably produce 77-100% mortality in 2- to 3-day-old C57BL/6 mice (n = 4 litters of 5-9 mice per litter; 109 CFU: 3.1% ± 6.3% survival, mean ± standard deviation; 108 CFU: 23.5% ± 22.3% survival). Furthermore, C57BL/6 mice demonstrated an age-dependent susceptibility to infection, where 2- and 5-day-old pups exhibited 100% (7/7) and 50% (4/8) mortality following PO exposure to K. pneumoniae B5055, respectively. K. pneumoniae was found in the blood, spleen, liver, and intestines of infected neonates. In contrast, 30- and 60-day-old C57BL/6 mice exhibited low susceptibility to infection with K. pneumoniae B5055 administered PO (1/10 and 1/10, respectively) and low bacterial colonization (1/10 and 0/10, respectively). We are currently evaluating 7-, 10- and 15-day-old mice. In conclusion, we have developed a neonatal sepsis model which consists of PO infection of neonatal C57BL/6 mice with K. pneumoniae B5055. We continue to characterize this model.

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## FIRST REPORT OF OXA-181-PRODUCING ENTEROBACTERALES IN LATIN AMERICA

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Carbapenemase-producing Enterobacteriaceae (CPE) infections are a growing threat contributing to the global burden of antimicrobial resistance (AMR). Although carbapenemase enzymes KPC, NDM and IMP are the most common enzymes worldwide, β-lactamase type OXA-48-like(oxacillinases) are on the rise. The blaOXA-181(a variant of OXA-48) enzymes show a high level of hydrolytic activity against penicillins and a low level of hydrolysis of carbapenems, with a strong preference for imipenem, which is challenging for laboratory diagnosis and may misguide treatment strategies. CPE blaOXA-181 has been reported in human, animal and environmental samples, but has not yet been reported in Latin America. We characterized five CPE clinical isolates from two healthcare facilities in Lima, Peru. The isolates were identified as Klebsiella pneumoniae (n=3),

Citrobacter portucalensis, and Escherichia coli, all presenting multidrug-resistant phenotypes. Illumina sequencing revealed the presence of the blaOXA-181 gene as the only carbapenemase in all isolates. Genes associated with resistance to aminoglycosides, quinolones, amphenicols, fosfomycins, macrolides, tetracyclines, sulfonamides, and trimethoprim were also found.The plasmid group IncX3 was identified in all genomes in a truncated Tn6361 transposon flanked by ΔIS26 insertion sequences. The qnrS1 gene was found downstream of blaOXA-181, conferring fluoroquinolone resistance to all isolates. The emergence of blaOXA-181 in association with the qnrS1 gene in IncX3-type plasmids may represent the primary vector for the spread of blaOXA-181 in Latin America. Genomic surveillance of atypical AMR patterns in clinical and non-clinical isolates is needed to elucidate the transmission of this CPE genotype, as routine clinical laboratory diagnoses fail to detect these new OXA variants.

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## THE RELATIONSHIP BETWEEN CO-MORBID MALNUTRITION AND DIARRHEAL ILLNESS AMONG HOSPITALIZED TANZANIAN CHILDREN UNDER FIVE YEARS OF AGE

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There is a bidirectional association between malnutrition and diarrheal illness wherein malnutrition increases the risk, frequency, and duration of diarrhea while diarrhea further exacerbates malnutrition through nutrient depletion. We analyzed preliminary data on 120 (females: n=47, 39.1%; mean age: 14 ± 10 months) children enrolled in the Diarrhea Etiology and Malnutrition study who were hospitalized with diarrhea at Haydom Lutheran Hospital in Haydom, Tanzania from July 2022 to February 2023. There were 25 (20.8%) severe/moderate malnourished children based on doctor report. An infectious etiology was identified for 70/94 (74.5%) children infected (Ct < 30) with at least one of the top 10 pathogenic causes of diarrhea found in the MAL-ED study (n=28 bacterial and viral, n=22 bacterial, n=19 viral, n=1 parasitic). Log binomial and linear regression analyses adjusted for sex were used to determine the relationship between malnutrition and diarrhea etiology, length of stay (LOS), and respiratory illnesses. There were no associations between malnutrition and identification of an infectious etiology of diarrhea (RR: 0.87; 95% CI: 0.63, 1.21). Children with malnutrition were not more likely to have a bacterial etiology (RR: 0.97, 95% CI: 0.61, 1.53) or viral etiology (RR: 0.51, 95% CI: 0.25, 1.03). Malnourished children with diarrheal illness had significantly longer hospital stays (LOS difference: 8.23 days; 95% CI: 5.26-11.19 days) than children who were not malnourished. No differences in LOS between bacterial and non-bacterial diarrhea were observed (LOS difference: 0.93 days; 95% CI: -2.33, 4.19). Malnourished children were more likely to have co-morbid respiratory symptoms (RR: 1.47; 95% CI: 0.89, 2.46) compared to children who were not malnourished. In conclusion, although malnourished children were not at greater risk for specific diarrhea etiologies, they were more likely to have longer hospitalizations. These data suggest malnutrition may contribute to poor outcomes of hospitalized diarrhea independent of diarrhea etiology.

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## ETIOPATHOLOGY OF STUNTING: INFANT GUT CHARACTERIZATION AND MICROBIAL INFLUX ROUTES IN A MOTHER-INFANT COHORT IN CENTRAL-AFRICA

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Background : Environmental enteric dysfunction (EED) is an enigmatic disease of the small intestine intimately associated with child undernutrition, a pressing global health problem with over 149 million children affected globally. The etiopathology of EDD has remained elusive for decades. Recent evidence showed that stunting was associated with small intestinal bacterial overgrowth dominated by bacteria that normally reside in the oropharyngeal cavity in children 2-5 years. However, evidence is limited on how the oropharyngeal cavity and the gut are colonized in the stages preceding the appearance of stunting in a context of a high burden of stunting and a highly microbial polluted environment. Methods : We followed a cohort of 50 infants from birth until 6 months of life in Bangui (Central-African Republic). We performed metagenomic analyses of oral and stool microbiota at birth, and at 1, 4, 11, 18, and 25 weeks. We also analyzed breastmilk microbiota starting at 1 week. We gathered complete socio-economic and clinical data, anthropometric measures and 24-hour recalls and food-consumption questionnaires for diet assessment at each visit. Results : Stunting was significantly associated with stool microbiota of the infants at 6 months (P value of the Permanova = 0.04). The relative abundance of Rothia SGB16985, Streptococcus parasanguinis, and Veillonella dispar was significantly higher among non-stunted infants in both oral and stool samples. Even if there were no significant differences in the breastmilk microbiota depending on stunting status of the infant, 6/10 of the species with the highest relative abundance in breastmilk (Rothia SGB16985, Streptococcus thermophilus, Streptococcus peroris, Streptococcus pneumoniae, Staphylococcus hominis, and Streptococcus salivarius) were significantly associated with stunting in oral and stool samples. Conclusion : The ectopic colonization of oral bacteria in the gut occured also in the absence of stunting. The role of the breastmilk microbiota should be further investigated in the context of stunting and a highly microbial polluted environment.

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## DISCOVERY OF A SMALL MOLECULE THAT MIMICS A UNIQUE ZIKA-NEUTRALIZING EPITOPE FROM A LARGE LIBRARY OF RANDOM MOLECULAR SHAPES

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Antigenic similarities between Zika virus and other flaviviruses pose challenges to the development of virus-specific diagnostic tools and effective vaccines. We screened a DNA-encoded one-bead-one-compound combinatorial library of 508,032 synthetic, non-natural oligomers, for compounds that mimic ZIKV-specific epitopes. High-throughput FACS-based screening was used to select molecules that bound IgG from ZIKV-immune but not from dengue-immune sera. Deep sequencing of the encoding tags on beads that retained high levels of antibodies to ZIKV-immune serum and clustering analysis of structurally homologous

hits identified 40 candidate molecular structures. A lead candidate small molecule "CZV1-1" was selected that correctly identifies serum specimens from Zika-experienced patients with high sensitivity and specificity (78.9% and 98.9%, respectively). Affinity chromatography using immobilized CZV1-1 resulted in a ≈ 600-fold enrichment of the antibodies from the serum that recognizes this synthetic molecule. Binding assays revealed that these enriched anti-CZV1-1 IgGs recognize the domain III (DIII) of the ZIKV envelope protein and show minimal reactivity to DIII of dengue virus envelope proteins. Binding competition studies of purified anti-CZV1-1 IgG against known ZIKV-specific monoclonal antibodies (mAbs) showed that CZV1-1 mimics a nonlinear neutralizing conformational epitope in DIII of the ZIKV envelope. Purified anti-CZV1-1 IgG neutralized infection of distinct strains of ZIKV in cell cultures with potencies comparable to highly specific ZIKV-neutralizing monoclonal antibodies. Molecular dynamics simulations revealed that anti-CZV1-1 IgG binds to ZV48, a highly specific ZIKV mAb that targets the C- C' loop epitope of ZIKV envelope DIII, with high affinity and displays a higher rate of native contacts, hydrogen bonds, and configurational stability within the binding site with ZV48 than other mAbs. Collectively, our findings reveal that systematic mining of 'antigenically agnostic' libraries of small molecules can be used to discover biomarker correlates of virus neutralization.

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## PHARMACOKINETICS, TOLERABILITY AND SAFETY OF FAVIPIRAVIR COMPARED TO RIBAVIRIN FOR THE TREATMENT OF LASSA FEVER: A RANDOMIZED CONTROLLED OPEN LABEL PHASE II CLINICAL TRIAL

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Lassa fever (LF) is a severe re-emerging infectious disease and defined as priority disease for urgent research and development by the World Health Organization. It is caused by the Lassa virus (LV) which belongs to the segmented negative strand RNA viruses of the family Arenaviridae. Transmission occurs primarily by spill-over from the animal reservoir but secondary cases with human to human transmission are common. LF affects a large number of countries in West Africa, with Nigeria carrying the highest case burden in the world. So far, treatment options are limited to supportive care and the antiviral ribavirin. Evidence for the efficacy of ribavirin in LF is, however, poor. A recent study conducted in Nigeria showed that in vivo plasma concentrations do not suffice to exert a relevant antiviral effect. New drugs for LF treatment are therefore urgently needed but no therapeutic trials have been conducted for this indication in the past decades. Favipiravir is a broad-spectrum antiviral registered for pandemic influenza that has previously been evaluated for Ebola virus disease and Covid-19. It has potent activity against LV in pre-clinical studies. The aim of this phase II clinical trial was to explore the pharmacokinetics, pharmacodynamics, safety and tolerability of favipiravir as repurposed drug in the treatment of LF. The trial was conducted at the Irrua Specialist Teaching Hospital and the Federal Medical Centre of Owo in Nigeria - the worldwide largest LF treatment centres. Blood samples for pharmacokinetic analyses were collected at 0.5, 1, 3, 5, 8, 12 and 24 hours after the first dose and at 0, 1 and 4 hours after drug administration on day 7. Besides clinical parameters, further sampling for virological, serological and immunological analyses as well as hematology and biochemistry safety was done on days 1, 2, and then every other day until the end of the study. Between 2021 and 2022, 40 LF patients were included in the trial. Trial results on cure rates, pharmacokinetics, safety and tolerability of this first GCP compliant phase II clinical trial will be presented to provide first insights into prospects of this new treatment candidate for LF.

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## A BEAD-BASED MULTIPLEX SAMPLE-SPARING ANTIBODY ASSAY FOR DETECTING CURRENT AND PAST DENGUE AND ZIKA VIRUS INFECTIONS

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Serological assays to identify recent or past flavivirus infections are needed for surveillance and predicting the risk of severe disease. The four dengue virus (DENV) serotypes and Zika virus (ZIKV) can co-circulate in the population. Prior immunity to one DENV serotype or ZIKV is a risk factor for severe disease during secondary DENV infection. Detecting viral RNA or non-structural protein during acute infection does not identify all acute infections due to brief viremia. Paired serology using the whole virus antigen is sensitive but lacks specificity to distinguish between DENV and ZIKV infections. To address this, we developed a multiplex immunoassay using beads coupled to DENV1-4 and ZIKV EDIII antigens to detect and distinguish acute and past infections. We tested 204 paired blood samples collected at acute and convalescent stages from febrile patients (2-77 years) during the 2016 Zika (n=30) and the 2018 DENV2 outbreaks (n=174) in León, Nicaragua. We developed an algorithm to detect and distinguish acute infections based on increased of EDIII antibody levels from acute to convalescence. Using EDIII binding pattern to the acute sample and our earlier validated algorithm for detecting past infection, we stratified acute infection by prior exposure. Compared to paired serology using whole viruses, the multiplex assay demonstrated a sensitivity of 90.1% and a specificity of 88.2% for detecting acute infections. Despite DENV and ZIKV background immunity being 85.7%, the multiplex assay correctly classified acute DENV or ZIKV infection for 91.9% and was indiscernible for 8.1% of individuals with >2 past DENV infections. The accuracy among the 14.3% naïve individuals for identifying DENV serotype or ZIKV infection was 100%. By comparison, RT-PCR missed one for every two symptomatic cases tested. Thus, the multiplex EDIII assay is robust, requires a small sample volume, and does not require handling virus to determine acute infection and past exposure to DENV and ZIKV. It offers a significant benefit for surveillance, estimating disease burden, and implementing control measures to reduce DENV and ZIKV transmission.

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## DEVELOPMENT AND EVALUATION OF NOVEL NANOBODIES AGAINST ZIKA VIRUS INFECTION

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Zika virus (ZIKV) is a human-pathogenic flavivirus that has raised global concerns in recent years as it caused an unprecedented large-scale epidemic of congenital microcephaly and malformations. While the case number of ZIKV infection has seemingly decreased in the past few years, the actual incidence rate may be largely underestimated and ZIKV remains an important public health threat. Despite the clinical importance of ZIKV, treatment and prophylaxis options remain limited. Over 70 therapeutic anti-ZIKV monoclonal antibodies (mAbs) with moderate to high neutralizing activities have been reported, with some demonstrating antiviral effects in preclinical animal models. However, a major concern of these anti-ZIKV mAbs is the potential to induce antibody-dependent enhancement (ADE) of infection. The possibility of exacerbating disease progression by ADE has limited the clinical application of these anti-ZIKV mAbs. In this study, we constructed a nanobody (single-domain antibody) library by immunizing alpacas (Illama pacos) with recombinant ZIKV proteins and

identified a number of nanobodies with high affinity against ZIKV. Among them, a number of nanobodies targeting the ZIKV envelope (E) protein or nonstructural protein 1 (NS1) demonstrated potent anti-ZIKV activity in vitro in viral load reduction, plaque reduction, and cytopathic effect inhibition assays. Type I interferon receptor-deficient A129 mice treated with a single dose of any one of these nanobodies either as prophylaxis (before virus challenge) or treatment (after virus challenge) demonstrated significantly higher survival rate at 10-14 days post-infection (70-100% vs 0%, P<0.05) and lower viral burden in brain tissues. Importantly, ADE assay showed that these nanobodies did not induce ADE. Mechanistically, these nanobodies significantly downregulated interleukin-1-beta in vitro and in mice. Taken together, these results showed that nanobodies are effective antivirals against ZIKV infection without inducing ADE. Clinical trials should be considered to assess the prophylactic and therapeutic effects of anti-ZIKV nanobodies in endemic regions.

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## GENERATION OF THERAPEUTIC HUMAN MONOCLONAL ANTIBODIES AGAINST HANTAVIRUSES FROM HUMAN-IMMUNE-SYSTEM HUMANIZED DRAGA MICE

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Hantaviruses are rodent-transmitted viruses that cause Hantavirus Cardiopulmonary Syndrome (HCPS) and Hemorrhagic Fever with Renal Syndrome (HFRS) around the globe. Most hantavirus infections in Asia are caused by Hantaan (HTNV) and Seoul (SEOV), in America by Andes (ANDV) and Sin Nombre (SNV), and in Europe by Puumala (PUUV) and Dobrava-Belgrade (DOBV) virus strains. Collectively, 150,000-200,000 cases of hantavirus disease are reported annually, with a case fatality rate of 12% for HFRS and up to 40% for HCPS. Recent studies in Argentina and Chile reported that the hantavirus strain ANDV can potentially be spread between human through direct and close contact with an infected person. Moreover, the lack of effective vaccines and therapeutics makes hantavirus infections dangerous and raising concern for potential hantavirus pandemics. Humanized DRAGA mice infused with human hematopoietic stem cells from cord blood reconstitute a long-term functional human immune system and demonstrated as a rapid platform for generation of human monoclonal antibodies (hmAbs) against infectious diseases. Here, we primarily describe to identify, characterize and develop therapeutics antibodies against glycoprotein complex (Gn/Gc, M segment) of ANDV from DRAGA mice immunized with DNA-encoding vaccine. Using hybridoma technology, 1026 human B cell hybridoma clones were obtained from splenic human B cells fused with K6H6 myeloma cells. Among them, 63 (6%) hmAb clones showed significant binding affinities for ANDV Gn/Gc protein as determined by ELISA. Nine (14 %) of the ANDV specific hmAbs also showed bind to HTNV Gn/Gc protein; suggesting small pool of generated ANDV-specific hmAbs able to cross-react against two different strains (ANDV & HTNV). Ongoing studies are aimed at immune-characterization, by assessing cross-reactivity and neutralizing activity of these hmAbs with the goal of testing their protective and therapeutic efficacy against different strains of Hantaviruses in animal models. Highly cross-reactive hmAbs have potential as “universal” immunotherapeutics for the prevention of and treatment of hantavirus infections in humans.

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## A NON-WHOLE GENOME SEQUENCING APPROACH FOR MONITORING SARS-COV-2 VARIANTS IN BURKINA FASO & KENYA

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The rapid emergence and global dissemination of SARS-CoV-2 highlighted a need for robust, adaptable surveillance systems. However, financial and infrastructure requirements for whole genome sequencing (WGS) mean most surveillance data has come from higher-resource geographies. Consequently, the molecular epidemiology of SARS-CoV-2 in low- and middle-income countries (LMICs) is limited, and there is a need for more cost-accessible technologies in LMICs to help close data gaps in variant surveillance. To address this, we developed a high-resolution melt curve (HRM) method targeting key variant-defining mutations in the SARS-CoV-2 genome, which give unique signature profiles that define different SARS-CoV-2 variants of concern (VOCs). Nasopharyngeal-oropharyngeal swabs from 178 participants (112 in Burkina Faso, 66 in Kenya) on the day of enrolment in the MALCOV (Malaria as a risk factor for COVID-19 in western Kenya and Burkina Faso) cohort study were used in the evaluation of the HRM assay and compared to WGS. A SARS-CoV-2 variant could be determined by both HRM and WGS for 74.7% of specimens. Overall, eleven distinct genotypes of SARS-CoV-2 were identified among Burkinabè and Kenyan cohorts, with Delta VOC most frequently detected. Additionally, the 19A strain, VOCs Alpha and Omicron, and other non-VOCs, including Eta, and those belonging to 19B, 20A, 20B, and 20E were detected, as was evidence of recombinant genomes. The sensitivity and specificity of the HRM assay compared to WGS were 100% and 94.6-100%, respectively, but varied by VOC. HRM-based assays can provide a lower-cost approach (<$1 per test) to conducting molecular epidemiology as part of wider surveillance strategies, particularly in settings where access to WGS is absent or limited. The HRM assay can be implemented on most modern real-time PCR instruments, which are already be available in most diagnostic facilities. Such assays can often be implemented with little capital investment. Additionally, the assay is readily adaptable and can focus on local epidemiological surveillance needs or be updated quickly to accommodate the emergence of a novel variant.

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## DETECTION OF BLOOD BIOMARKERS OF NEUROLOGICAL INJURY IN HUMAN CASES OF VIRAL ENCEPHALITIS AND SEVERE DISEASE

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Neurotropic viral infection and the ensuant immune response are a significant cause of morbidity and mortality worldwide, which can range in severity from mild to permanent central nervous system (CNS) damage and death. Encephalitic alphaviruses of military and public health concern include Venezuelan and eastern equine encephalitis viruses (VEEV and EEEV) and Madariaga virus (MADV; Alphavirus; Togaviridae), which are mosquito-borne viruses in the Americas that cause CNS disease in humans and equids. Injury to the CNS is an important determinant of poor outcome

and tools to predict this outcome are lacking. Glial fibrillary acidic protein (GFAP) and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) are proteins that when detected in the serum, signal astrocyte and neuronal injury, respectively. These biomarkers of CNS tissue injury could serve to better assess injury severity, monitor disease progression, direct treatment, and as reliable endpoints to help develop novel medical countermeasures. Recent advances in the use of blood-based biomarkers for diagnosis of traumatic brain injury (TBI) which have FDA approved assays have provided a scientific foundation for expanding the biomarker technology to brain damage caused by other CNS pathologies like viral encephalitis. Here we evaluated the ability to detect these biomarkers in the serum from multiple human cohorts with evidence of viral encephalitis or severe disease. Samples were collected from: 1) human cases infected with the alphaviruses VEEV subtype ID or MADV in Panama; 2) human cases of suspected sepsis in Cambodia and Ghana that were severely ill or diagnosed with encephalitis of unknown origin; 3) hospitalized patients with severe coronavirus disease (COVID-19). We found higher levels of GFAP and/or UCH-L1 in all cohorts and in some cases detection of these biomarkers could predict later developing cognitive impairment. Collectively, our results suggest that the detection of these blood-based biomarkers with an already FDA approved assay may be a good indicator for brain injury resulting from viral or other infections causing severe disease.

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## PATTERNS OF SARS-COV-2 ACTIVE INFECTIONS AMONG HUMANS AND COHABITATING DOMESTIC ANIMALS OF EAST CENTRAL TEXAS DURING THE EARLY OMICRON WAVE

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Given that SARS-CoV-2 is now known to infect a wide range of mammals, sustained animal surveillance is a critical tool to help protect public health and support early detection on emerging mutations and variants. We conducted a longitudinal One Health study in 47 households with active human COVID-19 cases in east central Texas during January-July 2022. We evaluated people (n=104; range 1-7 per house) and animals (n=101; 1-12 per house), by RT-qPCR, for the presence of SARS-CoV-2 at three sequential sampling points, each 1-2 weeks apart, with the first visit occurring 0-5 days from detection of the first person with COVID-19. Our study spanned the peak of the BA.1 Omicron wave to the BA.2/BA.5 wave. Household animals were predominantly dogs (n=57) and cats (n=29) but also included goats, horses, pigs, a donkey, a rabbit, a gecko and a tortoise (n=14). People tested positive (64%) by PCR in all but 4 houses. Positivity was highest (60%) in the first sampling than the second (48.5%) and third (20.4%). Serology from dried blood spots revealed antibodies against the spike glycoprotein in 95.7% of the people, indicating either vaccination or natural exposure. Only 5.3% (3/57) of dogs tested PCR-positive, including one with a PCR-positive food bowl, at days 2-9 after diagnosis of the first person in the household. All other animals were negative. Sequencing revealed the BA.2 Omicron in one dog. Positivity among dogs was similar to our prior, pre-Omicron study (n = 396; 6.3%; $\chi 2$ , $P > 0.5$) but was higher among cats pre-Omicron (n = 158; 15.2%; $P < 0.05$) despite efforts toward early and multiple sampling in this study. Our longitudinal study found no evidence of onward transmission from the three infected dogs, as no infections were detected in coinhabiting animals at subsequent sampling points. Our results show that fewer animals became infected with Omicron despite its higher transmissibility among humans, suggesting that each variant may interact differently with animals in terms of susceptibility and transmissibility. These differences among variants emphasize the need for continued active surveillance in animal populations.

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## REAL-TIME DATA COLLECTION FOR EFFICIENT MICROPLANNING AND MONITORING OF NATIONAL DOG RABIES VACCINATION IN BANGLADESH

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Rabies, the world's deadliest disease with 100% fatality, creates significant public health difficulties in the world's poorest regions. Bangladesh, has the third highest number of human deaths (2000-2500 annually) among all rabies endemic countries. The country's goal was to reduce the number of rabies cases by 90% by 2015 and to eliminate the disease entirely by 2030. The main approach to achieve this goal was to vaccinate dogs which initiated a mass dog vaccination program aiming to deliver 3 rounds vaccination across the country. To do this, microplanning was applied to reach every community to maximize vaccination coverage. For efficient monitoring and microplanning, KoboToolbox was used to collect real-time data of vaccinated dogs. Aggregated daily dog vaccination data were collected on paper forms since 2012 and from 2018 this was converted to electronic data collection using KoboCollect where at the first part, dog vaccination was reported daily in aggregation and later at individual level in the same year with geolocation and photos. From 2018 to 2022, information on 1.6 million dogs was collected with 58.6% data containing the geo-locational data of dogs and 41.3% had no geo-locational data. Average vaccinated dog coverage was 81.6% covering the whole country. Correlation between reported rabies cases and vaccination coverage from 2012 to 2022 including aggregated and individual level gave a coefficient of -0.769 which demonstrates the effectiveness of the program. The shift from aggregate to individual level helped in improving vaccine delivery in progressive rounds as the increasingly granular data was used to identify locations with lower coverage, thus improving possible planning for future rounds to ensure maximum vaccination coverage. Although the system is easy to run and maintain but still there are data with no geo-location. To address this additional training and improved technical support are needed to maximize data completeness for optimal geo-enabled microplanning.

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## GENETIC ADAPTATION OF NONTYPHOIDAL SALMONELLA IN HUMANS, ANIMALS AND IN THE ENVIRONMENT-ANTHROPONOTIC TRANSMISSION?

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Nontyphoidal Salmonella (NTS) causes more than 1.2 million annual deaths worldwide, the majority in resource-limited countries such as sub-Saharan Africa (SSA). NTS have also become increasingly resistant to antibiotics and are the most frequent cause of bacteraemia in SSA. Recent data suggests that this typically livestock- associated pathogen has genetically developed and adapted to different hosts and environments, proposing anthroponotic transmission. Within this study, we collected Salmonella from humans (stool and blood), animals and the environment (dust and soil), in Tanzania and in Ghana. Strains were identified by biochemical methods and confirmed using the VITEK 2 System. Serotyping and antibiotic susceptibility testing was performed. Further, isolates were subjected to sequencing using a

NextSeq 500 Illumina machine.9,099 samples were collected. From these, 222 NTS were identified comprising 58 serovars. The highest level of resistance was in humans with fluroquinolone resistance on the increase and multidrug resistance (MDR) highest in isolates from blood cultures (24%, n/N=11/46). Of the invasive strains, MLST analysis confirmed the serovars and sequence types S. Typhimurium (ST313/ST19) being most common followed by S. Enteritidis (ST11/ST1479) and S. Dublin (ST10). A sequence type overlap amongst humans and livestock or environmental strains was detected for ST19.Our study demonstrates a broad serovar distribution of Salmonella from livestock and the environment not typically associated with human infections. The substantially high level of MDR and emerging fluoroquinolone resistance seen in the invasive NTS poses a challenge to current treatment strategies. Interestingly, we found ST19 more common in invasive human disease but also prevalent in samples from livestock compared to ST313, only seen in human samples. These findings are not in line with previous results, mainly from East Africa where ST313 was identified as the dominant sequence type in disseminated human disease, strongly indicating anthroponotic transmission of ST313 but not of ST19 in SSA.

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## MOLECULAR AND SEROLOGICAL EVIDENCE OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS IN LIVESTOCK AND TICKS IN CAMEROON

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Crimean-Congo haemorrhagic fever (CCHF) is a widespread tick-borne zoonotic disease, responsible of haemorrhagic symptoms in humans, with a reported case fatality rate of up to 40%. This disease is caused by Crimean-Congo haemorrhagic fever orthonairovirus (CCHFV). Little is known about the occurrence of CCHFV in ticks and animals in Cameroon. Hence this study aimed to determine the prevalence of CCHFV in domestic ruminants and identify its potential tick vector in Cameroon. A cross-sectional study was carried out in two livestock markets of Yaoundé in 2019, 2020, and 2021. Blood and ticks were collected from cattle, sheep, and goats. Anti-CCHFV specific antibodies were detected in plasma using a commercial double antigen Enzyme-Linked Immunosorbent Assay (DA-ELISA) assay. DA-ELISA- positive and a subset of negative samples were further tested by a seroneutralization assay for confirmation. Ticks were screened for the presence of orthonairoviruses by amplification of a fragment of the L segment and the genetic evolution of the virus was inferred by maximum likelihood. Overall, 756 plasma samples were collected from 441 cattle, 168 goats, and 147 sheep. The seroprevalence of anti-CCHFV antibody was 61.77% for all animals, with the highest rate in cattle (433/441, 98.18%) followed by sheep (23/147, 15.65%), and goats (11/168, 6.55%), (p-value < 0.0001). The highest seroprevalence rate was found in cattle from the Sahelian region of Cameroon (100%). Overall, 1500 ticks of the Rhipicephalus (773/1500, 51.53%), Amblyomma (341/1500, 22.73%), and Hyalomma (386/1500, 25.73%) genera were screened. CCHFV was identified in one Hyalomma truncatum pool collected from cattle. Phylogenetic analysis of the L segment classified this CCHFV strain within the African genotype III. These findings undoubtedly demonstrate for the very first time the circulation of CCHFV in Cameroon with the identification of genotype III. The results also highlight the necessity for more studies on CCHFV using a One Health approach and targeting at-risk human and animal populations, as well as ticks in high-risk areas of the country.

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## MOLECULAR CHARACTERIZATION OF EXTENDED - SPECTRUM BETA - LACTAMASE PRODUCING KLEBSIELLA PNEUMONIAE AMONG CHILDREN AND LIVESTOCK IN RURAL KOROGWE, TANZANIA

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Extended-spectrum beta lactamase- (ESBL) producing Klebsiella spp. are a global concern, and are on the increase, worldwide. This is mainly attributed to the overuse of antibiotics not only in human medicine but also in animal farming, also in Tanzania. Studies have been conducted on the prevalence of ESBL in children in Tanzania, but few studies have examined Klebsiella in livestock as a possible transmission reservoir. Using a one-health approach, this study investigated the frequency of ESBL-KP and antibiotic resistance in humans and livestock in rural Tanzania. This cross-sectional study was conducted from February 2019 to July 2020 and enrolled study participants at the outpatient department of Magunga Hospital (Korogwe). Stool samples were collected from children aged five and younger with and without diarrhea. Faecal specimens from livestock were sampled at commercial farms or smallscale farms in communities within the Korogwe District. Strains were identified using a chromogenic agar and confirmed using the VITEK2 Compact System. Antimicrobial susceptibility testing was performed by disc diffusion, and isolates subjected to whole genome sequencing using the NextSeQ500 Illumina machine. Of 258 asymptomatic and 259 children with diarrhoea, 16 (6%) and 32 (12%) tested positive for ESBL-KP, respectively. Furthermore, ESBL-KP was detected in 54 (7%) samples from chicken, one (3%) sample from pigs, and one (4%) sample from goats. All isolates were resistant to beta-lactams and cepholosporins and susceptible to carbapenems and tetracyclines. Whole genome analysis revealed ST17 to be the most common sequence type, and phylogenetic analysis revealed thirteen very closely related human and livestock isolate clusters. The results demonstrate the presence of ESBL-KP in human and chicken populations with ESBL-KP being closely related in the two reservoirs under investigation. Livestock represent a potential reservoir for transmission of ESBL-KP to humans. It is therefore essential to implement measures using a one health approach in order to control the spread and transmission of this pathogen.

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## RECONSTRUCTING RODENT CONTACT NETWORKS FROM TRAPPING DATA TO UNDERSTAND LASSA FEVER TRANSMISSION NETWORKS

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Lassa fever (caused by Lassa mammarenavirus) is a zoonotic infectious disease endemic to West Africa. Outbreaks of rodent-to-human infections are regularly reported from Nigeria, Sierra Leone and Liberia with sporadic outbreaks reported from elsewhere in the region. The primary rodent reservoir of this pathogen is Mastomys natalensis although evidence of infection has been identified in eleven other rodent species across

the region. The role of these other rodent species in the maintenance and transmission of viral populations among rodent communities is not understood. It is likely the spatially heterogeneous outbreaks of Lassa fever observed in human populations are indicative of complex viral and host population dynamics among rodent communities. Here we use rodent trapping data and rodent serology from a two year study in a Lassa fever endemic region of Sierra Leone, comprising more than 40,000 trap nights and 650 rodent detections to describe rodent communities across landuse gradients and to reconstruct rodent contact networks that will determine viral transmission. We find that rodent communities are structured along gradients of anthropogenic landuse disturbance moderating the hazard of exposure to reservoir species'. We further find that the primary reservoir of Lassa fever displays differential inter- and intra-specific contact rates across these landuse types which will affect the potential of viral persistance in these communities. These findings are important for understanding the dynamic risk of pathogen spillover in human communities. Findings from this study can guide future One Health focussed interventions and are of particular interest when conducting research attempting to assess the prevalence of Lassa fever and risk of spillover.

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## HEALTHY CHILDREN, HEALTHY CHIMPS: A RESEARCH-PRACTICE PARTNERSHIP FOR REDUCING RESPIRATORY DISEASE TRANSMISSION FROM HUMANS TO CHIMPANZEES IN UGANDA

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Respiratory disease is a major cause of morbidity and mortality among people in the developing world and also threatens great apes across Sub-Saharan Africa. Our studies of wild chimpanzees in Kibale National Park, Uganda, have identified the causative agents of respiratory disease outbreaks as "common cold" pediatric human pathogens, but reverse zoonotic transmission pathways have remained unclear. Between May 2019 and July 2022, we collected approximately 2,000 paired respiratory symptoms surveys and nasopharyngeal swabs from 264 people (local children and forest workers) and over 700 fecal samples from 141 chimpanzees as part of a prospective cohort study. We characterized respiratory pathogens using a multiplex PCR panel and metagenomic DNA sequencing and examined the transmission risk of various pathogen types, seasons, social factors, and the individual characteristics of humans and chimpanzees. Children exhibited high incidence rates and symptom severities, whereas adults were largely asymptomatic. COVID-19 lockdown in 2020-2021 significantly decreased respiratory disease incidence. Human symptoms peaked in February. In chimpanzees, the most common month for respiratory disease outbreaks was March. Rhinovirus, which caused a 2013 outbreak that killed 10% of chimpanzees in a Kibale community, was the most prevalent human pathogen throughout the study. Rhinovirus was also most prevalent during February and was the pathogen most likely to be carried asymptomatically by people. Our data suggest that respiratory pathogens circulate in children living near Kibale, and that adults in the same communities become asymptomatically infected and may carry the pathogens into the forest and infect chimpanzees. The "Healthy Children, Healthy Chimps" program reflects our hope that reverse zoonotic disease transmission to chimpanzees can be mitigated through a One Health approach that considers the health of chimpanzees and local people to be linked.

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## ASSESSING SHIFTS IN BITING PATTERNS OF ANOPHELES GAMBIAE AND AN. FUNESTUS, THE MAJOR MALARIA VECTORS IN SOUTHEASTERN TANZANIA

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Long-lasting Insecticidal Nets (LLINs) and Indoor Residual Spraying (IRS) are key vector control strategies in malaria control initiatives in Africa, including Tanzania. However, the prolonged use of LLINs and IRS has led to mosquitoes developing physiological and behavioral resilience to insecticides, resulting in increased residual malaria transmission that threatens malaria elimination efforts. To assess how mosquito-biting behavior changes could impact malaria epidemiology in Tanzania's southeastern area, a study was conducted using the mosquito electric trap (MET), collecting Anopheles mosquitoes weekly (18:00 - 06:00) from 22 villages from November 2019 to September 2020 in the Rufiji, Kilwa, and Kibiti districts. For each house, two METs were employed; one trap was set inside the house and the other was positioned 15 meters away outside the house. Each trap included a volunteer. A total of 3,586 An. mosquitoes were collected, 1,912 (53.32%) An. gambiae, 1,666 (46.46%) An. funestus, 7 (0.2%) An. coustan, and 1 (0.03%) An. pharoensis. An. gambiae exhibited a greater preference for outdoor biting, at a rate of 0.32 bites per person per hour during 20:00-21:00hr, increasing progressively through the night to reach a peak of 0.48 bites per person per hour during 00:00-01:00hr, while An. funestus showed a higher preference for indoor biting at a rate of 0.35 bites per person per hour indoors and 0.29 bites per person per hour outdoors. This study revealed, for the first time, that An. gambiae density is higher outdoors than indoors in southeastern Tanzania's Kibiti, Rufiji, and Kilwa districts, indicating a behavioral shift of this crucial malaria vector from primarily indoor biting to outdoor biting. These findings highlight the necessity of promptly implementing supplementary interventions to control outdoor biting malaria vectors with the goal of managing residual malaria transmission and ultimately achieving complete elimination.

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## THE ROLE OF SEROTONIN IN MOSQUITO SWARMING AND AUDITORY PERCEPTION OF MATES

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Mating is an important aspect of vector biology and is crucial for current control strategies, such as SIT and Gene Drive. It also represents a target for novel control tools. Anopheles mosquitoes mate in swarms at dusk. Within these noisy swarms, males locate females by listening for their wingbeat frequency. However, the molecular underpinning of this process is poorly understood, and greater insight could benefit vector biology and even identify new targets for vector control. We are investigating the contribution biogenic amines to the regulation of this behaviour. One such amine, serotonin, has many well documented roles, for example in human depression. However, serotonergic neurotransmission isn't unique to humans and is found throughout the animal kingdom, particularly in efferent signalling systems, where signals are carried away from the central nervous system to tune peripheral effectors. Mosquitoes have highly complex hearing and are the only documented insect with an auditory efferent system. Our research suggests that this efferent input modulates both swarming itself, and hearing within the swarm. We have found that levels of serotonin show distinct periodic cycles, peaking around dusk. By chemically altering serotonin levels, we see changes in dusk-associated activity - a central feature of mosquito swarming. Furthermore, 3/6 serotonin receptors identified in A. gambiae are highly expressed in the male ear. All three are

G-protein-coupled receptors - highly “druggable” pharmacological targets. Using CRISPR, we have disrupted these vector control targets, and begun characterising their role in audition, swarming, and ultimately reproduction. Initial results show at least two have male-mating phenotypes. We are currently investigating the basis of these phenotypes by interrogating mutant phonotactic behaviour (male attraction to female tones) as well as their biophysical and electrophysiological responses. Thus, in addition to its broad role in swarming, we are beginning to understand the molecular underpinnings of serotonergic signalling within the context of hearing and partner-detection inside the swarm.

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## A SEMI-FIELD SYSTEM TO DEFINE THE CHEMOSENSORY BASIS OF MALARIA TRANSMISSION AT HIGH DEFINITION

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Heterogeneity in mosquito biting risk is a key factor influencing malaria epidemiology. To gain insight into the chemosensory basis of mosquito attraction towards the scent of some humans versus others in a malaria endemic setting, we developed a large-scale, multi-choice system that quantifies mosquito olfactory preferences under naturalistic semi-field conditions in Zambia. Using infrared video tracking in an expansive flight cage arena, we engineered a high-content mosquito behavioral assay that tracks landing preferences of the African malaria mosquito Anopheles gambiae towards heated targets mimicking human skin temperature that are baited with whole body odor from sleeping humans or other host-related olfactory stimuli. Using this multi-choice system, we determined that An. gambiae prefers to land on heated targets baited with CO2 emissions reflective of a large human over environmental air, body odor from one human over CO2, and the scent of one human over another. When simultaneously presented with a choice between the scent of six humans, we identified individuals at both ends of the attractiveness spectrum who are consistently more or less attractive relative to other humans over replicate nightly trials. Applying integrative whole body volatilomics across these humans, we have identified a panel of airborne compounds including specific volatile carboxylic acids putatively associated with modulating human attractiveness to this prolific malaria vector. The comparative power of this multichoice preference assay may now readily be used to define the chemosensory basis of malaria transmission at high definition.

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## IS THE INVASION AND SPREAD OF THE URBAN MALARIA VECTOR ANOPHELES STEPHENSI INTO AND ACROSS AFRICA MEDIATED BY WINDBORNE MIGRATION?

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The invasion of the urban malaria vector Anopheles stephensi into Africa is a serious public-health threat and a challenge to malaria elimination. Whereas malaria in Africa has been primarily a rural problem, the recent establishment and expansion of the invasive urban Asian vector Anopheles stephensi will likely drastically increase the risk in Africa, elevating urban malaria. It is widely believed that this incursion and subsequent spread was mediated by transport on ships, airplanes, and cars. Here, we examine the geographic, genetic, and related data and propose that the invasion and spread of An. stephensi in Africa has been mediated primarily by high-altitude windborne migration. The key evidence supporting windborne invasion and spread include i) the gradual range expansion over serval decades exhibits an unmistakable diffusion process, ii) distribution that does not concentrate near major sea ports, airports, or even main highways, but is correlated with predominant winds, iii) genetic evidence of high diversity that is incompatible with a single introduction such as by a ship and similarity between populations from southern Arabia (Yemen) with those in Africa, and iv) low tolerance of An. stephensi eggs to desiccation limiting their capacity for long travel, but strong capacity of gravid Anopheles females to to be carried by wind for tens or hundreds of kilometers. To our knowledge, no An. stephensi mosquitoes were intercepted on ships or other vehicles, nor in high altitude (>100 m above ground); however, sampling should be carried out to evaluate both possible modes of transport. The possibility of windborne migrations of gravid An. stephensi should be incorporated in surveillance efforts while testing this and the transport of these mosquitoes in human vehicles in tandem. Understanding the contributions of the different mechanisms of spread is critical to mitigating the impact of An. stephensi and other invasive vectors in future.

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## VECTOR AND HOST DIVERSITY SHAPE WEST NILE VIRUS TRANSMISSION IN URBAN GREEN SPACES ALONG AN URBAN-RURAL TRANSECT

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Urban green space is associated with a wide range of societal benefits, including for conservation, human well-being, and to mitigate effects of climate change. Relatively little however is known regarding the importance of urban green spaces for West Nile virus transmission dynamics and whether this role changes as one moves further away from the urban core. To elucidate this, we set out traps at 10 sites in the Greater Chicago area that spanned a transect from the heart of the city to the rural edge. Specimens were collected weekly between June and October, identified to species, and pools of Culex pipiens, Cx. restuans, and Cx. salinarius tested for the presence of WNV. The host species from which blood meals were obtained in blood-fed females was determined through MiSeq sequencing. We monitored the relative activity of medium- and large mammals with camera traps and estimated avian abundance by performing point counts at three times during the summer at each site. Blood-meal origins for Cx. pipiens and Cx. restuans females were predominantly from avian species such as American robins and Northern cardinals and did not differ strongly between more urban and more rural sites. For Cx. salinarius, a possible bridge vector, white-tailed deer and humans were the most common hosts, but the relative frequency of blood meals taken on humans increased in the more urban locations. West Nile infection rates differed significantly among vector species and sites. To explain variation in prevalence we assessed the impact of land use and environmental parameters. The amount of impervious surface around the collection site itself was only weakly associated with WNV infection rates, but the proportion of land consisting of turf grass had a significant positive relationship. WNV prevalence was also significantly associated with the community competence index, as well as with abundance of species including American robins and house sparrows. Together, this work highlights that urban green space with certain characteristics that provide ample suitable habitat for important avian host species with repercussions for risk of WNV exposure in humans.

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## MOSQUITO GENE SURVEILLANCE (MGSURVE): A FRAMEWORK TO OPTIMIZE TRAP PLACEMENT FOR GENETIC SURVEILLANCE OF MOSQUITO POPULATIONS

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Genetic surveillance of mosquito populations is becoming increasingly relevant as genetics-based mosquito control strategies advance from laboratory to field testing. Especially applicable are mosquito gene drive projects, the potential scale of which leads monitoring to be a significant cost driver. For these projects, monitoring will be required to detect unintended spread of gene drive mosquitoes beyond field sites, and the emergence of alternative alleles, such as drive-resistant alleles or non-functional effector genes, within intervention sites. This entails the need to distribute mosquito traps efficiently such that an allele of interest is detected as quickly as possible - ideally when remediation is still possible. Additionally, insecticide-based tools such as bednets are compromised by insecticide-resistance alleles for which there is also a need to detect as quickly as possible. To this end, we present MGSurvE (Mosquito Gene SurveillancE): a computational framework that optimizes trap placement for genetic surveillance of mosquito populations such that the time to detection of an allele of interest is minimized. A key strength of MGSurvE is that it allows important biological features of mosquitoes and the landscapes they inhabit to be accounted for, namely: i) resources required by mosquitoes (e.g., food sources and aquatic breeding sites) can be explicitly distributed through a landscape, ii) movement of mosquitoes may depend on their sex, the current state of their gonotrophic cycle (if female) and resource attractiveness, and iii) traps may differ in their attractiveness profile. Example MGSurvE analyses are presented to demonstrate optimal trap placement for: i) an Aedes aegypti population in a suburban landscape in Queensland, Australia, and ii) an Anopheles gambiae population on the island of São Tomé, São Tomé and Príncipe. Further documentation and use examples are provided in the vignettes at the project's repository. MGSurvE is freely available as an open-source Python package on pypi. It is intended as a resource for both field and computational researchers interested in mosquito gene surveillance.

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## DESIGN AND PRELIMINARY FIELD VALIDATION OF A HANDHELD TOOL FOR AUTOMATED MORPHOLOGICAL IDENTIFICATION OF MOSQUITO SPECIES, SEX, AND ABDOMINAL STATUS BY VILLAGE HEALTH TEAMS IN UGANDA, FOR COMMUNITY-BASED VECTOR SURVEILLANCE

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Vector surveillance, a critical pillar of malaria control and elimination strategy, is limited by the global lack of trained medical entomologists and Vector Control Officers (VCOs), especially in low- and middle-income countries. We present the design of VectorCam, a novel Artificial Intelligence-enabled handheld field tool that automatically identifies the species, sex, and abdomen status of wild-caught mosquitos. The system consists of a low-cost smartphone running an Android application attached to low-cost hardware that can uniformly magnify and illuminate at a set distance. The system can effectively task-shift vector surveillance to Village Health Teams (VHTs), allowing them to rapidly image a batch of specimens and pack them into individual Eppendorf tubes with unique identifiers for quality assurance. This tool was designed with the iterative feedback of over 60 VHTs and 10 VCOs in Uganda through multiple formative usability studies that finalized the design and performance parameters. The core algorithm uses a Convolutional Neural Network architecture run on smartphones without internet connectivity. The classification accuracy against molecular identification using wild-caught mosquito specimens was as follows: Anopheles funestus s.l. (96%), An. gambiae s.l. (94%), other Anophelines (91%), Culex sp. (97%), and other genera (97%), with an overall F-1 macro score of 95 ±3%. A recent addition to the algorithm included An. stephensi, an invasive species of concern in Africa. With colony-bred specimens of An. stephensi, we achieved a classification accuracy of 98%. Accuracy for sex and abdominal status against morphological labeling by VCOs presented as 95±1%, and 86±4%, respectively. VHTs were placed in pairs of imaging and loading roles to evaluate their effectiveness in using VectorCam. They were trained by VCOs for an average of four hours, resulting in an average imaging speed of 1:11 ± 0:38 minutes per mosquito. VectorCam is a novel system that could help task-shift a major expertise bottleneck in Africa and Asia, allowing for a community-based approach towards vector surveillance.

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## ELUCIDATING THE INTERACTIONS OF PFCRT AND PLASMEPSINS 2/3 IN MODULATING FITNESS AND RESISTANCE IN PLASMODIUM FALCIPARUM TO PIPERAQUINE AND OTHER ARTEMISININ PARTNER DRUGS

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Failure of dihydroartemisinin and piperaquine (DHA+PPQ) combination therapy (ACT) in Southeast Asia has been linked to the Plasmodium falciparum KEL1/PLA1 co-lineage, which harbors the DHA resistant Kelch13 (k13) C580Y mutation and PPQ-resistant marker multicopy plasmepsins 2 and 3 (pm2/3). PPQ resistance-associated novel mutations in the P. falciparum chloroquine resistance transporter (pfcrt) including F145I or M343L, have also emerged on KEL1/PLA1 parasites expressing the Southeast Asian Dd2 pfcrt allele. However, it is unclear whether multicopy pm2/3 or mutant pfcrt is the major determinant of PPQ resistance in clinical isolates, and how they impact the efficacy of other ACT partner drugs. To examine the contributions of these PPQ-resistant markers, we generated an isogenic panel of CRISPR/Cas9 pfcrt-edited parasites harboring 1 to 3 copies of pm2/3 from progeny derived from the genetic cross of NF54 and RF7, a KEL1/PLA1 parasite. PPQ dose assays revealed that pfcrt is epistatic to pm2/3 in conferring high-level PPQ resistance, and both pfcrt M343L and pm2/3 amplification were necessary to produce a biphasic response at >1 μM PPQ. In addition, we identified Dd2 pfcrt and multicopy pm2/3 as key determinants of resistance to MMV675939, a preclinical compound. In contrast, the African 3D7 pfcrt allele led to a ~3-fold increased sensitivity to ACT drugs, lumefantrine and mefloquine, when compared to the Southeast Asian Dd2+M343L isoform. We also performed long-term competitive fitness assays between isogenic RF7 clones harboring 1 or 3 copies of pm2/3 to investigate fitness costs. Our results revealed that multicopy pm2/3 led to a moderate parasite fitness defect in the absence of drug pressure. Overall, our findings suggest that PPQ selects for pm2/3 amplification in mutant pfcrt parasites, and the poor fitness associated with multicopy pm2/3 may explain the loss of pm2/3 amplification in clinical isolates upon the switch from DHA+PPQ to artesunate+mefloquine. Furthermore, we demonstrate higher sensitivity of PPQ-resistant pfcrt alleles to lumefantrine and mefloquine, thus reinforcing their appeal as effective ACT drugs in Southeast Asia.

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## UNDERSTANDING LEAD DISCOVERY ANTIMALARIAL DRUGS RESISTANCE TRANSLATION FROM LAB TO FIELD PARASITES TOWARD SUSTAINABLE MALARIA ELIMINATION

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Not anticipated chloroquine resistance has had serious consequences for public health worldwide. It has subsequently led to the use of artemisinin-based combination therapies (ACTs) as first-line treatments for Plasmodium falciparum malaria. The aim of drug combinations was to anticipate appearance and spread of artemisinin resistant parasite on the field by protecting short-life artemisinin with long lasting existing antimalarial drug. Thus,for chloroquine, atovaquone, pyrimethamine and the current frontline artemisinin, major investigations have not been made at early discovery stage to identify which gene, mutations or conditions will cause drug resistance and can translate into field parasites. It has taken years to figure out the molecular determinant driving the resistance until complete loss of the drug.The discovery process of new alternative molecules to anticipate loss of ACTs should subsequently investigative their drug resistance markers that can translate from lab to field parasites and select partner drug accordingly for combination to ensure long lasting use of new antimalarial drug after their approval and field deployment. In a proof of concept study, we demonstrated that some key mutations found in lab parasites translate into field parasite conferring field parasites drug resistance Merck M5717 antimalarial drug candidate. With the great vision to ensure efficient long-term use M5717, we applied mathematical modelling to isobologram data to show that Pyronaridine is a viable partner for M5717. We extended this study to one of the most advanced lead discovery antimalarial, Novartis KAF156 which is currently in phase IIb clinical trial entering to phase III. We report that when exposed to GNF179 (Imidazolopiperazine: IPZ), close analogue of KAF156, recrudescent field parasites were detected which is being investigated for resistance purpose. This approach allows us to study the presence of resistant genes in field strains and predict resistance to antimalarial drugs, in order to anticipate therapeutic combinations before the new molecules are deployed.

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## CONFIRMED ARTEMISININ PARTIAL RESISTANCE AND HIGH EFFICACY OF ARTEMETHER - LUMEFANTRINE AND ARTESUNATE - AMODIAQUINE FOR THE TREATMENT OF UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA IN NORTH-WESTERN TANZANIA

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This study was conducted from April to September 2022 to assess efficacy and safety of artemether-lumefantrine (AL) and artesunate-amodiaquine (ASAQ) for the treatment of uncomplicated falciparum malaria Tanzania due to reports of artemisinin partial resistance in Rwanda and Uganda. It was a single-arm prospective study that recruited 176 patients aged 6 months to 10 years who were treated with AL and ASAQ. Capillary sequencing was used to determine the prevalence of single nucleotide polymorphisms in Pf kelch 13 and multi-drug resistance 1 (mdr1) genes PCR corrected cure rates, safety of the two drugs, parasite clearance time and molecular makers of resistance in k-13 and mdr1 genes were assessed. PCR corrected adequate clinical and parasitological response (ACPR) for AL and ASAQ was 96.6% for AL and 100% for ASAQ. Among patients treated with AL and ASAQ; 11/88 (12.5%) and 17/88(19.3%) had parasitaemia beyond 72 hours, respectively. The slope half-life was <3.9hrs in both groups but it was significantly higher (>6.5hrs) in patients with day 3 parasitaemia and/or k-13 mutations at enrolment. In both groups, ≥23% of the patients had 561H mutations at enrolment. The day 3 patients with parasitaemia, 9.1% in AL and 11.4% in ASAQ had 561H mutations. The Mutations in k-13 gene were significantly associated with day 3 parasitaemia but they were not associated with recrudescent or recurrent infections in AL. Common adverse events reported were cough, runny nose, abdominal pain and fever; and not related to the study drugs. Confirmed artemisinin partial resistance, high cure rates and adequate safety were observed with 96.6% ACPR for AL and 100% for ASAQ. Containment strategies are urgently needed to prevent mutated parasites from spreading to other parts of the region and the entire country. 1

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## A RAPID DECLINING OF MULTIDRUG RESISTANT KEL1/PLA1 PLASMODIUM FALCIPARUM PARASITE IN VIETNAM DURING 2020-2022, A RESULT OF DRUG POLICY CHANGE

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Genetic surveillance has been proved as a useful tool to monitor drug resistance and provided support evidences for the National Malaria Control Programs in decision making including drug policy change. In collaboration with the Vietnam NMCPs, genetic surveillance has been conducted in twelve malaria endemic provinces located in Central Highland and Southern of Vietnam since 2017. Our continuous results from six years surveillance have timely informed and alarmed the NMCPs about the efficacy of current first-line treatment and joined supported the change of National Treatment Guideline in 2020 in areas confirmed multi drug-resistant malaria (including Binh Phuoc, Dak Nong, Dak Lak, Gia Lai, Phu Yen provinces). Over 400 Plasmodium falciparum samples have been collected in those provinces during 2020-2022. The parasites have been genotyped follow transfered Amplicon Sequencing procedure from Welcome Sanger Institute and GenRe Mekong project. Our preliminary results show a declining of multidrug resistance KEL1/PLA1 P. falciparum parasites after national drug policy changed. The P. falciparum parasites carried C580Y Kelch13 mutation without associated piperaquine and mefloquine resistance (evidenced by amplification in the plasmepsin2/3 and pfmdr1 genes), has been gradually predominant in those malaria provinces. This indicated that the reversal back to a single plasmepsin2/3 genotype was the result of the change in first line therapy away from dihydroartemisinin-piperaquine in Vietnam.

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## ASSOCIATIONS BETWEEN SULFADOXINE-PYRIMETHAMINE+AMODIAQUINE CONCENTRATIONS, MALARIA INCIDENCE, AND RESISTANCE MARKERS IN CHILDREN RECEIVING SEASONAL MALARIA CHEMOPREVENTION IN BURKINA FASO

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Seasonal malaria chemoprevention (SMC) with sulfadoxine-pyrimethamine+amodiaquine (SP-AQ) is given monthly during the transmission season to prevent childhood malaria in the sub-Sahel region of Africa. Optimal SP-AQ concentrations to confer protection against malaria and prevent selection of SP-AQ resistance are unknown. We conducted a prospective cohort study of children in Sourkoudougou, Burkina Faso 3-59 months old and eligible to receive SMC. 89% of the children were enrolled prior to the first SMC cycle, and all were followed longitudinally through the malaria transmission season (July to November, 2022). SMC was administered in four cycles: July 9-12, August 7-10, September 5-8, and October 4-7. Plasma levels of sulfadoxine, pyrimethamine, amodiaquine and its metabolite desethylamodiaquine were collected on days 7 and 28 following SMC administration and additionally on days 3 and 14 in a sub-cohort of 57 children for whom all three doses of SMC were directly observed. Incidence of malaria infection was assessed during routine and unscheduled clinic visits by blood smear. Dried blood samples were collected for assessment of genotypes. Among the 178 children enrolled, 103 incident malaria infections were recorded (incidence rate = 1.82 episodes per person-years). Across SMC cycles, malaria infection incidence was highest at four weeks after SMC administration (just before the next SMC cycle). Mean parasite prevalence was 5.9% [range: 4.0%-8.3%] on day 7 and 8.1% [range: 4.8%-10.8%] on day 28 after treatment. Compared to the full cohort, parasite prevalence was significantly lower among participants of the sub-cohort (whose SMC regimens were directly observed) on day 7 (1.9% [range: 0%-3.9%]; p=0.001), but not on day 28 (5.8% [range: 0%-9.1%]; p=0.09). Assessments of SP-AQ drug levels and drug resistance genotypes are ongoing. With these data we will perform population pharmacokinetic/pharmacodynamic modelling to characterize associations between drug exposure, malaria infection incidence, and drug resistance selection.

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## POTENTIAL SUITABILITY OF SULFADOXINE-PYRIMETHAMINE PLUS AMODIAQUINE FOR SEASONAL MALARIA CHEMOPREVENTION IN AREAS OF HIGH, PRE-EXISTING DRUG RESISTANCE

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Seasonal malaria chemoprevention (SMC) targets the burden of malaria in children under five in areas of seasonal malaria transmission. Previously, the WHO recommended SMC only in the Sahel region which has low levels of sulfadoxine-pyrimethamine (SP) drug resistance, one of the drugs used for the intervention. However, in 2022 geographic restrictions were removed from WHO guidelines prompting new countries to consider SMC as a possible intervention. There is a need to understand whether SP-AQ would still be effective in areas with high SP resistance to guide the scale-up of SMC to new geographies. Here, we utilize data from the first randomized controlled trials of SMC outside of the Sahel region to estimate the protection provided by SMC which demonstrated a day 28 protective efficacy of 77%, even in areas with established high-level SP resistance. We use Bayesian inference methods to estimate the probability that SMC using SP-AQ would prevent an infection given time since drug administration, seasonality, baseline transmission and existing frequency of SP-resistance conferring mutations. We are currently using these results within an existing, extensively validated individual-based Plasmodium falciparum transmission model to estimate the potential impact of implementing SMC under a variety of scenarios, including exploring the number of cycles, their timing, and the suitable age range. Initial results suggest that in an area with 64.7% of clinical cases in 4 months and established dhfr-dhps quintuple mutation, SMC using four cycles of SP-AQ could prevent 51.1% (95% CI: 37.0 - 65.2%) of annual clinical P. falciparum cases in children under five years. Despite the high drug resistance already present in east and southern Africa, we predict that SMC has the potential to be a highly effective malaria intervention and could help avert some of the substantial malaria burden in young children in these geographies. It will be important to consider the impact of scaling up SMC on driving resistance to SP and AQ in areas where SP resistance is already relatively high.

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## WUCHERERIA BANCROFTI MICROFILARIAE POSITIVE INDIVIDUALS SHOW AN INCREASED HUMAN IMMUNODEFICIENCY VIRUS INCIDENCE IN A GENERAL POPULATION STUDY IN SOUTHWEST TANZANIA

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As part of a large prospective general population study, our group had described a 2.3-fold increase in HIV incidence among individuals with Wuchereria bancrofti infection, as measured by the circulating filarial antigen of the adult worm. However, during the first study no night-blood was collected, which is essential for measuring of microfilariae (MF) of W. bancrofti was taken, because the focus of this activity was on soil-transmitted helminths and malaria. A new study presented here aimed to retrospectively determine the microfilariae status of the participants to reveal whether the increased HIV susceptibility previously described was associated with patent (MF-positive) or latent (MF-negative) filarial infection. Circulating filarial antigen(CFA)-positive biobanked human blood samples (n = 350) were analysed for W. bancrofti MF chitinase by real time PCR. The PCR provided a positive signal in 12/350 (3.4%) samples. During the four-years of follow-up period (1109 person years (PY)), 22 study participants acquired HIV infection. Three new HIV infections occurred in 39 PY of W. bancrofti MF chitinase positive individuals (7.8 cases per 100 PY), in contrast to 19 seroconversions in 1070 PY of W. bancrofti MF chitinase negative individuals (1.8 cases per 100 PY, p = 0.014). Immunomodulation induced by W. bancrofti appears to be more pronounced in microfilariae-positive individuals, as the HIV incidence observed in this subgroup

exceeded the moderately increased HIV risk previously described in all individuals with W. bancrofti (regardless of MF status) compared with uninfected persons from the same area.

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## DEVELOPING THE NATURAL PRODUCT CORALLOPYRONIN A TO TREAT FILARIASIS, STIS AND STAPHYLOCOCCI

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Corallopyronin A (CorA) inhibits bacterial DNA-dependent RNA polymerase at a different binding site to rifampicin. Thus, it kills rifampicin-resistant Staphylococcus aureus. CorA also kills Gram-negative Wolbachia endobacteria of filarial nematodes that cause onchocerciasis (river blindness) and lymphatic filariasis (elephantiasis). Depleting the essential endosymbionts causes worm sterility and slow adult worm killing. We demonstrated CorA activity against Neisseria gonorrhoeae and multi-resistant S. aureus. At 4x MIC no CorA resistant N. gonorrhoeae could be selected (predicted frequency of mutation of ≤10-10). CorA also has activity against established S. aureus biofilms and prevent their formation. With its excellent biodistribution into bone, we have received funding to investigate CorA as a new antibiotic class to treat osteomyelitis and S. aureus biofilms. To develop CorA as a novel solution to several targets of the WHO Priority Pathogen List for which new antibiotics are needed, we conducted standard non-GLP ADMET studies. In-vitro toxicity tests (off-target, AMES, micronucleus, hERG, phototoxicity) demonstrated that it is non-toxic and pharmacologically safe; in vivo toxicity studies in rats and dogs measured a maximal tolerated dose (MTD) in both species of 1000 mg/kg. Seven-day repeated dose studies in rats and dogs demonstrated no prohibitive safety issues: predicted NOAEL=150 mg/kg/d; predicted HED=4 mg/kg. CorA drug substance is heterologously produced in genetically modified Myxococcus xanthus. Up-scale to industrial scale (15m3) was achieved in 2022 at Bio Base Europe Pilot Plant (Belgium). The Helmholtz Centre for Infection Research purified this large amount of material, achieving 90-95% pure HQ-RGM material. With amorphous solid dispersion formulation principles, two solid oral formulations were developed that increased stability (>3 months at 30 °C, >6 months at 5 °C) and oral bioavailability (mouse >59%, dog>53%) compared to neat CorA. We are establishing drug product production at GMP facilities. After finalization of the pre-clinical work, we plan to enter the clinical phase I in 2025/2026.

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## ANIMALS AS RESERVOIR OF BRUGIA MALAYI IN BELITUNG DISTRICT, INDONESIA, AS A POTENTIAL THREAD FOR THE ELIMINATION OF LYMPHATIC FILARIASIS IN HUMANS

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In Indonesia, Brugia malayi is the most common filarial parasite in Sumatra, Kalimantan, Sulawesi, and adjacent islands. B. malayi occurs in two ecotypes; the zoophilic type that infects both humans and animals, and the anthropophilic type that is only found in humans. Belitung district was known to be endemic for zoophilic B. malayi but received 5 rounds of mass drug administration (MDA) with DEC and albendazole between 2006 and 2010 at high coverage, passed 3 transmission assessment surveys (TAS) and was assumed to be free of infection in 2017. In 2021 a surveillance survey in 7 villages found that 5 villages were endemic for B. malayi with an average microfilaria (Mf) rate of 2.1% (40/1910 adults). Mf positive subjects were treated using ivermectin, DEC and albendazole, and a more extensive survey in the entire district was performed in 2022 that found an average Mf rate of 1.3% (87/6898 participants). There are several reasons that could explain the presence of B. malayi despite of MDA and passed TAS. For example, TAS is based on schoolchildren, and may not detect ongoing transmission in adults or an animal reservoir could be responsible for reintroduction of parasites into the human population. To test the later hypothesis, we collected blood from 291 cats and 41 dogs in villages where Mf positive humans were found. Microscopic examination found B. malayi Mf in 3 dogs and 4 cats, most of them from a single village. Dirofilaria immitis was found in 13 dogs while Brugia pahangi was found in 11 cats and 3 dogs. Screening of monkeys is still ongoing, but Mf have been found in 6 of 28 macaques. Molecular species confirmation by qPCR and whole genome sequencing of B. malayi Mf for population genomics is currently underway. Results showed that in Belitung B. malayi is present in animals and could be the source for its reintroduction into the human population. Further studies have to demonstrate gene flow between the B. malayi populations in animals and humans to confirm the role of an animal reservoir. For the elimination of lymphatic filariasis, B. malayi areas with an animal reservoir may need to be declared as special zones with intensified intervention and surveillance.

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## SPATIAL ANALYSIS OF THE RELATIONSHIP OF ONCHOCERCA VOLVULUS EXPOSURE BETWEEN HUMANS AND BLACK FLIES IN ETHIOPIA

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Onchocerciasis is a parasitic disease caused by Onchocerca volvulus that spreads through the bite of infectious Simulium flies, which breed in rapidly flowing rivers. Ethiopia has one of the highest burdens of onchocerciasis worldwide. Two key indicators of impact are Ov16 antibody prevalence in children, detected in dried blood spots (DBS) by ELISA, and the presence of infectious O. volvulus larvae in flies, detected through O-150 PCR. However, the quantitative relationship between the measures and their degree of spatial dependence is unclear. We explored associations between O-150-positive pools of flies and the prevalence of human antibodies to Ov16 in temporally and spatially proximal samples. The relationship was explored among villages in 8 zones in southern Ethiopia where S. damnosum sl is the principal vector. Data include DBS from 907 villages with data from 2022, and flies from 22 sites collected from 2021-2022. The study areas had received 4-20 years of ivermectin treatment by then. Clustering of residuals from an intercept-only log-binomial model of DBS locations was assessed using Moran's I and global colocation quotient, and the impact on spatial autocorrelation was measured after adding a covariate for O-150 fly pool positivity at the nearest breeding site within a threshold distance. Threshold distances were tested at 64km, 33km, 20km, and 10km according to the literature and common practice in the program. Results suggested that the relationship between nearby fly site positivity and village parasite exposure may be significant if the fly site is within 33km, with the association growing stronger as the threshold distance decreases. However, estimate precision became weaker as the threshold lowered due to data sparsity. Similarly, examination of residual spatial autocorrelation showed that incorporating fly site positivity to the model began to lower residual clustering at the 33km threshold. These findings will direct the collection and testing of flies to better elucidate the relationship between parasite prevalence in flies and humans across diverse geographies, informing cost-effective surveillance.

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## CHALLENGES OF APPLICATION OF THE WHO ONCHOCERCIASIS TECHNICAL ADVISORY SUBGROUP-PROPOSED THRESHOLD FOR INITIATING MASS DRUG ADMINISTRATION AGAINST ONCHOCERCIASIS IN ETHIOPIA

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Mapping is a prerequisite for determining onchocerciasis endemicity and the need for interventions to eliminate transmission. Ethiopia has made nationwide mapping part of its elimination strategy. National guidelines were developed in 2015, in which a woreda (district), the implementation unit in Ethiopia, was considered endemic if the mean Ov16 antibody prevalence in 300 adults (100 from each of three villages) was ≥2%. In 2017, the Onchocerciasis Technical Advisory Subgroup (OTS) of the World Health Organization proposed sampling 500 adults in 5 communities and commencing mass drug administration (MDA) in an implementation unit if Ov16 prevalence in at least one community was ≥2%. Ethiopia embarked on mapping the 671 previously untreated districts between 2015 and 2019 using a combination of entomological and Ov16-serology studies, prioritizing areas adjacent to those already under treatment. Desk review was used first to exclude areas ecologically unsuitable for supporting vector flies and thus O. volvulus transmission. Next, entomologists prospected river systems during the rainy season to confirm these exclusions and to identify a minimum of three first-line communities near suitable vector breeding sites. Investigators excluded 181 districts based on unsuitable ecological conditions for vector breeding; dried blood spot (DBS) sampling was performed in the remaining 490 districts. To date, ELISA analysis for Ov16 has been analyzed for 122,405 DBS from 427 districts. Of these, 53 districts (12.4%) are considered endemic and in need of MDA by Ethiopia's guidelines. An additional 102 districts (23.9%), encompassing 15 million people, would be considered endemic by OTS guidelines. This discrepancy has dramatic resource (cost and labor) implications. We recommend further investigation to determine if OTS thresholds reliably identify district-wide transmission.

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## MONITORING IMPACT OF THREE ROUNDS OF MASS DRUG ADMINISTRATION IN EIGHT HIGH-RISK VILLAGES USING A THREE-DRUG REGIMEN ON LYMPHATIC FILARIASIS IN AMERICAN SAMOA

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In 2018, 2019 and 2021, the American Samoa Department of Health (DOH) conducted mass drug administration (MDA) for lymphatic filariasis (LF) with ivermectin, diethylcarbamazine and albendazole (IDA) and conducted impact surveys after each MDA. COVID-19 prevented MDA in 2020. Post-MDA 1, circulating filarial antigen (CFA) and microfilariae (Mf) prevalence in 8 high-risk villages (defined as having ≥3 CFA positive and/or ≥1 Mf positive people in 2019) were 4.3% (95% confidence interval (CI) 2.8-6.4) and 1.3% (95% CI 0.6-2.6), respectively. In 2020, CFA prevalence was 2.0% (95% CI 1.2-3.0) and Mf was 1.1% (95% CI 0.5-2.1). MDA coverage was 78.3% (MDA 1) and 90.1% (MDA 2). In Sep-Oct 2022, DOH sampled 550 people (≥5 years) in the 8 high-risk villages using a sampling strategy based on history of LF and village size. We asked about participation in all 3 MDAs and fingerstick blood samples were screened for CFA; CFA positive persons were tested for Mf and offered IDA. Mf positive persons were examined 7 days after treatment for Mf clearance. We weighted analyses based on the proportion of sampled data over village population. Mean CFA and Mf prevalence in 2022 were 2.2% (95% CI 0.7-3.6) and 0.1% (95% CI 0.0-0.1), respectively. Of participants, 83.4% (95% CI 69.2-97.5) reported taking the medicines in MDA 3, and 76.2% (95% CI 61.6-90.7) participated in all 3 MDAs. Of the 48 CFA positive persons, 64.0% (95% CI 0.1-1.9) were male and most were aged 20-39 years (49.4%; 95% CI 0.1-3.8); 90.1% (95% CI 0.1-1.3) reported swallowing pills in MDA 3. Eight (9.9%) reported never participating in MDA; 7/8 were male (mean age 52.9 years). The 2 Mf positive persons were both males aged 59 years, neither reporting ever taking the medicines. DOH followed up and treated 46/48 CFA positive persons, including the 8 never treated. High MDA coverage (≥65%) and a low proportion of never treated led to a decline in Mf prevalence in the defined villages. Despite Mf prevalence meeting the stop MDA target (<0.5%), high CFA prevalence, vector efficiency and frequent movement between American Samoa and LF-endemic Samoa, supports the need for another MDA, including social mobilization targeting men.

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## RATE OF ONCHOCERCA VOLVULUS MICROFILARIAE IN NODULE CARRIERS IN VILLAGES UNDER MASS DRUG ADMINISTRATION IN FUAMAH DISTRICT, LIBERIA

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Onchocerciasis or riverblindness is endemic in most parts of Liberia. In 2021 it was estimated that 3.24 million people required mass drug administration (MDA) with ivermectin to control and eliminate onchocerciasis, and it was reported that 2.3 million (71%) received treatment. Although the entire country is covered by MDA, the coverage rates vary considerably by area/clan. Fuamah district (Bong County) has a population of 56,000 belonging to 5 clans living along the St. Paul river. Before the civil war, the district was a successful mining community, but now the infrastructure has degraded, the population consists mostly of retired mining workers, and subsidence farmers and their families and villages are difficult to reach. Although the district receives annual MDA through the Neglected Tropical Disease Department of the Ministry of Health, onchocerciasis is still highly endemic with Onchocerca volvulus nodule carrier rates in adults of up to 40%. Simulium vector densities are high, but the prevalence of microfilariae (Mf) in the human reservoir is unknown. In order to estimate the prevalence of O. volvulus Mf in the human population and to determine whether the area is suitable for clinical trials to improve the treatment for onchocerciasis, we performed a survey in the entire district. We examined 888 adults (25% women) with palpable nodules by skin snip (2 snips from the iliac crest and 2 snips from the calf) 12 months after the last MDA. The prevalence of Mf in nodule carriers varied between clans from 4.9% to 49.4%. The sensitivity to detect Mf in snips taken from the iliac crest and the calf was similar (94.5%, mean 10 Mf/mg vs 94.3%, 9 Mf/mg, respectively). A total of 353 subjects with palpable nodules and a Mf density ≥3 Mf/mg suitable for a clinical trial were identified. Subjects were pretreated with ivermectin to clear Mf from the eye and to enable future trials with regimens that contain the drug DEC. These results show that despite widespread MDA, subjects with O. volvulus nodules and microfiladermia are still prevalent in Fuamah and clinical trials can be performed in the same communities that will later benefit from new treatment strategies.

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## WHOLE-GENOME SCAN OF AFRICAN SNAIL VECTORS IDENTIFIES GENES ASSOCIATED WITH RESISTANCE TO INFECTION BY SCHISTOSOMES

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Schistosomiasis is a chronic inflammatory disease afflicting hundreds of millions of people worldwide. Schistosomes are helminths transmitted by aquatic snails, and the disease can potentially be controlled by blocking transmission at the snail stage. Most cases of intestinal schistosomiasis occur in sub-Saharan Africa, where they are caused by Schistosoma mansoni, transmitted by the snail Biomphalaria sudanica and related species. In contrast to the better-studied neotropical vector B. glabrata, there has been little genomic work on these African snails and the genetic basis of snail-parasite interaction is completely unknown. Identifying snail genes that convey resistance to infection may facilitate ways to leverage these immune mechanisms and disrupt the parasite's life cycle. To uncover such immunogenetic pathways, we have generated an annotated genome assembly of B. sudanica and used it to support a genome-wide association study. We exposed F1 offspring of wild-caught Kenyan B. sudanica, originating from Lake Victoria, to S. mansoni and recorded infection status through cercarial shedding. Pools of infected (N = 493) and uninfected (N = 295) snails were then sequenced across the whole genome at a mean per-individual coverage of over 1x. We have identified several loci associated with infection status, including genomic regions known to influence parasite resistance in B. glabrata as well as previously uncharacterized genes. These results provide a first glimpse into genes of the innate immune system of the major vector B. sudanica and will help inform schistosomiasis control strategies aimed at predicting or manipulating the vector competence of the snail host, particularly in the African communities most severely affected by this disease.

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## GENOMIC EPIDEMIOLOGY OF THE CARCINOGENIC LIVER FLUKE OPISTHORCHIS VIVERRINI

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Transmission of the carcinogenic trematode Opisthorchis viverrini is ongoing in Southeast Asia despite decades of control efforts. The resulting bile duct cancer (cholangiocarcinoma) is one of the leading causes of death in regions of Thailand, Lao PDR and Cambodia. Developing evidence-based programs for control require a mechanistic understanding of parasite transmission, however there are several unresolved epidemiological questions including the extent of parasite migration between neighboring countries and whether animals, primarily domestic cats, act as reservoir hosts. To address these knowledge gaps and to stimulate further research into the molecular epidemiology of O. viverrini, we present an improved reference genome which incorporates long read sequencing to give chromosome-level resolution. We then developed methodologies to whole-genome sequence adult worm and egg stages of flukes isolated from infected individuals to understand the pattern of genetic variation in natural parasite populations. Finally we sequenced O. viverrini from Thailand and Lao PDR and compared sequence data between human and animal infective parasites to determine the relatedness between populations and the extent of zoonotic transmission. Our study provides an unprecedented understanding of the molecular epidemiology underlying the transmission of a medically significant, yet highly neglected, human parasite.

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## TEST-TREAT-TRACK-TEST-TREAT (5T) APPROACH FOR BREAKING SCHISTOSOMIASIS TRANSMISSION

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The World Health Organization has set the goal to eliminate schistosomiasis as a public health problem worldwide by 2030. Pemba Island, Tanzania, achieved this goal in 2017 and is now aiming for interruption of transmission. In most parts of Pemba, the Schistosoma haematobium prevalence is below 3% and mass drug administration of praziquantel no longer seems justified. As an alternative, we investigate a test-treat-track-test-treat (5T) approach to prevent recrudescence and accelerate elimination. In the 4-year SchistoBreak project implemented in two districts in the North of Pemba, schoolchildren are screened for microhematuria as a proxy for S. haematobium infection at the point-of-care. Positive children are treated with praziquantel and tracked to their homes and the waterbodies they use. Test-and-treat is offered to household members and people present at waterbodies. Additionally, urine samples are examined for S. haematobium eggs by urine filtration. Annual cross-sectional school- and household-surveys are conducted to monitor the prevalence and impact of the intervention. In 2021, 5.5% (239/4347) of the children screened in schools were microhematuria-positive and tracked to 199 households. Among their household members, 21.3% (60/282) were microhematuria-positive and 11.0% (31/282) egg-positive. At 77 waterbodies, 22.4% (30/134) of individuals tested microhematuria-positive and 4.5% (6/134) were egg-positive. The cross-sectional surveys in schools revealed a microhematuria prevalence of 2.4% (47/1933) in 2021 and of 6.3% (111/1767) in 2022. In households, the microhematuria prevalence was 5.6% (162/2972) in 2021 and 13.2% (387/2921) in 2022; the S. haematobium prevalence was 0.5% (14/2971) in 2021 and 0.6% (19/2929) in 2022. Our results show that 5T is an excellent approach to identify and treat individuals with urogenital schistosomiasis in low-prevalence areas. While microhematuria levels increased from 2021 to 2022, the S. haematobium prevalence remained stable. Future study years will confirm if 5T is indeed a suitable intervention to maintain current gains or to accelerate interruption of transmission.

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## CHARACTERIZATION AND PROCESS DEVELOPMENT OF SERINE PROTEASE INHIBITOR: A NEXT GENERATION TRANSMISSION-BLOCKING VETERINARY MRNA VACCINE FOR ASIATIC SCHISTOSOMIASIS

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Asiatic schistosomiasis caused by Schistosoma japonicum is a neglected tropical disease resulting in significant morbidity to both humans and animals - particularly bovines - in endemic areas. Infection with this parasite leads to less healthy herds, causing problems in communities which rely on bovines for farming, milk and meat production. Additionally, excretion of parasite eggs in feces perpetuates the life cycle and can

lead to human infection. We endeavored to develop an inexpensive and effective mRNA vaccine based on secretory serine protease inhibitor (serpin) from S. japonicum (Sj-B6). In pathogens, serpins are believed to have evolved specifically to limit host immune responses by interfering with the host immune-stimulatory signals. Transcriptional profiling and proteomics demonstrated that Sj-B6 is expressed in the intra-mammalian life cycle stages but particularly in the eggs, suggesting a possible role in disease transmission. Recombinant Sj-B6 inhibited host pancreatic elastase in a dose-dependent manner and was strongly recognized by experimentally infected rat (naturally-resistant hosts) sera when compared to chronically-infected mouse counterparts, indicating that rSj-B6 is not only highly immunogenic, but critically involved in disease resistance. This study presents a comprehensive functional characterization of Sj-B6 supporting its further development as a vaccine candidate against Asiatic schistosomiasis. A pilot study evaluating the efficacy of a novel Sj-B6 mRNA vaccine using a proprietary technology (HDT-301) invented by our partners at HDT Bio is now underway. The HDT-301 platform consists of a self-replicating RNA (repRNA) adsorbed and stabilized on a Lipid InOrganic Nanoparticle (LION™) carrier. The repRNA/ LION™ vaccine stabilizes the RNA in vivo allowing it to persist longer. A successful veterinary vaccine would play a major role in reducing pathogen transmission to humans by interrupting the parasite life cycle and improving quality of life for people living in endemic countries.

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## SCHISTOSOMA JAPONICUM CHALLENGE INFECTION MODEL IN CARABAOS (PHILIPPINE WATER BUFFALO) FOR THE PLACEBO-CONTROLLED TRIAL OF THE SJ97 AND SJ68 VACCINE CANDIDATES

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Schistosomiasis japonica is a zoonosis that persists as a public health problem in Asia particularly the Philippines, where hotspots remain despite decades of mass drug administration. Novel interventions such as vaccines are warranted to control the infection sustainably. Schistosoma japonicum infects over 40 mammals, with water buffalos as major animal reservoirs. We reported the development of an S. japonicum challenge model in carabao (native Philippine water buffalo) to evaluate vaccine candidates. Locally collected snails from endemic areas in Leyte were initially used as the source of cercaria. However, the perfused worm count is much lower than in our previous vaccine-challenge experiments in China, with a mean patency of 2.6%. The carabaos require a four-fold higher infectious dose, necessitating laboratory-reared snails. Weekly trickle infections in juvenile carabaos produced the highest yields upon sacrifice and worm enumeration by perfusion. To demonstrate the utility of the carabao challenge model, we conducted a placebo-controlled trial of the Sj97 and Sj97+Sj68 vaccine adjuvanted in ISA206. Carabaos (N=8 per group) were vaccinated with 3 doses of 500 ug Sj97, Sj97+Sj68, or saline placebo, adjuvanted with ISA206, and administered subcutaneously every 4 weeks. A half-dose booster was given 3 months after the last dose due to pandemic-associated delays. Vaccination induced robust antigen-specific IgG1, IgG2, total IgG responses, and Sj97-specific IFN gamma. Trickle infection with 2,000 S. japonicum cercaria yielded a mean of 251.8 in the adjuvant ISA206-only group upon perfusion 8 weeks post-infection. Carabaos in the Sj97+Sj68 vaccinated group had lower worm burdens than the placebo. Due to the significant variance in worm counts, the 40-70% protection by vaccination didn't reach statistical significance (Mann-Whitney test, p=0.6 for rSj97-rSj68 vs. ISA206; p= 0.5 for rSj97 vs. ISA206). We expect to report on the larger placebo-controlled Sj97+Sj68 vaccine trial with N=20 carabaos per group in the near future.

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## TWO KEY ACTINOBACTERIA GENERA BIFIDOBACTERIUM AND COLLINSELLA IN THE HUMAN GUT MICROBIOTA ARE DIFFERENTIALLY ASSOCIATED WITH SCHISTOSOMA MANSONI INFECTION BURDEN

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Among the soil-transmitted helminths schistosomiasis is the least amenable to control due to the ease of reinfection and the difficulty of administration of praziquantel en masse, due to its side effects. The overall goal of the study was to identify the key microbial taxa associated with human gut dysbiosis during Schistosoma mansoni infection and to obtain orthologs information that could form the basis for an alternate schistosomiasis control development. Twenty schistosomiasis positives stool samples and an equal number of age-sex matched negatives from a -endemic rural community, Nyive, in Ghana were studied. The Kato-Katz method was used and the infection intensity scored as egg count per gram (e.p.g.) and positive stool samples further stratified as surrogates of chronic (<400 e.p.g., n=15) and acute (>400 e.p.g., n=5) infections. The composition and biodiversity of the gut microbiota and potential biomarkers associated with S. mansoni infection intensity were determined from 16S rRNA amplicon sequencing method and QIIME2 analytical software. A significant increase in Beta diversity (a measure of similarity) seen in the positives compared to the negatives (ANOSIM R = 0.06, p = 0.0386). There was a significant increased abundance of Bifidobacterium among chronic cases (p = 0.0025), whilst Collinsella was significantly elevated in acute infection samples (p = 0.039). The pathobionts Escherichia-Shigella was significantly reduced in the acute cases (p = 0.027). The KEGG pathway analysis revealed significant enrichment of 31 significant orthologs (p < 0.001, False Discovery Rate (FDR) < 10%) in positive samples. Bifidobacterium is a known probiotic shown to reduce malaria intensity in mice and the identified orthologs would enable exploitation for drug discovery.

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## PREVALENCE AND DISTRIBUTION OF FEMALE GENITAL SCHISTOSOMIASIS ACROSS THREE ENDEMIC COUNTRIES, TIMELINE, AND AGE GROUPS

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Schistosomiasis is the world's second-deadliest parasitic disease, affecting 220 million people and causing approximately 200,000 deaths. It is caused by a trematode parasite of the genus Schistosoma, three species of which frequently infect humans: Schistosoma japonicum, S. haematobium, and S. mansoni. Female Genital Schistosomiasis (FGS) is caused by S. haematobium and is predominant in young girls between 12-15 years old. In Lower Middle-Income Countries (LMIC), like Zambia, Ghana, and Tanzania where schistosomiasis is endemic, knowledge regarding FGS is incomplete and often confused with other sexually transmitted diseases. FGS remains largely overlooked within the national health systems and Neglected Tropical Diseases (NTDs) programs, which causes a chronic gynecological condition that leads to substantial morbidity, infertility, cervical cancer, and syphilis disease. It modifies the immunological response to increasing the risk of contracting human papillomavirus (HPV), and human immunodeficiency virus (HIV). The objective of the study is to determine the prevalence of FGS based on the presence of parasite DNA and diagnostic tests in females across age groups from a database of field-acquired human samples from Zambia, Tanzania, and Ghana over multiple years. During the analysis for Ghana, 39 out of 90 samples were females of which 31 (79.5%) were positives and 8 (20.5%) were negatives. In Zambia (2016), 80 out of 133 samples were females of which 46 (57.5%) tested positive and 34 (42.5%) were negative. For Zambia 2017, 60 out of 110 samples were females of which 45 (75%) tested positive and 15 (25%) tested negative. In Tanzania, 70 out of 104 samples were females of which

43 (61.4%) tested positive and 27 (38.6%) tested negative. The outcome highlights that FGS is predominant among females in different endemic countries. This study will help determine and explore the burden of FGS to develop strategies to control FGS and improve current intervention measurements for Schistosomiasis.

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## EFFICACY AND SAFETY OF BUTANTAN-DV LIVE-ATTENUATED TETRAVALENT DENGUE VACCINE FROM A PHASE 3 CLINICAL TRIAL IN CHILDREN, ADOLESCENTS, AND ADULTS

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Butantan-DV is a live-attenuated tetravalent dengue vaccine produced by Instituto Butantan. We assessed the efficacy and safety of Butantan-DV. Participants were stratified by age (2-6, 7-17, and 18-59 years old) and randomized 2:1 to receive a single dose of Butantan-DV or placebo in an ongoing, phase III, double-blind trial conducted in 16 sites across Brazil, with projected five years follow-up (NCT02406729). 16,235 participants were enrolled and received Butantan-DV (10,259) or placebo (5,976) between 2016 and 2019; 46.5% of participants were dengue-naïve. Safety was evaluated as the frequency of participants with solicited (local and systemic) vaccine-related adverse events (AEs). Vaccine efficacy (VE) to prevent symptomatic virologically confirmed dengue (VCD) by RT-PCR after Day 28 postvaccination to any dengue virus (DENV) serotype was determined. Secondary objectives, VE by baseline serostatus, serotype, age, and against severe disease/dengue with warning signs, regardless of hospitalization, were also evaluated. Non-serious, solicited systemic vaccine-related AEs were observed in a slightly higher proportion of overall participants receiving Butantan-DV (58.3%) compared to placebo (45.6%) within 21 days postvaccination. The proportion of participants with AEs within each age group was generally comparable to what was observed in the overall population. After two years of follow-up, the overall VE was 79.6% (95% CI:70.0%-86.3%) and was 80.1% (95% CI:66.0%-88.4%) in ages 2-6, 77.8% (95% CI:55.6.0%-89.6%) in ages 7-17, and 90.0% (95% CI 68.2%-97.5%) in ages 18-59. Serotype-specific VE was 89.5% (95% CI:78.7%-95.0%) against DENV1 and 69.6% (95% CI:50.8%-81.5%) against DENV2 in the overall population. Through the extended follow-up period, which included between 2-5 years of follow-up for all participants, there were no cases of DENV3 or DENV4 and VE against dengue with warning signs/severe dengue was 88.2% (95% CI: 50.8-98.2%). In summary, Butantan-DV was generally well tolerated and efficacious against DENV1 and DENV2 symptomatic VCD, regardless of dengue baseline serostatus or age, through the follow-up.

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## A PHASE 1 OPEN LABEL TRIAL ASSESSMENT OF A DENGUE HUMAN INFECTION MODEL USING A DENGUE VIRUS SEROTYPE 4 LIVE VIRUS CHALLENGE

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Dengue Human Infection Models (DHIM) are critically needed in dengue vaccine and pharmacologic development and can provide a tool for understanding viral-host immunology in a controlled setting. We conducted a phase 1 open labeled, dose-escalation study evaluating the safety and dose of an attenuated Dengue-4-Virus-Live Virus Human Challenge (DENV-4-LVHC) strain intended for DHIM studies. Ten healthy adult volunteers were challenged with a single subcutaneous dose (95 PFU) of DENV-4-LVHC strain H-241 and followed daily from Days 4-16 (or until RNAemia resolved) with quantitative PCR used for detection and solicited adverse events assessed. All ten volunteers developed detectable RNAemia with mean onset at 4.2 days (range of 4-6 days) and a mean duration of 8.7 days (range of 7-11 days). RNAemia peaked between Days 5-11 (mean 7.3 days) and ranged from 5.28 x 105 to 7.60 x 106 copies/µL. All ten volunteers developed symptoms consistent with self-limited, mild dengue infection. The most common symptoms were fatigue, rash, and headache. All developed a nonpruritic, nontender, morbilliform rash, of which 8/10 involved >50% of their body surface area. The majority of other dengue symptoms were mild to moderate, with one volunteer reporting transient grade 3 (severe) headache and fatigue/malaise. Three of 10 developed mild to moderate fevers (38.1-38.8 oC). Laboratory abnormalities were seen in 8 of 10 volunteers, most common being elevated AST and leukopenia - all spontaneously resolved by Day 28. None developed thrombocytopenia. No serious adverse events were observed, and dose escalation was not required. Compared to a previously optimized DENV-1-LVHC, DHIM-4 resulted in an earlier onset (mean 4.2 vs. 8.3 days, $p < 0.0001$), earlier peak (mean Day 7.3 vs. 13.0, $p < 0.0001$), and shorter duration (mean 8.7 vs. 13.0 days, $p = 0.0002$) of RNAemia. In addition, rashes were more prominent in DHIM-4 than in the previous DHIM-1. Our findings support the safety of low-dose DENV-4-LVHC, resulting in uncomplicated mild dengue infection, and is suitable for use in future DHIM evaluation for vaccine and therapeutic development.

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## SAFETY AND IMMUNOGENICITY OF A SYNTHETIC NANOPARTICLE-BASED, T CELL PRIMING PEPTIDE VACCINE AGAINST DENGUE IN HEALTHY ADULTS IN SWITZERLAND: A DOUBLE-BLIND, RANDOMIZED, VEHICLE-CONTROLLED, PHASE 1 STUDY

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Vaccine types other than current antibody-inducing ones are needed to address the global health threat posed by dengue. This study, the first of its kind, assessed the safety and immunogenicity of a CD8+ T cell priming, gold nanoparticle (GNP)-based, multi-valent, synthetic peptide dengue vaccine, designed to provide protective cellular immunity without inducing antibodies. In this randomized, double-blind, vehicle-controlled, phase 1 trial (NCT04935801), healthy individuals aged 18-45 years recruited at the Centre for primary care and public health, Lausanne, Switzerland, were randomly assigned to receive the vaccine candidate (PepGNP-Dengue) or a comparator (GNP without peptides [vehicle-GNP]). Randomization was stratified into four groups based on a risk-minimising dose-escalation strategy (low dose [LD] and high dose [HD], pioneers and followers), allocation was double-blind for participants and investigators. Two doses

were administered by intradermal microneedle injection 21 days apart. Primary outcome was safety, secondary outcome immunogenicity. 26 participants were enrolled (Aug–Sep 2021) to receive PepGNP-Dengue (LD or HD, n=10 each) or vehicle-GNP (LD or HD, n=3 each). No vaccine-related serious adverse events occurred. Most (90%) related adverse events were mild; injection site pain and transient discoloration were most frequently reported. Injection site erythema occurred in 58% of participants. As expected, PepGNP-Dengue did not elicit anti-DENV antibodies of significance. Significant increases were observed in specific CD8+ T cells and dengue dextramer+ memory cell subsets in the LD PepGNP-Dengue but not in the HD PepGNP-Dengue or Vehicle-GNP groups, specifically PepGNP-activated CD137+CD69+CD8+ T cells (day 90, p= 0·046), differentiated effector memory (TemRA) and central memory (Tcm) CD8+ T cells (day 35, p=0·014 and p=0·024, respectively). Results provide proof of concept that a synthetic nanoparticle-based peptide vaccine can successfully induce virus-specific CD8+ T cells. The favourable safety profile and cellular responses observed support further development of PepGNP-Dengue.

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## CHIKUNGUNYA VACCINE VLA1553 INDUCES CROSS-NEUTRALIZATION AGAINST DIFFERENT CHIKV GENOTYPES

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Chikungunya virus (CHIKV) is a small spherical RNA virus and a member of the Alphavirus genus in the family Togaviridae. The virus is vectored by the daytime-biting Aedes aegypti and Ae. albopictus mosquitoes and spread in over 100 countries with more than 2.6 million suspected cases alone in the Americas since 2013. CHIKV is classified into three genotypes including the West African, the East-Central and South African (ESCA) and the Asian lineage. Currently, neither specific antiviral treatment nor a vaccine is available to prevent CHIKV infection. Valneva´s attenuated CHIKV single-dose vaccine candidate, VLA1553, comprises a large deletion in the non-structural replicase complex protein nsP3, which leads to attenuation of the virus in vivo. The vaccine is based on the La Reunion (LR) strain belonging to the Indian Ocean sublineage of the ECSA genotype. Cross-neutralization testing with other lineages is of importance to provide information on a broad neutralization activity by the vaccine. For this purpose, a panel of human sera (n=72) collected at day 1, 29, 85 or 180 from a phase 3 clinical study has been tested by a classical PRNT against wt CHIKV strains from different lineages. The tested strains include the La Reunion strain (LR2006_OPY-1) of the Indian Ocean/ECSA lineage, strain 37997 from the West African lineage and the Caribbean M109 strain from the Asian lineage. Neutralizing antibodies were detected against all three CHIKV strains in sera 28 days after a single dose of VLA1553. The PRNT50 titers of CHIKV baseline-negative participants increased on day 29, and sustained neutralization was measured on days 85, and 180 post-vaccination. The high titer vaccine sera reached neutralization titers comparable to convalescent sera (collected during outbreaks in Latin America 2015-2016) tested in the same PRNT against the various wt CHIKV strains. In summary, analysis of human serum from the phase 3 clinical trial of Valneva´s live-attenuated vaccine demonstrated a broad spectrum of neutralizing antibody activity against all major CHIKV genotypes.

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## IMMUNOGENICITY OF AN EXTENDED DOSE INTERVAL FOR THE AD26.ZEBOV, MVA-BN-FILO PROPHYLACTIC EBOLA VIRUS VACCINE REGIMEN IN ADULTS AND CHILDREN IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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During the 2018-2020 Ebola virus disease (EVD) epidemic in north-eastern Democratic Republic of the Congo, we undertook a large, population-based Phase 3 trial of the two-dose Ad26.ZEBOV, MVA-BN-Filo vaccine regimen in healthy participants aged ≥1 year in Goma (Protocol DRC-EB-001; NCT04152486). However, all face-to-face activities were suspended five months into the study due to the COVID-19 pandemic, resuming five months later. As a result, 6,043 out of 20,408 participants received dose 2 outside the recommended 56-day (-14/+28 days) window. We reconsented adults (≥18 years), adolescents (12-17 years), and children (4-11 years) who received dose 1 but not dose 2 and returned after the study suspension and enrolled them into an immunogenicity subset to assess the impact of receiving dose 2 outside the recommended window. We collected blood samples before dose 2 and 21 days later and tested them for IgG binding antibodies against Ebola virus glycoprotein by the Filovirus Animal Non-Clinical Group (FANG) ELISA. No sample was taken from participants who received two doses with a 56-day interval in this trial. Results were available for 49 adults, 32 adolescents and 52 children. The median interval between dose 1 and dose 2 was 9.3 months. The pre-dose 2 antibody geometric mean concentration (GMC) in ELISA Units (EU)/mL was 217 (95% CI 157-301) in adults, 378 (95% CI 281-510) in adolescents and 558 EU/mL (95% CI 471-661) in children. At 21 days post-dose 2, the GMC increased to 22,194 (95% CI 16,726-29,449) in adults, 37,896 (95% 29,985-47,893) in adolescents and 34,652 (95% CI 27,906-43,028) in children. The post-dose 2 GMCs were higher than those noted in previous African trials using a 56-day regimen (VAC52150EBL2002 and VAC52150EBL3001), but similar to GMCs in participants who received a delayed dose 2 in these trials. We conclude that extending the two-dose interval from two to nine months may increase vaccine immunogenicity. These results support the practical deployment of the Ad26.ZEBOV, MVA-BN-Filo EVD vaccine, which might require vaccination campaigns in remote areas with logistical challenges that could delay dose 2 delivery.

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## DETERMINANTS AND DURABILITY OF ANTIBODY RESPONSE TO RVSVΔG-ZEBOV-GP AND AD26.ZEBOV,MVA-BN-FILO EBOLA VIRUS DISEASE VACCINES: A MODELLING STUDY FROM THE PREVAC RANDOMIZED TRIAL

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Both rVSVΔG-ZEBOV-GP and Ad26.ZEBOV,MVA-BN-Filo vaccines against Ebola virus disease (EVD) have been approved by regulatory agencies and prequalified by the WHO. In the absence of the ability to measure long-term clinical protection, the evaluation of kinetics of the humoral immune response after vaccination is crucial. We used data from PREVAC's large phase 2 randomized double-blind clinical trial, which assessed three vaccination regimens against placebo in four Western African countries in children older than 1 year and adults. Linear mixed-effect regression models were used to evaluate the waning of anti-Ebola virus glycoprotein (GP1,2) binding antibody concentrations after rVSVΔG-ZEBOV-GP or Ad26.ZEBOV,MVA-BN-Filo vaccination protocols, and their potential determinants. The models included data from 1572 (781 vaccine, 791 placebo) and 1565 (779 vaccine, 786 placebo) participants, respectively. After a post-vaccination peak, each vaccination regimen was associated with a decrease of antibody concentrations with distinct kinetics. One year after dose one, the EBOV GP binding antibody concentrations were higher in children compared to adults for both vaccines. However, different effect sizes were identified, with the following ratios of mean antibody concentrations [95% confidence intervals] for rVSVΔG-ZEBOV-GP at 1 yr compared to adults: from 1.4 [1.1; 1.6] for the 1–4 yr group to 1.4 [1.2; 1.6] for the 12–17 yr group. For Ad26.ZEBOV,MVA-BN-Filo, the ratios were from 3.1 [2.6; 3.7] for the 1–4 yr group and 1.7 [1.5; 2.0] for the 12–17 yr group. Antibody concentrations also differed according to geographical location, pre-vaccination antibody concentration, and sex. Our findings show distinct dynamics of the antibody responses after either of the two vaccines, with age and several other determinants having an effect, especially for Ad26.ZEBOV,MVA-BN-Filo. In combination with information on memory response to the EBOV GP antigen, characterization of the major determinants of the immune response durability of the two licensed vaccination protocols may guide future EVD control strategies.

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## HIGH DIMENSIONAL IMMUNOPHENOTYPING OF ACUTE EBOLA VIRUS INFECTED NONHUMAN PRIMATES

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Ebola Virus Disease (EVD), caused by the filoviruses of the genus Ebolavirus, is a hemorrhagic fever with a high mortality rate. Research on the immune response during acute EVD has been limited by limited samples and the necessity of high biosafety level practices. Here we report the use of high dimensional spectral cytometry to broadly phenotype the immune response during acute EVD in rhesus macaques. Rhesus macaques (n=2) were infected with 1300 PFU of the Makona strain of Zaire Ebolavirus and supported in an ICU model of care under BSL-4 conditions. Daily blood samples were drawn and peripheral blood mononuclear cells (PBMCs) isolated and cryopreserved, then analyzed with a 29-color immunophenotyping panel using a spectral cytometer. We identified key transitional steps in the immune response including loss of CD14 and HLA-DR from monocytes starting at day 4 post infection, progressive upregulation of the hemoglobin scavenger CD163 through day 5, and collapse of the monocyte lineage at day 6. Among lymphocytes we observed selective loss of CXCR3 positive B and T cells starting on day 4, expansion of naïve B cells, and activation of NK cells starting on day 5. Changes particularly in monocytes correlated with progression of sepsis physiology. We demonstrate here that significant changes in immune phenotype occur as early as day 4 post infection and continue through disease progression, as well the demonstrate the feasibility of performing broad immunophenotyping on Zaire Ebolavirus positive specimens with spectral cytometry. This detailed timeline of acute EVD will assist in future work to treat this highly fatal disease.

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## CHARACTERIZING THE ROLE OF TICK SPECIES IN POWASSAN VIRUS FITNESS AND EVOLUTION

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Powassan virus (POWV, Flaviviridae) is a reemerging tickborne virus endemic in North America and Russia. POWV was first isolated in 1958 from a fatal encephalitic case in Canada. In 1997, a POWV-like agent was isolated from Ixodes scapularis in New England and determined to be genetically distinct from the original POWV isolate. This revealed the existence of two lineages: lineage 1, prototype Powassan (POWV-1) and lineage 2, deer tick virus (DTV). Each lineage is maintained in separate enzootic cycles with POWV-1 thought to be primarily maintained between I. cookei and woodchucks and I. marxi and squirrels, while DTV is primarily maintained between I. scapularis and small mammal hosts. POWV-1/DTV, however, have been detected in a range of tick genera. In New York State (NYS) between 2018-2022, POWV-1 was isolated for the first time from I. scapularis and detected in Dermancentor variabilis and DTV was isolated from Ambylomma americanum. These novel findings suggest POWV-1/DTV circulation in a broader range of tick hosts which is further supported by the overlapping and expanding geographic and mammalian host ranges of these genera. The propensity for POWV-1/DTV to further adapt to new tick hosts and transmission cycles following these rare spillover events is unknown but could facilitate the emergence of increasingly transmissable strains. To understand the potential for adaptation of POWV-1 and DTV to distinct tick genera, we conducted experimental evolution of recently isolated POWV-1 and DTV strains from NYS in I. scapularis, D. variablis, A. ambylomma, and Haemaephysalis longicornus. Experimentally infected ticks were collected at 20-day intervals for 100 days. Infection rates, viral kinetics, and full genome sequences of viral outputs were conducted to assess changes in the viral population. Early timepoints suggest introduction of POWV-1 and DTV into noncanonical tick vectors results in viral diversification and emergence of mutations potentially involved in invertebrate host immune evasion. These data suggest the capacity for POWV vector expansion and demonstrate the need for expanded viral surveillance of non- Ixodes ticks.

**5786**

## ARE THE BITES OF NON-INFECTED SAND FLIES IMPORTANT FOR THE MAINTENANCE OF CUTANEOUS LEISHMANIASIS ANIMAL RESERVOIRS?

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Sand flies transmit several pathogens, of which, Leishmania parasites are the most prominent. More than 20 Leishmania species cause disease in humans worldwide. Strikingly, all but one are zoonotic agents; animal reservoirs play an essential epidemiological role in leishmaniasis. However, our understanding of such animal reservoirs, and particularly, their interactions with sand flies is still limited. Since in the wild, rodents were incriminated as reservoirs of L. major parasites, here, we took advantage of two rodent models of cutaneous leishmaniasis (CL) to try to understand

whether the bites of non-infected Phlebotomus duboscqi sand flies impact disease progression. In the susceptible model, a single exposure event in the context of CL active lesions - three weeks after the infection of BALB/c mice with 1000 L. major metacyclics - did not impact disease progression, as per the similar ear thickness measurements recorded in exposed versus non-exposed animals. This translated into similar ear parasite burdens throughout the follow-up period in exposed BALB/c versus non-exposed control animals. Similar results were obtained in the context of a single exposure of CL active lesions to the bites of P. duboscqi sand flies in the self-healing mouse model (C57BL/6 mice). However, when we performed a single exposure to non-infected sandfly bites in the context of healed CL lesions (C57BL/6 self-healing model; 9 weeks post-infection), a transient pathological response was observed, as per the increase in the ear thickness of exposed versus control animals. Importantly, this phenotype was accompanied by a significant increase in the ear parasite burden of exposed versus control animals. Of note, pathological changes of greater magnitude were observed in the context of multiple exposures of healed CL lesions to the bites of non-infected P. duboscqi sand flies. These preliminary results may suggest a role of sand flies in the maintenance of competent Leishmania reservoirs, in line with a previous study reporting that sand fly bites favor the transmissibility of the anthroponotic L. donovani parasites by infected hosts.

**5787**

## THE HUMAN SKIN MICROBIOTA CHANGES IN RESPONSE TO SCABIES INFESTATION, WITH AN INCREASE IN OPPORTUNISTIC PATHOGENS

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Scabies is amongst the most common dermatological diseases worldwide with an estimated prevalence of 300 million cases and is recognised as a neglected tropical disease. Prevalence is high in tropical regions where a link with secondary bacterial infections has been established through clinical epidemiological studies. This research has demonstrated that scabies mites promote opportunistic bacterial infections with Staphylococcus aureus and S. pyogenes. Recent molecular data has also linked Acinetobacter baumannii, another extensively drug resistant nosocomial pathogen, to scabies infections. Our aim is to provide the fundamental molecular evidence of how scabies interferes with the host microbiome, to better understand the role scabies mites play in severe secondary bacterial infections and to improve treatment outcomes. We present here a first dataset from a collaborative multi-national study that collected skin scrapings from scabies infected patients from three countries (India, France and Australia), representing a diverse climate and socio-economic range. Microbial DNA was extracted from 751 samples, and 16s full-length rRNA and ITS long-read amplicon PacBio sequencing was performed. Using an established bioinformatics pipeline in R, we have analysed the data to determine the microbial profiles present during scabies infection. We have assessed samples from 134 patients from 3 different countries, and preliminary data demonstrates that there is an increase in opportunistic pathogenic bacteria in scabies lesions. This study is the first to quantify the scabies associated microbiome at the molecular level, and address how it might differ globally.

**5788**

## EVALUATION OF THE EFFECT OF LONG-LASTING INSECTICIDE IMPREGNATED BED NETS ON PHLEBOTOMUS ARGENTIPES EXPOSURE USING SALIVARY BIOMARKERS: AN EARLY ANALYSIS AFTER SIX MONTHS

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Salivary proteins are useful serological markers of sand fly exposure. Long-lasting insecticidal-impregnated bed nets (LLINs) have been used for vector control interventions. However, their effectiveness against exposure to sand fly bites in Sri Lanka is unknown. The study aimed to evaluate the effectiveness of LLINs on exposure to Phlebotomus argentipes, a vector of CL in Sri Lanka using salivary biomarkers. A cluster randomized controlled trial was carried out in an endemic region of CL with a total of 600 (300 per group) individuals in intervention & control groups LLINs were given to the intervention group (at least 3 nets per house) & normal bed nets for the control group. 3cc blood was collected in the baseline survey from 310 individuals in the intervention group & 235 individuals in the control group prior to the intervention. In the post-intervention survey after 6 months, blood samples were collected from 259 & 208 individuals in the intervention & control groups respectively. A previously validated indirect ELISA assay against composite rPagSP02+rPagSP06 antigen of Ph. argentipes was used to measure the anti-saliva antibody levels of two groups & compared with the baseline sera. The geometric mean ELISA optical density (OD) of an intervention group in the baseline was $0.124 \pm 0.083$ whereas that was $0.138 \pm 0.100$ in the control group. The mean OD of an intervention group (post-intervention OD: $0.069 \pm 0.062$) declined significantly ($p < 0.05$) after 6 months in comparison to the baseline where that remained more or less constant in the control group (post-intervention OD: $0.139 \pm 0.070$). The difference in difference (DID) standard model showed that LLINs reduced exposure to Ph. argentipes by 43.54% at 6 months where the effect of the intervention was -0.054 at 95% CI. We observed a significant difference in serological markers of sand fly exposure in the intervention group in a tested cohort & need to combine the results with entomological data to validate the effect of LLINs mediated intervention.

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## EXPANDING TOOLBOX FOR ODOR-BASED TSETSE FLY CONTROL IN EAST AFRICA

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Tsetse fly - transmitted Human African Trypanosomiasis (HAT) and Animal African Trypanosomiasis (AAT) are among most neglected tropical diseases in sub-Sahara Africa. Tsetse fly control strategies constitute cornerstones efforts in suppression and eradication of HAT and AAT. Tsetse fly lures that attract the flies to traps/insecticide-treated targets and repellents that minimize contact between infective flies and their vertebrate hosts can augment the strategies. We formulated a Novel Attractant Blend (NAB) comprised of ε-nonalactone, nonanoic acid, 2-nonanone and acetone) and Novel Repellent Blend (NRB) (δ-nonalactone, heptanoic acid, 4-methylguaiacol and geranyl acetone) based on tsetse-refractory waterbuck odor constituents, their structural analogues and attractant buffalo odor. Using two-choice wind tunnel in the laboratory

and Latin square experimental design in the field, we establish that 1) NAB is 2.4 times as attractive to Glossina pallidipes tsetse flies as POCA (3-n-Propylphenol, 1-Octen-3-ol, 4-Cresol, and Acetone) blend routinely used in tsetse control and 2) NRB is two-folds more efficacious than current commercial repellent blend against most savannah species. We microencapsulated the optimized NRB into β-cyclodextrin nano particles by kneading technique, evaluated responses of G. pallidipes tsetse to the microencapsulated blend and established kinetic release rates from the microcapsules under field conditions. We established significantly ($p<0.05$) lower release rate (5.35mg/h) in microencapsulated blend than the un-encapsulated control (11.82 mg/h) and that the micro-capsulation did not significantly affect responses of the tsetse flies to traps. We assessed efficacy of NRB in livestock protection using randomized block experimental design and established at least 95% repellence of G. pallidipes from oxen by NRB. We successfully masked the NRB in fragrance for odor appeal (for potential use in security and hospitality industries) and are developing NAB and NRB into semiochemical prototypes for integrated push-pull deployment in areawide control of tsetse flies in Eastern Africa.

**5790**

## ONCHOCERCIASIS TRANSMISSION IN BENIN: BITING AND PAROUS RATE OF SIMULIUM DAMNOSUM COMPLEX ALONG THE OUEME, SOTA AND ZOU RIVERS

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Having up to date knowledge of the entomological indices of onchocerciasis vectors is critical for countries targeting the elimination of the disease as recommended by the WHO roadmap. This entomological study targeted breeding sites located in the former Special Intervention Zones (ZIS) as previously described by the Onchocercias Control Programme (OCP) where mass drug administration with ivermectin has being implemented for several decades in Benin. Females of Simulium damnosum complex were collected using human land catch method from August to the mid-November 2022 on 13 breeding sites: Agonlin Pahou, Sokpounta, Bethel, Bouri, Fonkpodji, Samiondji, Ayissakpo, Bétérou, Kika Barrage, Oubérou, Térou, Bétécoucou, and Atchérigbé in the districts of Kétou, Ouèssè, Glazoué, Gogounou, Djidja, Zangnanado, Tchaourou, and Dassa. These sites are in savannah areas distributed along the Ouémé, Sota, Okpara and Zou rivers. The blackflies were collected at fixed points located within 100 to 200 metres of the riverbanks from 7am to 6pm by two collectors who alternate every hour. The vectors were identified morphologically using characters based on the Meredith and Townson key. While the collection expanded over four months, the dissection was only conducted during two months, when the flies density was high, in the middle of the rain season. The average biting rate in the study areas was 119 bites/man/day (bp/day), significantly higher than the tolerable threshold of 30 bites/man/day ($p<0.001$). The monthly parous rate at the evaluated sites is an average 79.23% ±1.94% (95%CI). The parous rate varies from 46% [32.19 - 59.81] to 98% [94.12 - 100.00] during the second month, to 48% [34.15 - 61.85] to 98.75% [96.32 - 100.00] in the third month. Pending confirmation of the infectivity rate at these sites, these entomological data indicate ongoing intense transmission in the study area. Molecular identification of the different species collected and cytotaxonomy on larvae from these sites to further clarify vector competence among the Simulium sibling specie would help NTD programme to refine their strategy toward elimination.

**5791**

## VERTICALLY INFECTED DOGS AS A RESERVOIR FOR LEISHMANIA INFANTUM IN AN ENDEMIC AREA FOR VISCERAL LEISHMANIASIS

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Leishmania infantum (Li) transmission to mammals occurs mainly via sand flies. However, vertical Li transmission has been demonstrated in locations with no apparent competent vectors, as well as in humans. The aims of this study were (1) to evaluate the Li infection rate in sand flies from an area endemic for human and canine visceral leishmaniasis (VL), and (2) to determine whether oligosymptomatic dogs vertically infected with Li are a reservoir of infection in Brazil. Weekly sand fly capture was performed for 2 years using CDC light traps in the peridomestic areas of 18 houses around Natal, Brazil. Eight puppies born to naturally Li-infected dogs were maintained in a kennel protected by insecticide-impregnated screens. These pups were followed for clinical progression. A Lutzomyia longipalpis colony was established and female sand flies at 5th and 6th generation were used for the xenodiagnoses. After ~18 months, xenodiagnosis was used to determine whether these pups could transmit Li to sand flies. Li infection of sand flies was measured by qPCR with a kDNA target. A total of 215 female sand flies were captured near the 18 houses and the Li infection rate in the endemic areas was 9.3%. Xenodiagnoseis using sand flies raised in the insectary showed that 6 out of 8 (75%) of offspring born from infected dams transmitted Li to sand flies. At the time of xenodiagnosis these dogs were subclinical with negative Leishmania serology and negative blood qPCR. 21.7% of sand flies fed on those dogs were Li positive by kDNA. vertical transmission in dogs may be important for maintaining Li in this region. Furthermore, although xenodiagnosis is not easy to implement, it may be the best way to identify Li reservoirs.

**5792**

## ASSOCIATION OF HIV/AIDS WITH PSYCHIATRIC ILLNESS AMONG TRANSGENDER POPULATION IN A LOW HIV PREVALENCE COUNTRY

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In Bangladesh transgenders are high-risk group for HIV infection. Gender identity crisis and psychosocial stressors contribute developing psychiatric illness. We aimed to identify association of psychiatric disorders in transgenders by comparing the HIV status in Dhaka city. A cross-sectional study of transgenders aged >18 years was interviewed after taking written informed consent between July 21-June 2022. Sociodemographic, personal, co-morbid condition and psychosocial stressor variables were collected. Self-Reporting Questionnaire 20 was applied for psychiatric evaluation. Psychiatric illness was diagnosed using Mini International Neuropsychiatric Interview 7.0.0. The primary outcome of the study was to detect psychiatric illness among transgender adults irrespective of HIV status. Ethical clearance was taken. Of 157 transgender adults, 34 (22%) were HIV positive. Fifty-nine percent participants were between the 18-30 years of age group. Male biological sex was predominant (97%). Gay or lesbian sexual orientation was predominant (50% vs. 17%; $p<0.001$) and statistically significant and HIV positive transgender people. Fifty-

six percent belong to rural birthplace. Failed suicidal attempts (12% vs. 2%; p<0.001), chronic medical conditions (91% vs. 15%; p<0.001), any romantic relationship (67%vs 36%; p<0.001)) and satisfaction in the quality of life (64% vs. 45%; p=0.04) were significantly associated with HIV positive transgender than HIV negative peers. Among 34 HIV-positive transgender 22 (65%) were diagnosed as having psychiatric illnesses. Generalized anxiety disorder (41%) was the predominant psychiatric illness next to Minor depressive disorder (32%) and obsessive-compulsive disorder (9%). After adjusting the confounding variables, chronic medical condition [OR 98 (9.93-98.18; p<0.001] and life satisfaction [OR 8.7 (1.02-74.3); p=0.048] were significantly associated with psychiatric illness. Psychosocial stressor-based national screening program for early identification of psychiatric disease need to be developed for transgenders with HIV.

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## SYNTHESIS OF FINDINGS FROM THE LITERATURE AND A QUALITATIVE RESEARCH STUDY ON THE IMPACTS OF GENDER, DISABILITY, AND ETHNICITY IN NEGLECTED TROPICAL DISEASES PROGRAMMING

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Act to End Neglected Tropical Diseases (NTDs) West, a USAID-funded program that seeks to eliminate or control five NTDs in West Africa, conducted a gender and social inclusion analysis to determine how NTDs differentially impact different population groups and how gender and social norms impact NTD programs. The study used a mixed methods approach, including a literature review; qualitative data collection in Côte d'Ivoire, Sierra Leone, and Ghana with a total of 477 participants; and quantitative analysis of programmatic data. Women and girls face additional health risks as well as social and economic impacts as a result of NTD infection compared to men and boys. Men are somewhat less likely to participate in mass drug administrations (MDAs) due to lack of information about campaigns, lack of access due to being out of the community when MDAs are conducted, and concerns and misconceptions about side effects. Pregnant and breastfeeding women are sometimes excluded from certain types of MDAs for which they are eligible or choose not to participate due to misinformation. MDA training rates for community drug distributors (CDDs) and supervisors are almost universally higher for men than women, even though feedback on the effectiveness of female CDDs is overwhelmingly positive, and female CDDs often have more access to women in conservative households. The role of CDDs can lead to career and social opportunities for both men and women. However, challenges faced by CDDs are seen as a greater barrier for women, including transportation, safety, household responsibilities, and low or lack of wages. Finally, people with disabilities and marginalized ethnic groups may sometimes be excluded from or exclude themselves from MDA. NTD programs can promote gender equity by increasing women's participation in MDA activities and providing financial compensation to CDDs. Additionally, programs should prioritize inclusive training for CDDs, and inclusive messaging about NTDs and MDA for communities. For example, in Sierra Leone, Act | West is now conducting rumor tracking to address misconceptions and collecting data on CDD sex for advocacy purposes.

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## NEEDS AND PREFERENCE FOR COMMUNITY HEALTH WORKER SERVICES IN CAMBODIA: A COMMUNITY SURVEY

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Despite ongoing efforts towards universal health coverage, many LMICs still suffer from shortages in health professionals, lack of infrastructure, and inequities in health protection, particularly in remote rural areas, where access to health care and health information are inadequate and where poor communities bear the greatest health and economic burden of disease. In Cambodia, CHWs are identified as key to local health promotion and as a critical link between health centres and the community. However, research on the needs and preferences of communities regarding CHW services is limited.As part of a wider RAI3E operational research project on expanding the role of and integrating village malaria workers we conducted a questionnaire-based community survey in 6 endemic communes of Kravanh District, Pursat province, Cambodia during March and April 2022 with 174 community members (72 males and 102 females; aged 22-71). Community members were familiar with the local community health workers; almost half of respondents had visited a CHW in their community for health services in the past 12 months. Community members and their family's most common health problems included common cold (81%), malaria (72%), unspecified fever (43%), non-communicable diseases (NCDs i.e. diabetes, hypertension, hyperlipidemia, 32%), and dengue (30%). NCDs (35%), influenza (20%), pneumonia (25%), dengue (12%), and malaria (11%) were reported as their greatest health concerns. Over half (54.5%) of respondents preferred home visits from a CHW. Almost two-thirds described a willingness to pay for additional CHW services and, of those, 59% preferred paying 10 USD or less. Nonetheless, a similar proportion of all respondents reported difficulties paying for their healthcare.In summary, there was a varied demand for primary healthcare at the community level in Cambodia, covering infectious and non-infectious diseases. Well-known and accessible, CHWs are well-positioned to meet some of these needs. Ensuring basic equipment for CHWs is key, as are providing adequate capacity building and supervision, particularly if their role is to be expanded.

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## ARMED CONFLICT REFUGEES' RESILIENCE: TRANSDISCIPLINARY STUDY ON A DIALOG FOR HEALTH PREVENTION IN THE EASTERN DEMOCRATIC REPUBLIC OF CONGO

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In DR Congo, the resurgence of violence by armed groups in 2023 caused the displacement of more than 5.8 million people. Parasitic infections, malaria, mental health, sexual and gender-based violence are considered more prevalent among refugees. This work argues the ability of refugees to persist, adapt and transform face to sanitary challenges might be limited given that it their context involves the loss of all their basic resources, the effectiveness or not of subsidies and the transitional state of displacement. This work aimed to set a dialog with key actors as to contribute to the characterization of infectious diseases (epidemiological investigation and microorganism identification), of psychosocial risks and to explore the ability of resilience in the context of refugee's camps in the region of Nyiragongo in Eastern DR Congo. Data were collected in a period of January and

February 2023 with socio-anthropological and microbial methods integrating "transdisciplinary approaches" that consisted in co-producing the objectives with stakeholders. The local knowledge was integrated through exploratory survey with key actors. Forty-six (46) sites sample where collected for microbiological analysis. In total, 104 persons were interviewed and 11 FG each consisting of 6 to 9 actors were organized. The "inductive thematic data analysis" was applied. Key findings are attitudes and behavior risks related to infectious diseases, sanitation and the psychosocial burden. Total coliforms, Vibrio ssp., Salmonella ssp., Shigella ssp., and Enterobacteria were identified from camps sites samples. The ability of refuges to persist, adapt and transform are analyzed into dynamic of the actors, resources, interactions context. In perceptive of actions, the results are translated into a grid of powers and interests in relation to the mobilization of resources for the prevention infectious and psychosocial burden. In addition, an analysis of the ability of refugees to persist, adapt and transform as to prevent health risks.

**5796**

## LIVING WITH HANSEN'S DISEASE IN MALAYSIA: A TRANSDISCIPLINARY RESEARCH APPROACH

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Hansen's Disease (HD) is a chronic bacterial infection that primarily affects the skin, nerves, and other parts of the body. The disease can cause physical deformities if left untreated, which can make daily activities more difficult. While multi-drug therapy is effective at curing HD, allowing many to lead fulfilling lives, stigma still surrounds it. The application of a transdisciplinary research approach can facilitate a mutual understanding of the challenges faced by HD-affected people to identify their needs and concerns and develop solutions that address their unique challenges to reintegrate them into society and become contributing members. Stakeholder identification and mapping were conducted to identify twenty HD-affected individuals and twenty key people and decision-makers from governmental agencies, non-governmental organizations, and community and family members involved in their care (n=40). Participants were purposively sampled. Semi-structured interviews were conducted with each participant, and their responses were transcribed and analyzed with NVIVO software. A stakeholder engagement workshop was held where the problems were discussed and ranked, and solutions were co-created for the problems identified. An interagency meeting followed to discuss the implementation of the agreed interventions. The four main themes that emerged regarding the impact of HD on the affected individuals were biophysical, psychological, social, and economic. The stakeholders' analysis showed that the main challenges were a lack of public and health workers' awareness and knowledge of HD, insufficient resources for prevention and control, and a lack of interagency engagement and communication. Another issue was access to employment and skills training for self-sufficiency. Details of the analysis will be presented. The transdisciplinary approach can facilitate the integration of traditional knowledge and expertise, promoting a more inclusive and participatory approach to research. This collaboration ensures that solutions are empowering and sustainable to enhance the standard of living of the affected people.

**5797**

## CHARACTERISTICS ASSOCIATED WITH SARS-COV-2 SEROPOSITIVITY IN CAMEROON

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It is important to study both symptomatic and asymptomatic infected persons to get a complete picture of SARS-CoV-2 in a population. The aim of this study was to identify characteristics (travel and testing history, exposure to crowded areas, contact with infected persons, household number) associated with SARS-COV-2 seropositivity in adults and in children in Cameroon. We used data from a SARS-CoV-2 country-wide seroprevalence survey conducted in Cameroon from November 2020 to February 2021 among males and females aged 5 to 100 years. A whole blood specimen was collected for SARS-COV-2 serology testing using ABBOTT Architect immunoglobulin G (IgG) and WANTAI total antibodies (Ab). A test was considered positive only if both tests were positive. Descriptive statistics, crude association between each covariate of interest and SARS-COV-2 seropositivity, and multivariable association (logistic regression) were used. Weighted counts (estimates) are presented. Data were available for 9836 participants. Less than 20% (17.53%) of the participants had symptoms of COVID-19 (1423/8116). The seroprevalence of SARS-COV-2 was 10.45% (8347/9321) and the seroprevalence was higher in those with symptoms 12.93 % than those without symptoms 9.97%. In univariate analyses, seropositivity was similar with increasing number of people in household (OR=0.98, 95%CI 0.76-1.28, p=0.90). Those who had travelled abroad had a higher risk of seropositivity than those who did not (OR=1.63, 95%CI 1.08-2.47, p=0.02). In the final multivariate model, seropositivity increased with age, after adjusting for sex, contact history, and testing history (AOR=1.61, 95%CI 1.29-2.01, p<0.001). The risk of seropositivity was higher in those with prior testing compared to those without prior testing (AOR=1.34 95%CI 1.04-1.72, p=0.02). Males had a higher seropositivity rate in both children and adolescents, (AOR=1.79 95% CI 1.24-2.58 p=0.003) and in adults (AOR=1.66, 95%CI 1.35-2.05, p<0.001). In conclusion, these data suggest an association between increasing age, male gender, previous SARS-COV-2 testing and seropositivity for SARS-COV-2.

**5798**

## CONCEPTUALIZING AND UNDERSTANDING STIGMA ASSOCIATED WITH CUTANEOUS LEISHMANIASIS IN A RURAL COMMUNITY OF SRI LANKA

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Stigma is a barrier to health-seeking and treatment adherence, affecting disease mortality, morbidity, a person's quality of life and social inclusion. Cutaneous Leishmaniasis (CL) is widely accepted as a stigmatising skin disease. This disease has been endemic in Sri Lanka for centuries. The drivers and facilitators of the stigma associated with CL are not understood properly compared to those of other stigmatising diseases, preventing the development of holistic interventions, research, and policy. This study aims to identify the drivers and manifestations of the stigma associated with CL in rural Sri Lanka. This study was conducted in 2021 in the Anuradhapura district, a district with one of the highest incidence of CL in Sri Lanka. A multimethod qualitative approach was used with two main components, 1) An ethnographic study; participant observation and an auto-ethnographic diary study, 2) A qualitative study on people with CL

using a Participant Experience Reflection Journal (PERJ) and Post-PERJ interviews. We observed indirect, hidden and unexpressed stigma. Most of the negative reactions were confined to people with notable wounds. Disgust, fear, myths and misconceptions of the disease and the notion that it is a dangerous/deadly disease are identified as potential drivers of stigma. Marginalisation, internalised, anticipated and social stigma were identified as stigma experiences. People with internalised stigma felt sad/disappointed/uneasy; they accepted the disease as bad Karma. People with big/visible wounds also anticipated stigma from others and took measures to cover up/hide the wounds. They had reduced engagement with society as they feared dismissal by others. Social stigma manifested as calling people with a wound 'a distortion' and 'ugly'. The wound was described as disgusting by community members. Ideally, stigma interventions should interrupt the process before the stigma is established. It is important that the drivers we have identified through this study are addressed through public health interventions and policy before the stigma is widely established.

**5799**

## INTEGRATING AND ACCESSING EQUITY IN GLOBAL HEALTH PROGRAM DESIGN

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PATH set out a five-year strategy focused on advancing health equity through innovation & partnerships. The strategy includes four change strategies for becoming a more equitable organization: equity in health, community-focused priorities, respectful partnerships, & inclusive innovation. PATH translated its change strategies into tactical benchmarks. The working group conducted a literature review, 17+ qualitative interviews with staff & partners, & held workshops with 60+ people. 11 project teams piloted & provided feedback on the first version of the Equity in Programming Benchmarks (EBP). The tool is a self-assessment measuring 12 indicators on a continuum from low to transformative & helps identify how each project can progress along the continuum. Project assessments are reviewed by PATH leaders. 35 proposals & projects have used the benchmarks to assess their work. The New Nets Project-a malaria vector control evaluation-used the benchmarks at the end of the project with partners to review results & consider ways to modify mosquito net distribution in the future. Highlights from this discussion include: Identifying structural changes to address barriers for institutions in malaria-endemic countries to access resources from large donors & build capacity; More consideration of systemic inequities within routine processes such as how to conduct & measure mass distribution campaigns of nets. The Technical Assistance Platform Project-a global health informatics technical assistance mechanism-used the EPB tool in annual work planning & made several adjustments including: Evolving the model so partners strengthen their organizational & leadership capacity & take on increasing project leadership, responsibilities, & autonomy over time; Ceding control over certain aspects of project management to colleagues & partners based in Africa & Asia. With the EPB, teams have better tools for understanding equity in their projects & can communicate the rationale for changes to donors & partners. We continue to gather feedback & iterate on the EPB so it meets the evolving needs of teams & the communities where the projects take place.

**5800**

## INCREASING ADOPTION OF MALARIA PREVENTION AND CONTROL USING MULTIPRONGED SOCIAL BEHAVIOR CHANGE APPROACHES

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Social behavior change interventions, in the USAID PMI Uganda Malaria Reduction Activity, implemented by JSI, are data-driven, gender sensitive and targets malaria high burden districts. Aided by behavioral science and human centered design approaches to understand why people make decisions the way they do to tailor interventions that trigger behavioral change. District led programming and working with already existing structures like community health workers or village health teams is the main strategy. Focus is on increasing demand for health services, improving mosquito net use, improving health care provider and client interactions, and influencing social norms for sustainability. A multi-sectoral approach was used to engage district leaders and other stakeholders to inspire change at community level. We engaged district health teams, opinion leaders, epidemic task forces for malaria and other implementing partners. The number of children between 2 months and 5 years old with fever that received malaria rapid diagnostic tests seen by village health teams reduced from 1,194,184 in April to June 2022 to 960,454 in October to December 2022. The number of sick children of the same age range with confirmed malaria decreased from 1,057,854 to 860,548.The number with fever and danger signs seen in the community reduced by 12% from 65,570 to 57,754 and the number seen by village health teams and treated within 24 hours for fever reduced by 51% from 881,890 to 428,374 between April to December 2022. Supporting the districts to identify their own strategies for mobilization empowers district leaders and creates ownership. Each district has its own dynamics and have their own strategies for community engagement that works for them like engaging elders in community mobilization, engaging positive deviants in giving testimonies during community mobilization events, using data to map hotspots, and door-to-door mobilization. The role of community engagement in the planning and execution of community led activities directly leads to better health outcomes and sustainability of the adopted positive behaviors.

**5801**

## EQUALITY IN AJTMH PUBLICATIONS FROM 1952 TO 2022: WHAT CAN WE LEARN TO MAKE GLOBAL HEALTH RESEARCH PUBLISHING MORE EQUITABLE? A BIBLIOMETRIC ANALYSIS

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The long overdue focus on decolonising global health has prompted various institutions to assess existing inequities in global health research partnership and resulting publications. Recent reviews have demonstrated inequities in published global health research between researchers from high-income countries and low- and middle-income countries in terms of authorship, gender and academic affiliations, among others. Reflecting the American Society of Tropical Medicine and Hygiene's aim to advance health equity globally, we propose to conduct a bibliometric analysis of the American Journal of Tropical Medicine and Hygiene (AJTMH) publications between 1952 and 2022. Specifically, we propose to assess the following: - Author order- Author affiliation(s), classified using World Bank country income classifications- Author gender, when available- Funding source- Study type- Study topic- Region of publication- Year of publication.Funding sources will be recorded primarily to identify main stakeholders for further dissemination of our findings. Data will be analysed using Student's t-tests and Chi-square, followed by logistic regression. Results from this review will 1) inform a widening participation strategy launched by the ASTMH in 2022, to reflect the current make-up of global health researchers worldwide and

2) strengthen the record of AJTMH as an innovative publication with not only its finger on the pulse of change, but also actively seeking to equalise the field of global health reporting. Finally, the authors will propose further direct collaboration with the AJTMH and its affiliates to update guidelines and prepare authorship guidelines describing the Journal's commitment to inclusivity, equality and fairness.

5802

## ESTABLISHING A RELATIONSHIP WITH THE SURVIVORS OF TORTURE CLINIC AND THE UNIVERSITY OF LOUISVILLE SCHOOL OF MEDICINE; AN INNOVATIVE ALLIANCE TO MENTOR AND ASSIST REFUGEES

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Kentucky ranks 5th in the nation for the number of refugees. A Physicians for Human Rights chapter was created in response to an unmet need in refugee support services. Students were recruited through an application process and were selected as vice president, secretary, treasurer, and community outreach chair. Our mission is to partner with the Survivors of Torture Clinic at the Univerisity of Louisville. The Survivors of Torture Resource Center offers comprehensive services to refugees and immigrants who have experienced torture in their home countries. They include direct mental health services, social and medical care coordination, and legal service referrals. Through our cooperative relationship, medical students can volunteer and serve as mentors to asylum seekers. Cultural differences often make it difficult for young refugees to adjust to a new country. Besides offering friendship, mentors can also assist them in navigating a foreign country. In addition to exposing them to different events in the city, they can provide them with a wide variety of restaurants to eat at or offer ways to foster their passions. Students will also help them navigate healthcare appointments and schedules. The US healthcare system is extremely daunting, and patients can feel intimidated when seeking to see a provider. A third-party mediator can help the patient explain their feelings to the doctor when there are cultural and linguistic barriers between the provider and patient. Additionally, students can assist refugees in finding transportation and organizing their medications. Through this program, students will gain exposure to working with marginalized populations and increase their cultural competence. Furthermore, refugees will receive much-needed peer support and have an easier time adjusting to life in the U.S.

5803

## GROW502: A POP-UP CLINIC TO TACKLE HEALTH DISPARITIES WITHIN THE HOUSELESS POPULATION

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At the height of the Covid-19 pandemic and racial justice movement, students created, Grow502. The goal was to pursue a healthier community by raising awareness which led to the co-creation of a curriculum focused on education, advocacy, community engagement, and creative media. In 2022, we focused on the Houseless community, Refugees, and folks living in the West End. Houseless communities experience disparities in chronic diseases, premature death, poverty, and unemployment; systemic injustice in housing, healthcare, nutrition, and the environment are significant contributors. To raise awareness in our community and provide resources, we launched a pop-up clinic for the Houseless on May 21st. Some of the service offerings at this pop-up clinic included Dental Assessments, Wound Care, Showers, Vaccination, Food, and a Donation Drive. Other services include infectious disease testing and enrollment into Medicare. We kickstarted this event by having an event where we had a case-based discussion breaking down health disparities among the unhoused and barriers to proper health care. Homeless folks were transported to the clinic by Feed Louisville. Flyers were given to shelters in the area. Grow502 acted as a connection between the houseless and organizations in the community. Recognition of the unique healthcare needs of homeless people has encouraged the developing of special services for them. Homeless individuals are unique in the multiplicity of needs they experience, the lack of support networks they face, and the difficulty of their daily activities. Our hope as an organization is that these pop-up clinics will continue to be a vital asset in our community through the coordination of multiple services. Participants saw the pop-up clinic as a means of building awareness, inspiring others to reduce health disparities, and strengthening the partnership between harm reduction, the homeless, and the medical field. Due to volume, we are planning another pop-up clinic.

5804

## NUTRITIONAL STATUS, DIETARY DIVERSITY AND FOOD INSECURITY AMONG WOMEN AND CHILDREN IN PERI-URBAN COMMUNITIES OF KARACHI, PAKISTAN

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Malnutrition is a serious public health problem in Pakistan. One of the causes is inadequate consumption of nutrients to support the requirements at various life stages. Undernutrition among women in the reproductive age leads to maternal morbidity and mortality, fetal growth restriction and childhood malnourishment. We conducted a survey in 7 peri-urban areas of Karachi, Pakistan to assess the nutritional status and dietary diversity among married women of reproductive age and their children under 2 years of age. We also assessed household food security. We used a two-stage systematic random technique to select households and enrolled 1470 mother-child dyads (210 at each site). To assess the nutritional status, we followed the 2018 FANTA guide for anthropometric measurements of participants and used the 10-item minimum dietary diversity scale for women and the 9-item minimum dietary diversity scale for children through 24-hour dietary recall. Household coping strategy and hunger tool were used to measure food insecurity. We found that slightly more than 45% women had normal BMI, while 16% were underweight. Among children, 24% were found to be 'wasted', 39% were underweight and 39% were stunted. We found that 22.5% (n=332) of women and 8% (n=188) of children met the standards for consuming 5 food groups. All households reported food insecurity. To cope, 20% (n=290) borrowed food; 30 % (n-438) limited their portion sizes; 31% (n=452) reduced the number of meals consumed, while 24% adults restricted their food consumption. We also found poor food access; 37% (n=549) of women reported not having food at home, 33% (n= 503) slept hungry, and 31% (n=463) reported atleast one day in the previous month in which they did not eat. These findings suggest that even in large cities like Karachi, the peri-urban population suffers from food insecurity and women and children are malnourished. Along with poverty alleviation at the macro level, there is a need for contextually designed interventions to improve the nutrition and health of women and growth trajectories of children.

5805

## A SYSTEMATIC REVIEW AND META-ANALYSIS: IMPACT OF THE COVID-19 PANDEMIC ONVIOLENCE AGAINST CHILDREN

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COVID-19 lockdowns caused interruption of child protection services and economic/psychological burdens on parents. It has become challenging for teachers or school counselors to detect and report signs of abuse. Therefore, in this systematic review and meta-analysis (SRMA), we aimed to identify the impact of the worldwide COVID-19 pandemic on violence against children by investigating the change in the prevalence of violence against children before and during COVID-19 lockdowns. The protocol of this study was registered in PROSPERO with the registration number CRD42022377660. We included any studies eligible for meta-analysis comparing violence against children before and during COVID-19. Eleven

electronic databases were systematically searched in March 2022. The meta-analysis was conducted using STATA, pooled odds ratios were calculated, and subgroups by countries and sex of children (when possible) were analyzed. A total of eleven publications were included in the meta-analysis. Overall, we found insufficient evidence to support that the COVID-19 pandemic impacted the prevalence or proportion of any type of violence against children, even after segregating the data to the country or sex levels. Furthermore, one article showed a significant decrease in reporters from school staff, which might be explained by an under-report of child violence. In conclusion, although our results suggest that the COVID-19 pandemic did not result in increased prevalence of child abuse, we should be cautious when interpreting these results because the number of violence incidents could have been under-reported as a result of the lockdown and school closure. Therefore, we believe that the concerns about an increase in child abuse cases during the pandemic –although not statistically evidenced– still hold.

**5806**

## A QUALITATIVE ASSESSMENT OF THE LANDSCAPE AND DYNAMICS OF CAPACITY STRENGTHENING INITIATIVES FOR MALARIA MODELING IN AFRICA

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Modelers in malaria-endemic countries face unique challenges in their pursuit of funding, research collaborations, and modeling training. Through in-depth interviews, this study aims to gathered malaria modeler perspectives on capacity strengthening and training initiatives, delved into the dynamics of implementing partner and modeler relationships with NMCPs and generated recommendations for equitable research collaborations and training initiatives. Preliminary fFindings revealed several opportunities for improving intercountry research collaborations, training, and communication with NMCPs. While there are were mixed perceptions about the role modelers should play in their collaborations with NMCPs, respondents largely agreed that modelers should be exploring questions that are relevant to NMCPs and technical support approaches should be more aligned with the needs of each NMCP. Respondents also highlighted that while communication with NMCPs can be challenging, maintaining flexibility, being highly responsive, and identifying multiple points of contact within NMCPs can help mitigate these challenges. As the study progresses, we will dive more deeply into the role of funders in creating better alignment among technical support and training activities. We will also dive more deeply into how technical support for NMCPs can be improved to generate better buy-in and greater sustainability.

**5807**

## EPIDEMIOLOGICAL PROFILE OF ASYLUM SEEKERS AT THE US-MEXICO BORDER: ASSESSMENT OF DISEASE BURDEN IN A MATAMOROS MIGRANT SETTLEMENT CAMP FROM NOVEMBER 2019 TO MARCH 2021

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Asylum seekers at the US-Mexico border face significant health challenges. While select humanitarian groups deliver primary care at the border, there is a paucity of population-level data on disease incidence among this vulnerable population. Understanding asylum seekers' epidemiological profile is important for migrant health delivery and anticipating health needs upon entry to the US. The aim of this retrospective study was to characterize disease incidence among asylum seekers in the Matamoros, Mexico, encampment. De-identified health records from Nov 2019 to Mar 2021 were obtained from the NGO-run camp clinic. Logistic regression models estimated associations between health outcomes and demographic factors, including age, sex, country of origin, and migration time (time since departing the country of origin). From Nov 2019 to Mar 2021, 11,307 unique patient encounters were recorded. Patients were mostly female (59.9%) and from Central America (67.6%), with a median age of 27.0 (IQR: 9.4-36.8) years and median migration time of 4 months (IQR: 0.1-0.7 years). Acute respiratory diseases were of highest incidence (10.1%; 95% CI: 9.6-10.7), with the highest risk among children (aOR = 1.87, 95% CI: 1.65-2.13) and patients with migration time under 0.5 years (aOR = 1.68, 95% CI: 1.46-1.93). Infectious and parasitic diseases, including mycoses, helminthiases, pediculosis, and protozoal diseases, were more likely to occur among patients with longer migration times (aOR = 1.59 (95% CI: 1.14-2.19) for migration time of 0.5-1 year; aOR = 2.00 (95% CI: 1.23-3.14) for migration time of 1-2 years). Among these, mycoses resulted in the highest incidence (0.7%, 95% CI: 0.6-0.9). Logistic regression analyses found that infection with mycoses was significantly associated with older age, while parasitic diseases, pediculosis, and acariasis were more significantly associated with children and younger adults. These findings have important implications for clinicians, NGOs, and policy makers working to provide healthcare to asylum seekers at the US-Mexico border, including targeted infectious disease reduction and treatment strategies.

**5808**

## EXPLORING THE POTENTIAL OF POLICY IMPLEMENTATION STRATEGIES AS HEALTH JUSTICE-MAKING TOOLS: AN ILLUSTRATIVE CASE OF NEGLECTED TROPICAL DISEASES MASTERPLAN IN ZAMBIA.

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Neglected tropical diseases (NTDs) pose a considerable challenge in Zambia, with more than 12 million people at risk of at least one NTD. Social factors play a critical role in disease transmission and effectiveness of control and elimination measures. National NTD Masterplans are the main policies used in endemic countries, outlining strategies that improve access to key interventions like mass drug administration that facilitate progress towards disease elimination. Implementation processes of Masterplans may result in relational inequities as policy strategies are translated across administrative levels and among different actors. Consequently, populations such as migrants and mobile populations are hardly reached by evidence informed interventions. Policy processes have had limited impact in reducing inequities as proposed options such as collaborative governance are simple yet inequities are a wicked, ambiguous and contested problems. The problematization of NTDs in policy has resulted in a biomedical focus that limits social and policy research, which address key issues like health inequities arising during implementation. Further, few policy studies have systematically evaluated implementation processes and strategies translating NTD Masterplans into accessible services within communities. We use a case study of the process of developing policy implementation strategies for Zambia's National NTD Masterplans to understand if these findings help in shifting our understanding of transformative implementation bringing us closer to realizing the goal of health equity and justice. We evaluate the potential of equity focused implementation strategies to promote transformative implementation of health policies in Zambia. We make a theoretical proposition that implementation strategies developed using Africana critical theories combined with implementation science frameworks can modify policy implementation processes to transform the social, economic and political contexts into which health policies are implemented, reducing health inequities and improving the fit of these policies in their contexts.

5809

## NATIONAL GUIDELINES AND LEGISLATION CONCERNING THE MANAGEMENT OF ZIKA VIRUS INFECTION IN PREGNANT WOMEN DURING THE 2015-2018 EPIDEMIC IN LATIN AMERICA

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During the 2015-18 Zika Virus (ZIKV) epidemic in Latin America, microcephaly and congenital abnormalities were shown to be associated with ZIKV infection in pregnancy. Given the multitude of malformations beyond microcephaly experienced with maternal ZIKV infection and the uncertainties associated with diagnosis, the challenges regarding policies for response to an infection in pregnancy including termination options became evident. We examined fourteen documents from eight selected Latin American countries containing the management and guidance for ZIKV infection during pregnancy. Per country, we concentrated on the availability of guidelines, laboratory diagnosis practices, ultrasound screening schedules for standard and at-risk pregnancies, communication of laboratory results of infection, antenatal checkup visits modifications, the legal framework around abortion and their approaches of adaptations as a reaction to the epidemic. All guidelines considered pregnant women with ZIKV infections as high-risk pregnancies that needed to follow stricter protocols and agreed to use molecular RT-PCR test as the diagnostic method of choice. There were discrepancies between country protocols of reporting PCR results to the pregnant women. All guidelines, except for Brazil and Bolivia, have specified their ultrasound scans (US) follow-up schedule in case of ZIKV infection during pregnancy. There was notable increased demand for access to legal abortions during the ZIKV epidemic in included countries, clandestine abortions potentially have substantially surpassed these numbers. The included examined guidelines, apart from Ecuador, have not included early termination issues in its explicitly stated ZIKV-related guidelines. The fear of ZIKV-related congenital abnormalities puts abortion at the center of moral concerns. Our study suggests that the impact of guideline and policy response is an important public health intervention, and early investigation of protocols and policy impacts in real-time during emerging disease events is recommended.

5810

## IMPACT EVALUATION OF SOCIAL MEDIA CAMPAIGN TO IMPROVE ATTITUDES AND BEHAVIORS ON COVID-19 VACCINE IN AFRICA: DIFFERENCE-IN-DIFFERENCE ANALYSIS USING TANZANIA AS A CASE STUDY

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In Tanzania, the One by One: Target COVID-19 campaign was launched nationally in July 2022 to address the prevalent vaccine hesitancy and lack of confidence in COVID-19 vaccines. The campaign mobilized influencers to use social media and viral content to increase COVID-19 vaccine confidence and reduce hesitancy, with the ultimate goal of increasing COVID-19 vaccine uptake in the country.We used programmatic data collected through an online survey before and after the campaign to empirically assess the impact of the campaign on three outcomes: vaccine confidence, vaccine hesitancy, and vaccination status. We conducted a difference-in-difference (DiD) analysis and performed a crude, adjusted, and propensity score-matched analysis for each study outcome. Lastly, to observe whether there was any differential impact of the campaign across age groups, we repeated the analyses on age-stratified subgroups. Data included 5,804 survey responses, with 3,443 and 2,362 responses collected before and after the campaign, respectively. Although there was only weak evidence of increased COVID-19 vaccine confidence in the campaign-exposed group compared to the control group across all age groups, we observed a differential impact among different age groups. While no significant change was observed among young adults aged 18-24 years, the campaign exposure led to a statistically significant increase in vaccine confidence (weighted/adjusted DiD coefficient=0.76; 95% CI: 0.06, 1.5; p-value=0.034) and vaccination uptake (weighted/adjusted DiD coefficient=1.69.; 95% CI: 1.02, 2.81; p-value=0.040) among young adults aged 25-34 years. Among adults aged 35 years and above, the campaign exposure led to a significant decrease in vaccine hesitancy (weighted/adjusted DiD coefficient=-15; 95% CI: -21, -8.3; p-value<0.001).The social media campaign successfully improved vaccine hesitancy, confidence, and uptake in the Tanzanian population, albeit to varying degrees across age groups. Our study provides valuable insights for the planning and evaluation of similar social media communication campaigns aiming to bolster vaccination efforts.

5811

## USING MALARIA SURVEILLANCE AT ANTENATAL CARE TO DECODE LOCAL PATTERNS IN SEASONAL TRANSMISSION TREND IN TANZANIA, 2014-2022

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Optimal deployment of many malaria control interventions relies upon accurately characterising seasonal peaks in transmission. In West Africa, there exist largely validated rubrics defining seasonality based on rainfall patterns. In East and Southern Africa, rainfall also often exhibits strong seasonality, but other factors, including temperature, land use and bodies of water, are substantially different to the Sahel. Meanwhile, interpreting seasonal transmission patterns in case-reporting systems remains challenging, especially in the context of chemoprevention efforts for children. Malaria screening at antenatal care (ANC) enrolment has been offered routinely to pregnant women throughout Tanzania since 2014. Here, we use monthly summary reports of these tests from 2,437 health facilities across all 184 districts in Tanzania from 1 January 2014 to 31 December 2022 to characterise seasonality patterns across the country. We fit an existing malaria model using particle Markov chain Monte Carlo to account for the lagged nature of prevalence and infer seasonal patterns in incidence across the country. Pearson correlation coefficients measured synchronization

between this estimated incidence and rainfall. Our results suggest the extent to which rainfall explains malaria seasonality varies across the country. Central regions of Dodoma and Iringa had high correlation (r = 0.77 and 0.62, respectively). In contrast, correlation in regions close to major lake or irrigation systems varied from positively (Rukwa, r = 0.76) and negatively (Geita, r = 0.41) correlated to no correlation (Mwanza, r = -0.005). Another motivation for incorporating an existing malaria model within our framework is to provide near-real-time sub-nationally-targeted estimates of the likely impact of current and future interventions – a use case we will demonstrate by projecting Seasonal Malaria Chemoprevention impact. These results highlight the wider value of ANC-based malaria screening data as a tool to inform responsive and locally tailored control strategies.

**5812**

## INTEGRATION, EXPLORATION AND REUSE OF CLINICAL AND EPIDEMIOLOGICAL DATASETS: A CASE STUDY USING MALARIA DATA ON THE CLINEPIDB PLATFORM

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Open access to data from epidemiological studies has tremendous potential to preserve research outputs, increase secondary data use, and accelerate discovery and translational impact. Data sharing is increasingly a requirement for funding and publication of epidemiological research, but it comes with technical and ethical challenges. We present a case study of successful data sharing from the clinical epidemiological database, ClinEpiDB.org, built in 2018 to facilitate access to de-identified data from large, high-quality global health studies. ClinEpiDB currently hosts data from over 1.2 million participants representing 37 global studies in three major domains - malaria, maternal, newborn & child health, and neglected tropical diseases. The PRISM2 team, an International Center of Excellence for Malaria Research (ICEMR), conducted a cohort study of malaria in Uganda and collected socioeconomic, demographic, clinical, entomological, and other data, and was interested in data sharing to maximize use and impact of their research and to meet funder and journal requirements. De-identified data was securely transferred to ClinEpiDB along with codebooks and other contextual metadata. PRISM2 variables were ontologically harmonized for increased interoperability, and after extensive quality checks, data was released on the free, open-access, online data platform ClinEpiDB.org. In their publication, the PRISM2 team included a link to recreate key findings of PRISM2 analyses on ClinEpiDB. Readers can follow the link to learn about PRISM2 cohort study methodologies, discover additional variables collected but not included in the published analysis, download data with no restrictions, and modify their copy of the published analysis to explore their own hypotheses in a point and click interface. Metrics reveal that PRISM2 data is being accessed regularly even three years after publication. The PRISM2 team gained visibility while retaining ownership of data and making all data access decisions. ClinEpiDB will expand in 2023 with integration of new datasets as well as enhanced visualization tools and the ability to derive variables.

**5813**

## IMPROVING EFFICIENCY IN DETECTING ANOMALIES IN HEALTH SUPPLY CHAIN DATA USING AN AUTOMATED CONSUMPTION ANOMALY DETECTION TOOL IN ZAMBIA

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Identifying data anomalies help correct data errors and improve accuracy in supply forecasting and planning for medicines. However, it is challenging to detect anomalies from over 3000 facilities and over 5000 health products through eLMIS, because of the lengthy and less accurate manual process given that the system is not designed to automatically generate such reports. In 2019, the USAID Global Health Supply Chain Program-Procurement and Supply Management (GHSC-PSM) project developed an automated data analysis tool for detecting consumption anomalies and establishing the causes of the data errors using Python and Applied Statistical Process Control (SPC). This tool uses eLMIS data over a period of 24 months to determine the moving average of consumption over a set threshold and then uses the result to detect anomalies monthly. The automated system streamlined the process and improved the accuracy of anomaly detection from less than 100 a month with a manual process to over 200 detections monthly, using about 25 minutes to produce a list of all anomalies for over 3000 facilities and over 5000 health commodities, thereby improving accuracy and speeding up decision-making for all health products, including malaria, commodities. The anomaly report is shared monthly with the Central Pharmacy Unit of the Ministry of Health and the Chief Provincial Pharmacists to investigate and address data quality issues. The National Drug Theft Task Force (NDTTF) and provincial task forces also use the report to investigate anomalies that are not linked to incorrect data entry through on-the-spot checks at health facilities. Since October 2019, GHSC-PSM has supported the MOH by using this tool to identify and correct several data quality issues such as incorrect use of units of measure and transpositions errors. The anomaly detection tool replaced a time-consuming and less accurate manual process, allowing supply chain actors to correct data quality issues quickly and adjust supply plans accordingly. The tool is open-source and can be easily adopted by other countries to detect anomalies or other quality issues.

**5814**

## COMBINING ORTHOGONAL ANALYTICAL TECHNIQUES TO IDENTIFY SUBSTANDARD OR FALSIFIED FORMULATIONS OF PHARMACEUTICALS

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Near infra-red (NIR) technology is a powerful tool to assess the quality of pharmaceutical dosage forms. The NIR spectrum is sensitive to multiple factors: the identity and concentration of analyte, but also the excipient materials, capsule opacity and color, and pill coatings. Most NIR instruments use libraries of authentic products to discriminate between good quality and bad quality products, but assembling and maintaining those libraries is difficult in many low- and middle-income countries because markets are not stable and there are unreported changes to product formulations. Therefore, we have been developing methods to identify substandard or falsified pharmaceuticals that do not rely on libraries of authentic products. In this poster, we will report on several aspects of this work. Machine learning models were trained on lab-formulated mixtures of a target API with different excipients. Although the ML models all have “blind spots”, a simple voting algorithm compensates for the blind spots. The algorithm was applied to 40 samples of good and bad quality acetaminophen dosage forms and was able to classify them with accuracy of 94%. Capsule opacity and color is known to interfere with in-situ NIR analysis of dosage forms. We probed twenty different empty gelatin capsules with scanning electron microscopy and X-ray fluorescence, finding different amounts of opaquing agents and dyes that could be distinguished by clustering analysis of the NIR spectra of materials inside these capsules. NIR methods can be complemented by other field analysis methods, such as chemical color tests applied using paper analytical devices (PADs). PADs are engineered micro-fluidic systems that analyze capsules or

tablets for chemical constituents by using stored reagents and water flow, generating unique color patterns. These color patterns were used to train a convolutional neural network and other machine learning tools, giving accurate classification of 20 types of APIs. Hybrid classification algorithms were developed to combine the data from both PADs and NIR, which significantly improved the results better than either method if used alone.

5815

## USING THE DISTRIBUTION OF CLINICAL DATA FROM ROUTINE USE OF AN ELECTRONIC CLINICAL DECISION SUPPORT ALGORITHM TO IDENTIFY CLINICAL SKILL GAPS IN PRIMARY CARE IN RWANDA: A RETROSPECTIVE ANALYSIS

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Clinical Decision Support Algorithms (CDSAs) that guide clinicians throughout the consultation have the potential to enhance quality of care in resource-limited outpatient settings. However, the extent of the improvement depends on the accuracy of the inputs entered, which depends on the clinical skills (ability to identify specific signs or symptoms). In this retrospective analysis, we used data from an electronic CDSA deployed in 16 health centers in Rwanda for one year to evaluate the clinical skills of primary care clinicians, identify and understand the gaps, with the goal of designing an eLearning platform to address them. We analyzed data from 20,204 consultations with children aged 1 day to 14 years, conducted between November 2021 and October 2022, focusing on a set of numerical variables: temperature, MUAC, weight, height, z-scores (MUAC for age, weight for age, and weight for height), respiratory rate, blood oxygen saturation, and heart rate. Based on statistical summary measures (median, IQR, distribution, % of missing values) and their variation in individual health centers as compared to the average, we identified 11 health centers with potential important clinical skill gaps, signaled by high frequency of skipping measurements, entering the same plausible value repeatedly, and entering implausible/likely incorrect values. We then observed 209 consultations in the problematic health facilities to understand the potential causes of errors. These field observations showed that 19% of measurements were skipped -respiratory rate behind the most problematic one (43%) - due to misplaced equipment, not considering it necessary, or the child being agitated. More frequently (57%), measurements were done incorrectly -the worst ones being weight (82%), MUAC (77%), temperature (75%), and height (69%). As a next step in the project, we are creating eLearning content that is tailored to the clinical skill gaps we observed. The eLearning modules will be introduced to the health workers in June 2023, and their impact evaluated through re-analysis of the CDSA data around August or September 2023.

5816

## ELECTRONIC DATA CAPTURE IMPLEMENTATION DOCUMENTING MASS DEWORMING CAMPAIGNS:

## PILOT ANALYSIS IN THE DOMINICAN REPUBLIC

## 2019-2021

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High-quality data is critical to evaluating outcomes and interventions in low- and middle-income countries (LMICs). Health information and communication tools (ICTs) are increasingly being used to improve data documentation. However, ICT evaluation remains limited. Without proper study, several pilots fail to scale due to lack of funding and stakeholder engagement. In partnership with the Children's Hospital of Philadelphia, the Niños Primeros en Salud (NPS) clinic in Consuelo, Dominican Republic implemented electronic data capture (EDC) to document and evaluate biannual deworming campaigns. This study evaluates the data quality, efficiency, and efficacy of this pilot, adding to the literature on ICT utilization in LMICs. EDC was implemented via tablet-based software CommCare. All children registered at NPS ages 2-5 years with no deworming in the prior 4 months were eligible. Providers visited nine neighborhoods and documented deworming during the visit. This is a retrospective post-implementation analysis of five distinct campaigns between 2019-2021. Descriptive statistics examining demographics and EDC data quality, efficiency, and efficacy were conducted. Analysis of 503 subjects showed 78.7% were dewormed at least once in similar proportions across target neighborhoods. 52.7% of subjects were dewormed 2-4 times, and 57.6% in half or more of campaigns during which they were eligible. Deworming rates per campaign ranged from 26.1% to 66.8%. Post-EDC implementation, 78.7% of subjects were dewormed compared to 64.4% pre-implementation, with more serial dewormings. Only two data fields were missing data. Median task completion time was 5.0 seconds. Median cloud upload time was 16.5 hours. EDC was an efficient, efficacious tool in community deworming campaigns in this rural setting. EDC ensured better data quality, surveillance, and outreach, with improved deworming rates compared to pre-implementation. While EDC holds promise for low-resource settings reliant on community campaigns, funding remains limited due to lack of study. Evaluation is critical to scaling promising pilots and bridging the digital divide.

5817

## UNDERSTANDING THE 'CONTEXT' IN TROPICAL DISEASE CONTROL COMMUNICATION: A SRI LANKAN EXPERIENCE

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Preventing NTDs in LMICs requires a collaborative, cross-disciplinary, culturally appropriate approach that goes beyond biomedical interventions. Effective communication is key to controlling tropical diseases, however in LMICs, communication strategies are scarcely informed by scientific evidence. Media ethnography may capture the context-specific communication channels used in everyday community life, including modern and traditional media. We conducted an ethnographic study by using multiple techniques, including participant observation, in-depth interviews, key informant interviews, and auto-ethnographic diaries, to explore community-specific communication pathways and techniques for preventing cutaneous leishmaniasis in rural Sri Lanka. We engaged with local communities to Explore their communication practices and networks and identified various communication types and forms. The modes of communication identified and discussed by the community for disease prevention activities included interpersonal, group, public, mass, and virtual methods of communication, as well as verbal, non-verbal, performing, written, audio, visual, formal, and informal forms of communication. We identified that rural communities prefer and perceive more effective traditional communication methods, such as Loudspeaker, Group communication, schools, door-to-door campaigns, posters, notice boards, leaflets, and street dramas, to mass media in disseminating health information. We also observed the critical role of community networks in gathering and sharing health information. Our study highlights the need for evidence-based communication strategies that are context-specific and tailored to local communities. Using ethnographic research methods, we can better understand rural communication practices and networks and develop context-specific health communication models to prevent NTDs.

5818

## MATCHING DATA FOR THE STATE PARTIES SELF-ASSESSMENT ANNUAL REPORTING (SPAR) TOOL FROM 2010 TO 2021

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Since 2010, States Parties have been required under the International Health Regulations (IHR) to submit self-assessment annual reporting tool (SPAR) to monitor, track and evaluate compliance on IHR's core capacities, as a key component to World Health Organization (WHO) monitoring and evaluation frameworks (MEFs). Each year data is collected, stored and made accessible in the Electronic State Parties Self-Assessment Annual Reporting Tool (e-SPAR), a web-based platform hosted by the WHO. These data can be accessed by both States Parties and the public to track progress and to identify priority areas for national action. Since its inception, the SPAR has undergone three revisions, most recently in 2021. With each revision, capacities and indicators to assess country health security capacity have changed to improve measurement towards compliance. The revisions, despite their value-added to governments and global health researchers, prevent comparative analysis between tools, hampering country ability to understand systemic challenges to sustained capacity building. This study aimed to develop a method to match data across the three revisions of the IHR self-assessment tool, and to provide a single source to access and compare national, regional and global annual self-assessment scores for IHR compliance. Annual reporting data from 2010 to 2021, for 196 States Parties, was collected and analyzed. From these analyses, we developed a matching framework and aligned MEF data, providing a robust assessment at the capacity level. Ultimately, these analyses have resulted in the creation of a publicly available tool which visualizes IHR MEF self-assessment scores from the period of 2010 to 2021 for all reporting countries with the ability to compare scores over the three MEF revisions for the first time, providing insights on health systems strengthening at the national, regional, and global levels.

5819

## RECONSIDERING THE INFECTION RISK OF JAPANESE ENCEPHALITIS VIRUS IN AUSTRALIA

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Japanese encephalitis virus is a leading cause of viral encephalitis worldwide, causing life-changing disability and death in humans. Historically, the disease has only been recognised in Australia in small outbreaks in far northern sub-tropical regions, fuelled by independent introductions. However, since early 2022, the disease has been identified in 35 people and at least 85 piggeries across the central and eastern Australian mainland, including the temperate far south. The transmission cycle of the mosquito-borne virus is complex, with many reservoir species. In this work, we model the geographic distributions of suspected wildlife host and vector species using environmental data. We use the predictions of these environmental niche models to visualise the potential spatial extent of disease transmission during the recent outbreak. By incorporating heterogeneous surveillance effort and detection data we also map the intensity of detected disease transmission. We consider how the outputs of our models and their associated uncertainties might quantitatively inform the spatial allocation of surveillance resources within a structured decision-making framework. This framework will support decision-makers to best respond to future outbreaks of Japanese encephalitis virus in Australia.

5820

## USING MACHINE LEARNING TO PREDICT SURGICAL OUTCOMES OF PATIENTS WITH HYDROCEPHALUS POST INTERVENTION AT CURE CHILDREN'S HOSPITAL OF UGANDA

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The major objective of this research is to predict an outcome after surgical intervention of an individual using a machine learning approach. This study was carried out at Cure Children's Hospital of Uganda which is found in the Eastern part of the country about 240 km from Kampala, the country's capital city. This hospital also serves as the country's referral hospital for patients suffering from pediatric hydrocephalus and spina bifida. The data was collected retrospectively from the medical records kept at the hospital premises. A well-trained clinician assisted in data extraction using a questionnaire that was well designed in Redcap, a web-based software used to create and manage research databases and the data extracted covered a period between 2015 and 2019. The variables extracted include; age at presentation to the hospital, surgical procedures carried out, age at the time of head increase, head circumference, place of birth, the season of birth, gestational age at birth, hydrocephalus etiology, child's weight, WBC, CSF volume and brain volume before surgery after carefully doing a literature search. The outcome variable is a binary outcome, a surgical failure or success after surgical intervention. Exploratory data analysis and feature selection were done. A predictive model was developed using a machine learning algorithm of random forests. This model uses mean square error for regression and mean Gini for classification. Model performance was evaluated on a successful surgical outcome by Area Under the ROC curve (AUC), accuracy (AC), specificity (SP), sensitivity (SN), negative predicted (NPV) and positive predicted value (PPV). The model showed a good performance (AUC = 0.773, AC = 0.72, SP = 0.659, SN = 0.751, NPV = 0.586, PPV = 0.772). The prediction outcome can be used by the surgeons to make informed decisions pertaining to patient monitoring and the need for surgery revision before discharging them from the hospital.

5821

## A SYSTEMATIC REVIEW OF THE DATA, METHODS AND ENVIRONMENTAL COVARIATES USED TO MAP AEDES-BORNE ARBOVIRUS RISK

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Aedes (Stegomyia)-borne arboviruses pose a global threat, but gaps in surveillance hinder comprehensive assessments of risk. Geostatistical models combine data from multiple locations and use links with

environmental and socioeconomic factors to make predictive risk maps, allowing for targeted interventions. We systematically reviewed past risk mapping approaches for these arboviruses from local to global scales to identify similarities and differences in data types, covariates, and models. We searched online databases for predictive risk mapping studies for dengue, Zika, chikungunya and yellow fever with no geographical or date restrictions. We included studies that needed to parameterize or fit their model to real-world data and make predictions to new spatial locations of some measure of the population-level risk of viral transmission. Study quality was assessed with a modified scoring criteria based on the EPIFORGE checklist. We found a methodological shift in risk mapping through time based on 183 papers. We found that earlier approaches to map risk pooled occurrence data and used high dimensional machine learning models to map suitability for transmission at global or continental scales. Following major epidemics of Zika and chikungunya in the Americas, mechanistic models fit to national-resolution incidence data have been increasingly used to track the dynamic spread of epidemics among countries. With improved dengue case-based surveillance systems now present in many countries, statistical mixed effects models applied at the subnational scale have become increasingly common. Half of the studies reviewed utilized temperature and rainfall as covariates, with human mobility increasingly considered. A robust variable selection procedure was performed in 33 out of 148 studies that did not use mechanistic models or only random effects. Our review shows that approaches to map risk for different arboviruses have diversified based on changing use cases, epidemiology, and data availability. Future studies should consider the purpose of the map; to maximize improvements in data quality and statistical methodologies.

**5822**

## ALTERNATIVE EPIDEMIC INDICATORS FOR COVID-19: A MODEL-BASED ASSESSMENT OF COVID-19 MORTALITY ASCERTAINMENT IN THREE CITIES IN LOW-INCOME COUNTRIES

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Not all COVID-19 deaths are officially reported and, particularly in low-income and humanitarian settings the magnitude of such reporting gaps remain sparsely characterised. Alternative data sources, including burial site worker reports, satellite imagery of cemeteries and social-media-conducted surveys of infection, may offer solutions. By merging these data with independently conducted, representative serological studies within a mathematical modelling framework, we aim to better understand the range of under-reporting using the example of three major cities: Addis Ababa (Ethiopia), Aden (Yemen) and Khartoum (Sudan) during 2020. We estimate 69% - 100%, 0.8% - 8.0% and 3.0% - 6.0% of COVID-19 deaths were reported in these three settings, respectively. In future epidemics, and in settings where vital registrations systems are absent or limited, using multiple alternative data sources could provide critically-needed, improved estimates of epidemic impact. However, ultimately, functioning vital registration systems are needed to ensure that, in contrast to COVID-19, the impact of future pandemics or other drivers of mortality are reported and understood worldwide.

**5823**

## GLOBAL HEALTH INFRASTRUCTURE DEVELOPMENT IN LOW AND MIDDLE INCOME COUNTRIES, THROUGH MEDICAL EQUIPMENT REMANUFACTURE: POTENTIALS, PROSPECTS AND CHALLENGES IN NIGERIA

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Sustainable healthcare delivery through provision of adequate medical equipment is a very critical component of global health. Remanufacture of medical equipment (ME) in low and middle-income countries (LMICs) can be a cost-effective and sustainable way to improve access to healthcare services. Remanufacturing and reverse logistics of remanufactured products are increasingly becoming popular in many developed countries due to several reported benefits, however, most developing countries are yet to fully adopt and integrate ME remanufacturing into their healthcare systems. The objective of the study was to identify the potentials, prospects and challenges impeding the adoption and implementation of ME remanufacture, including reverse logistics in a resource limited country such as Nigeria. The study identified and contacted various stakeholders from relevant professional backgrounds, such as clinicians, engineers, technicians, and policy makers, who are currently working in public and private sectors, academia, biomedical industries, and in healthcare facilities. Information was obtained using semi-structured interviews and focused group discussions allowing participants to give feedbacks which was analysed and ranked in order of significance The following were highlighted as key factors affecting the implementation of remanufacture in Nigeria; lack of adequate power supply, resource constraint, inadequate facilities and infrastructural deficit to support remanufacture, lack of database to keep track of medical equipment lifecycle, shortage of skills and expertise in remanufacture, lack of spare parts and poor coordination of raw materials extraction from equipment core. Overall, remanufacturing medical equipment in LMICs has the potential to improve access to healthcare services and reduce healthcare costs, while also promoting sustainability and reducing waste. However, it requires policies supporting significant investment in skills development and infrastructure, as well as ongoing monitoring and evaluation to ensure that the remanufactured equipment meets the necessary standards.

**5824**

## PLOTTING A PATH THROUGH THE PLASMODIUM VIVAX TREATMENT DILEMMA: A MODELLING STUDY INTEGRATING INDIVIDUAL-LEVEL OBSERVATIONS FROM PRIMAQUINE TRIALS AND POPULATION-LEVEL TREATMENT EFFECTS

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Upon primary infection, some Plasmodium vivax (Pv) parasites develop into hypnozoites that lie dormant in the liver for weeks to months before reactivating to cause relapses. Treatment of Pv thus calls for radical cure, a type of therapy that clears parasites in both blood flow and the liver. Several randomised control trials (RCTs) have compared drug regimens with the liver stage drugs primaquine (PQ) and tafenoquine (TQ). While it is possible to estimate the individual-level efficacy of these drug regimes, their population-level impact still must be evaluated in cluster randomised trials. Such trials are necessary to measure the non-linear effects on transmission due to lower, heterogeneous Pv circulation and decreased population immunity. We developed an infection risk model and fit it to IMPROV trial data (two PQ regimens and a control arm trialled in multiple locations) assuming relapse and biting rates to be location-specific, but

drug efficacy to remain constant across locations. Next, we estimated the population-level impact of introducing radical cure in case management using an existing Pv transmission model covering multiple scenarios that vary by transmission intensity, seasonality, Pv relapse rates and care-seeking behaviour. Combining our efficacy estimates with hazard ratios and adherence data from RTCs, we tested several radical cure regimens varying dosage and duration of administration. The efficacies estimated for the 7- and 14-day IMPROV PQ regimens (7 mg/kg total dose) were of 81% (95% CI: 66%-96%) and 86% (95% CI: 72%-99%), respectively. Introducing radical cure may make elimination feasible where transmission is already low (<2% PCR-prevalence). As transmission intensity increases, the efficacy of radical cure is vastly reduced and differences between regimens even out. To date PQ and TQ have been tested under trial conditions, while real-world implementations introduce many constraints that hamper their population-level impact. Rather than focusing on optimal dose and duration of administration, it might thus be more effective to increase adherence and care-seeking rates, and to widen eligibility criteria.

**5825**

## AN EXPLAINABLE MACHINE LEARNING APPROACH IN THE PREDICTION OF MORTALITY AMONG PEDIATRIC PATIENTS HOSPITALIZED WITH ACUTE GASTROENTERITIS IN WESTERN KENYA

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Diarrhea is the second leading cause of death in children <5 years globally. Mortality prediction scores for diarrhea are currently unavailable but could provide opportunity for early detection of patients at-risk of death following an episode of acute gastroenteritis (AGE). We built and evaluated machine learning (ML) models to predict mortality among pediatric patients admitted with AGE (≥3 loose stools and/or ≥ 1 episode of unexplained vomiting followed by loose stool within a 24-h period) at Siaya County Referral Hospital between 2010-2020. We employed 6 ML algorithms to predict mortality using de-identified data collected from the AGE cases. We evaluated the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and the area under the curve (AUC) for each of the models. We conducted explanatory model analysis (EMA) and business value evaluation (BVE) of the best model. During the study period, 12, 546 children aged <5 years from the Health and Demographic Surveillance System were admitted, of whom 2,271 (18.1%) had AGE and 164 (7.2%) subsequently died. The sensitivity ranged from 61.0%-78.0% across models, while the specificity and AUC ranged from 71.7%-78.7% and 74.3%-82.6%, respectively. The Random Forest (RF) was the best model achieving 78.0%, 76.6%, 20.6%, 97.8% % and 82.6% for sensitivity, specificity, PPV, NPV and AUC, respectively. The SHAP attributions for the top 5 most important features from the EMA for the RF model were chest indrawing (-0.09), nasal flaring (-0.07), wasting (0.03), stunting (-0.03) and malaria (-0.02). From the BVE, the RF model was able to identify 3 times higher number of deaths compared to a random selection if we picked the top-20% cases based on model probability, and it was able to select 60% of overall deaths from the same selection. We did not observe broad variations in the performance of the models. These findings demonstrate alternative algorithms for prediction of patients at risk of death for targeted interventions to increase chance for survival following an episode of AGE.

**5826**

## TROPICAL TROUBLES: NEURASTHENIA AMONG MISSIONARY EX-PATS IN AFRICA, 1900-1945

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Discussions of "tropical neurasthenia" (TN) abounded in early 20th century medical journals. TN provided specialists in the emerging field of tropical medicine with a diagnostic classification to explain the vague symptoms that North American and European ex-pats often complained of in tropical climates. The condition was also used to account for high rates of invaliding among colonial staff and missionaries. One 1913 report in the British Medical Journal estimated that NT invalided 20% of all missionaries at tropical posts. Climate-based theories concerning the aetiology of NT were common. Some experts blamed not simply "tropical light" itself but exposure to its different spectra. Others blamed climate, heat, humidity, and altitude, while others cited ex-pats' contacts with "diseased or depraved indigenous peoples or simply the sense of loneliness and despair." Symptoms included irregular heartbeat, irritability, loss of appetite, sexual dysfunction, depression, and even suicide. Relying primarily on archival and published primary sources, this presentation will examine the rise and fall of TN as a clinical entity during the first quarter of the 20th century.

**5827**

## LOCAL PERCEPTIONS OF YELLOW FEVER OUTBREAKS IN UGANDA: A QUALITATIVE STUDY

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Yellow fever (YF), a mosquito-borne viral hemorrhagic fever, is endemic in Uganda and causes frequent outbreaks. We investigated the largest YF outbreak to date in Uganda and three subsequent smaller outbreaks. The aim of this study was to explore local perceptions of YF outbreaks to inform future public health campaigns. In this qualitative study we conducted 43 semi-structured interviews, 4 focus group discussions and 10 expert interviews - in total 76 participants. Data were collected in six affected districts with yellow fever outbreaks in 2010 and 2016. We included vulnerable groups such as elderly people ≥ 65 years and pregnant women. Participants perceived YF as a deadly disease. Although signs and symptoms of YF were broadly known the disease was frequently confused with newborn icterus, severe malaria, and hepatitis. Despite excessive awareness campaigns participants believed YF could be contracted by multiple pathways such as mosquito bites, airborne, close contact, sexual intercourse, vertical transmission, lack of hygiene, and through excessive consumption of yellow foods. Furthermore, people in remote areas affected by YF outbreaks were frequently unaware of the cause of outbreak. If a disease and its transmission pathway is not understood preventive measures cannot be successfully implemented. Moreover, timely diagnosis and reactive outbreak measures may be delayed. Thus, we recommend improving health education in communities at risk through village health teams, education at health centers, schools, and trusted community members. Awareness campaigns should be in conformity with local language habits. Additionally, public participation could be an important strategy to improve awareness among communities at risk.

**5828**

## PREVALENCE AND PREDICTORS OF INTENTION TO USE TOBACCO AMONG ADOLESCENTS IN OYO STATE, NIGERIA

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Tobacco use is a major public health problem globally, especially in developing countries like Nigeria. Despite the well-known risks associated with tobacco use, many individuals still initiate and continue to use tobacco. The intention of tobacco use is an important predictor of the actual use of tobacco, so therefore, understanding the associated factors is critical to developing effective tobacco prevention interventions. The goal is to assess

the predictors of intention to use tobacco among school-going adolescents in Oyo State, Nigeria. This was a cross-sectional survey among randomly selected adolescents (2,590) attending 28 secondary schools across six Local Government Areas (LGAs) in Oyo State, Nigeria. The participants' sociodemographics, knowledge, attitude, harm perception, tobacco use, SHS exposure, and intention of tobacco use were assessed using the modified GYTS questionnaire. Data were analyzed with SPSS version 25 and P-value was <0.05. Participants' mean(±SD) age was 14.6(±1.3). The majority were females (48.1%) and schooled in urban LGAs (70.8%). The prevalence of intention to use tobacco was 11.7%. Some (2.2%) were past tobacco users, 9.8% owned a tobacco-branded item, and 22.5% were against banning tobacco adverts. Some had low tobacco harm perception (40.9%), perceived that it was easy to buy tobacco (22.5%), had poor attitude to tobacco use (35.3%), and 10.5% had smoking friends. Predictors of intention for tobacco use were: schooling in urban areas (aOR:1.95;95%CI:1.40-2.71), male gender (aOR:1.45;95%CI:1.11-1.90), past tobacco user (aOR:3.18;95%CI:1.69-6.00), owning a tobacco-branded item (aOR:1.65;95%CI:1.14-2.40) and being against the ban on tobacco adverts (aOR:1.82;95%CI:1.34-2.38). Others were low harm perception (aOR:1.76;95%CI:1.31-2.25), poor attitude towards tobacco use (aOR:1.69;95%CI:1.30-2.21), perceived ease of buying tobacco (aOR:1.37;95%CI:1.02-1.84), and having smoking friends (aOR:1.71;95%CI:1.17-2.48). The intention of tobacco use among the participants was 11.7%. The predictors included individual, interpersonal, and environmental factors.

**5829**

## COMMUNITY PERCEPTIONS OF INCENTIVES FOR MINIMALLY INVASIVE AUTOPSY IN CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE (CHAMPS) IN WESTERN KENYA

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Incentives play an important role in improving participation in research and establishing ethically acceptable incentives is essential to preserving research integrity. Child Health and Mortality Prevention Surveillance (CHAMPS) uses minimally invasive tissue sampling (MITS) and careful diagnostic testing to improve understanding and prevention of child mortality. Incentives offered to participants in Kenya include transportation of the body for burial, a contribution of $40 towards funeral expenses, and payment of mortuary bills for up to 5 days. This study explored how community members perceive CHAMPS incentives. Qualitative key informant interviews (n=29), semi-structured interviews (n=11), and focus group discussions (n=5) were conducted with community members, community leaders, healthcare workers, and caregivers. Participants provided verbal consent prior to discussions. Data were analyzed using a thematic approach. Participants reported positive perceptions towards the incentives they believed were appropriate within their cultural context. While some respondents perceived them as a motivating factor, others expressed more preference for non-monetary forms of incentives and indicated that the aim of finding a child's cause of death was an adequate incentive alone. CHAMPS incentives were perceived to be well-aligned with the cultural values attached to burial-related practices and expectations. Respondents believed the educational status of the participating family influences the perception of the incentives; better-educated parents were believed to be less suspicious and view the incentives positively. Some respondents believed that research incentives could raise suspicion among community members about the true intention of the research. Overall, the findings suggest that CHAMPS has achieved a positive balance of both cultural value and financial value for participation incentives. Increased community sensitization may be needed to address negative perceptions or rumors that may be associated with research incentives.

**5830**

## BLOOD PRESSURE VARIATIONS AND THEIR ASSOCIATION WITH SOCIAL DETERMINANTS AMONG MEN AND WOMEN IN BANGLADESH

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Hypertension (HTN) is a leading risk factor for cardiovascular disease, and it is increasing in Bangladesh. Therefore, this study aimed to see blood pressure (BP) variations and their association with social determinants among Bangladeshi adults. This study performed a secondary analysis of the Bangladesh demographic and health survey 2017-18 data. A total of 20,250 households were selected and from these households, one-fourth of the households were randomly selected for BP. All adults (18 years of age or older) living in these households were invited to participate in measuring BP. Among 14,704 eligible women and men, about 90% participated. Hypertension cutoffs classify a person as hypertensive with an SBP/DBP of 140/90 mmHg or more. BP status was categorized as normal, pre-HTN, stage I HTN, and stage II HTN. This study investigated age, gender, education level, marital status, wealth quintile, body mass index, and residence as potential determinants. Sample characteristics were examined and stratified by gender because of established gender differences in HTN prevalence and compared characteristics among men and women using chi-squared tests for categorical variables. Results were considered statistically significant at P values <0.05. A total of 13,128 participants were included; the mean age (±SE) was 39.7 (±0.07) years and 56% were female. Thirty-three percent of them had pre-HTN, 14% had stage-I HTN and 7% had stage-II HTN. In terms of social determinants, stage II HTN was significantly associated with older age whereas pre-HTN was associated with younger people, and males were more likely to be affected (P<0.001). Males who were married, overweight, and from the richest families suffered mainly from stage II HTN (P<0.001). A female with poor education who lives in rural areas was significantly associated with stage II HTN and pre-HTN (P<0.001). It is crucial to lower HTN prevalence and improve control among older adults and men. Lowering the incidence of overweight may lessen the long-term burden of HTN and any associated problems. It is essential to create awareness among younger people and to prioritize rural areas.

**5831**

## THE URGENT NEED OF MOLECULAR DIAGNOSTICS IN LOW RESOURCE SETTINGS. CASE STUDY: CURE CHILDREN'S HOSPITAL OF UGANDA

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Hydrocephalus is a major indication of neurosurgery in infants worldwide. Post-Infectious Hydrocephalus (PIH), the most common form of hydrocephalus in Uganda, is a sequela of neonatal sepsis. Its diagnosis is largely based on clinical history, radiological imaging, or surgical findings because microbiological investigations using traditional culturing of CSF for microbial organism detection and identification have been largely unsuccessful. In a prospective cohort study to discover the cause of PIH at CURE Children's Hospital of Uganda, we recruited 208 consecutive PIH patients below 3 months of age and subjected their CSF samples to on-site conventional culture and PCR testing (in the USA). PCR identified virulent Mbale strains of Paenibacillus Thiaminolyticus in 47% of the processed samples while on-site conventional culture recovered no organisms. This highlights the urgent need for molecular diagnostics at point-of-care in such settings. Unfortunately, these techniques although important, are not readily available in low-resource settings like Uganda due to the high costs of setting up the infrastructure, and training personnel to perform these tests. This has limited our ability to effectively treat neonatal infections leading to the large number of PIH cases we observe at our hospital. To

bridge the gap between microbiological diagnosis and treatment of PIH at CURE Hospital, efforts have been undertaken to set up a molecular laboratory to facilitate diagnosis. An experienced laboratory technologist was recruited and trained in molecular diagnostic techniques at Penn State University, USA. Benchmark for the design of a molecular laboratory was done in collaboration with Penn State University, Yale University, and Harvard University with support from the NIH. The major challenges include: outsourcing the required equipment and reagents as they are expensive locally leaving us with no option but to purchase them from abroad. When fully functional, this laboratory will aid in the point-of-care diagnosis of causes of PIH in infants, enabling targeted and optimized treatment and clinical trials to improve patient outcome.

**5832**

## SPATIOTEMPORAL CHARACTERIZATION OF CONGENITAL MYELOMENINGOCELE IN UGANDA

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Myelomeningocele (MMC) is the commonest form of spina bifida and the most severe congenital disability of the central nervous system. Without surveillance, Uganda lacks accurate data on incidence, prevalence or geographic variation in MMC risk to guide prevention. Characterization of MMC distribution in Uganda based on treated cases and knowledge of the effect of environmental factors that directly affect crop yields or natural folate availability on MMC occurrence would guide prevention. Here, we examined; the distribution of MMC in Uganda, the presence or absence of clustering within MMC cases compared to controls (under the hypothesis of uniform distribution in space) and, the association between MMC and selected environmental factors (village elevation, temperature, rainfall, and vegetation index) using secondary data from 2916 consecutive infant cases of MMC and controls between 2018 and 2001. The environmental factors 3 months before pregnancy and the first month of pregnancy were accumulated into sums giving a single value for every patient. We created Relative risk maps for being a case compared to control using village location mapped onto the Ugandan map. We examined clustering within cases compared to controls using Ripley's K function Plots with Chetwynd and Diggle method for global clustering and, explored associations between MMC and, environmental factors in terms of odds ratios using Generalized linear Mixed Models. We found a high relative risk of MMC in the Eastern and Southwestern subregions compared to other regions. Vegetation index (OR=0.13 p-value <0.001) had the biggest effect on the odds of being a case. A high vegetation index in the months studied was strongly protective. Other environmental factors with notable effects on the odds of being a case were elevation (OR= 1.14, p-value=0.008), and temperature (OR=0.87 p-value=0.009). These findings show that MMC is neither a random event nor uniformly distributed across space in Uganda. In the absence of surveillance, seasonal risk maps based on vegetation index can guide prevention.

**5833**

## IMPACT OF CONFLICT ON THE HEALTH SERVICES: OPPORTUNITIES FOR RESILIENCE IN NORTH SHEWA ZONE, ETHIOPIA

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Recurrent conflicts pose threats to health systems, creating disruption in service provision. The conflict which occurred in northern Ethiopia, escalated to additional parts of the country affecting the health system operations, resulting in fatalities, disruption of livelihoods, widespread food insecurities, and displacement of residents. Through a qualitative approach, this study aims to assess the impact of conflict on health service provision, particularly maternal and child health services, and explore areas of resiliency that may be strengthened to mitigate the risks of conflict in the future. Data were obtained from community and facility key informants through in-depth interviews and focus group discussions to understand the challenges during the recovery period with suggestions on potential solutions. Total five overarching themes were explored: disruption in health services, social consequences, psychological implications, health system response for community support, and mitigation strategies to improve health system resilience. Results indicate shortage of staff and transportation, increased home/forest deliveries, rapes and sexually transmitted diseases, theft and destruction of property, lack of funds and resources, and insufficient and delayed support from government and other organizations were some of the reasons for poor health service provision during and immediately after the conflict. There is a need to acknowledge the requirement for additional outreach, IDP sites and temporary clinics to manage the caseloads during conflict crises and to strengthen the collaboration with community and other organizations for disaster management/preparedness to ensure that the health system is capable of absorbing shocks under future stress situations.

**5834**

## EVIDENCE GENERATION FOR TOOLS FOR SEVERITY TRIAGE OF FEBRILE PATIENTS IN LOW-RESOURCE SETTINGS: A MIXED-METHODS STUDY

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The screening and triage practice that offers severity assessment of a patient is a crucial initial step in any healthcare cascade. Effective utilization of triage could help direct resources for life-saving procedures, and improve the distribution of resources for patients. Much less is understood about the triage process in healthcare settings of low- and middle-income countries (LMICs). The main objective of this study was to describe and evaluate the triage process in an emergency room (ER) of Tribhuvan University Teaching Hospital (TUTH), Kathmandu, Nepal. The mixed-methods observational study has commenced at the ER of TUTH. The study will review ~1500 randomly selected emergency tickets from Jan to Dec 2022. The retrospective review entails collating data from recorded information on the emergency tickets including the demographic characteristics, vital signs, symptoms, triage categorization, primary diagnosis, and discharge of patients. Qualitative methods will be used to document 'patient journey mapping' through in-depth interviews (IDIs) with patients attending the ER. A total of 15 febrile participants will be interviewed upon receiving written informed consent. The interview will explore the decision-making process on pre-visit, visit, and post-visit exploring details on barriers and facilitators related to patients' journey to the ER. In addition, 15 direct observation notes will be collected at the ER that will document the triage practice. Descriptive statistics will be used to present recorded information on emergency tickets and the association between the triage category and specific symptoms or vital signs will be explored using regression analysis. All transcripts and observation notes will be collated into NVivo and analyzed using thematic synthesis. The evidence generated through this study would inform the improvement in the triage process echoing the concept of the 'learning health system' and 'universal health coverage'.

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## COMMUNITY-LEVEL USE OF ANTIBIOTICS IN RURAL BURKINA FASO: A HOUSEHOLD-BASED SURVEY USING THE DRUG BAG METHOD

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In Burkina Faso, little is known about antibiotics' use (ABU) outside the formal health system. Assuming that ABU at community level might be overlooked, the purpose of this study was to generate knowledge about the types of antibiotics commonly used at community-level and their purpose. The survey was conducted from June to September 2021 in the rural health district of Nanoro in Burkina Faso. 423 households were purposively selected based on socio-economic status. An electronic questionnaire was used for data collection through the drug bag method, consisting of purchasing locally available antibiotics for respondents to identify and specify their use. Descriptive analysis was performed in R version 4.2.1. Households' main sources of drug procurement were the primary health care facilities (76.8%) and informal drugs sellers (61.5%). Among the 33 antibiotics inventoried, amoxicillin tablets (93.4%) and oxytetracycline tablets (86.5%) were the most recognized by the study participants. Descriptive features were their color and the diseases they were believed to treat, combined with locally shared names. Amoxicillin and oxytetracycline remained the most frequently used antibiotics. There was greater use of antibiotics for gastrointestinal disorders, wounds, musculoskeletal and connective tissue disorders and skin and subcutaneous tissue disorders. However, oxytetracycline (79.9%), metronidazole tablets (57.1%) and metronidazole suspension (56.7%) were largely used for gastrointestinal disorders while amoxicillin tablets (29%) and ampicillin tablets (34.9%) were used to treat wounds. This study provides an overview of the categories of antibiotics people identified as being valuable for domestic use. Access to and use of these medicines are facilitated by the influence of informal drugs providers coexisting with formal health care providers in the community. This raises concerns about people's access to quality medicines and the inadequacy of the health system.

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## IMPACT OF THE COVID-19 PANDEMIC ON THE SURVEILLANCE AND CONTROL OF NEGLECTED TROPICAL DISEASES (NTDS) IN BRAZIL

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The Covid-19 pandemic led healthcare services to adopt contingency protocols and direct their attention to suspected and confirmed cases of Covid-19. In addition to measures to restrict people's movement, patients also avoided seeking healthcare services for fear of becoming infected. This led to a reduction in care for other health problems. We sought to quantify the impact of the pandemic on the detection of some NTDs (dengue, chikungunya, leprosy, visceral leishmaniasis, trachoma, and lymphatic filariasis) in Brazil, in the pandemic period (2020 - 2021) compared to the previous period (2015 - 2019). The number of reported cases in the pandemic years was compared to the mean number of cases in the 5-year previous period. The number of reported dengue cases fell 5.7% in 2020, in comparison to the previous period, followed by a sharp reduction in 2021 (-48%). Chikungunya cases fell 42% in 2020, and 32% in 2020. Leprosy cases fell 35% in 2020, and 33% in 2021 Visceral leishmaniasis cases fell 41% in 2020, and 49% in 2021. The national trachoma prevalence survey detected a TF prevalence <1% in all evaluation units in non-indigenous population, and below 5% in four evaluation units in indigenous people surveyed so far (national survey is ongoing). No new cases of lymphatic filariasis were detected in the last remaining focus of transmission in the country. The reduction in the number of dengue and chikungunya cases may be related to changes in services resulting from the pandemic, but it may also reflect the reduction in transmission associated with reduced mobility of people. NTDs in the final phase of elimination, such as trachoma and lymphatic filariasis, seem not to have been affected by the pandemic. The decrease in leprosy and visceral leishmaniasis seems to be related to the disruption of primary care services due to the pandemic. The Brazilian public health needs to carry out additional efforts to recover the ground lost in the pandemic.

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## SYMPTOMOLOGY, OUTCOME AND PATTERN OF RISK FACTORS OF CORONARY ARTERY DISEASE IN TANZANIA CLUSTERING AND STRATIFICATION APPROACH

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Coronary artery disease is a significant public health concern worldwide. Its burden is alarming, mainly in Low- and middle-income countries, with 75% of premature deaths and 164 million disability-adjusted life years (DALYs). Tanzania has deployed various interventions to combat disease risk factors and their aftermath, however, its burden is still high. Here, a retrospective study is conducted to identify and evaluate the distribution of symptoms and risk factors of coronary artery disease (CAD) in Tanzania to recommend appropriately tailored interventions.1673 patients who underwent coronary angiography at Jakaya Kikwete Cardiac Institute (JKCI) are analyzed based on four clusters. The lesion was defined as luminal stenosis >50% by invasive angiography. Clusters were determined by the k prototype and stratification analysis was done. The findings indicate that the prevalence of CAD was 51.3% with 56% of patients over 60 years old. Male patients composed 63% and hypertension (92%) was the commonest risk factor. Four clusters of patients are identified with a) hypertensive, diabetes, and dyslipidemia symptoms of chest pain and a higher chance of double vessel lesion, b) prior myocardial infarction (MI) and percutaneous coronary intervention (PCI) with age above 60 years old that contributed to higher prevalence and risk of triple vessel disease, c) dominant risks of alcohol use, family history and smoking resulting to triple vessel disease more than other groups and d) more female patients with a higher level of obesity and smoking. Apart from patients above 60 years old, there is a percentage of premature coronary artery disease, patients below 45 years old with modifiable risk factors. Therefore, the interventions targeting youth and women should focus on reducing unhealthy diets, physical inactivity, smoking, and alcohol abuse- this is the same for the older generation. However, adding proper patient management and counseling may result in better outcomes, reducing repeated PCI and MIs.

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## A QUALITATIVE EXPLORATION OF WOMEN PERCEPTIONS AND EXPERIENCES OF ANTENATAL CARE ACCESS IN MOZAMBIQUE

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Despite numerous efforts, maternal and newborn mortality rates in Mozambique are among the highest in sub-Saharan Africa. Poor access to health services and antenatal care (ANC) and low rates of institutional delivery are associated with maternal and newborn deaths. This study aims to understand pregnant women's perceptions and experiences of access to ANC in Manhiça and Quelimane Districts, in Southern and Central Mozambique respectively. A qualitative rapid assessment was conducted in June and July 2022. Data was collected through in-depth interviews of pregnant women recruited from health facilities and communities. Interviews were digitally recorded, transcribed and systematically coded

in inductive and deductive categories. Data were thematically analyzed. A total of 18 in-depth interviews were performed. Almost all participants explained that pregnancy is not a disease and can be quietly managed at home; this influenced the low uptake for ANC in the early stage of pregnancy. Nevertheless, participants recognized the importance of ANC during pregnancy, mainly to protect the baby's health. Pregnant women highlighted the need to prioritize labor activities such as cultivating on farms and selling in markets over going to the healthcare facility for ANC. Further, participants stated the need to "hide their belly" to avoid the threat of the evil eye and witchcraft to their pregnancy. These factors led women to delay seeking ANC until the late stage of pregnancy. Long distances and waiting times during ANC were also mentioned as factors that influence access and the experiences of pregnant women. Participants perceive pregnancy as an easily managed condition at home, and this has impacted their ANC-seeking behavior, although they recognize its importance. Education is needed to improve women's knowledge of the importance of ANC at the first stage of pregnancy and mitigate beliefs about witchcraft in order to improve ANC-seeking behavior.

**5839**

## ASSOCIATIONS BETWEEN MATERNAL AND PATERNAL STRESS, MATERNAL DEPRESSION, MATERNAL EXPOSURE TO INTIMATE PARTNER VIOLENCE, AND CHILD STRESS

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Globally, many women experience intimate partner violence (IPV), depression, and stress, which have long-term maternal and child health consequences. We assessed the potential associations between maternal and paternal perceived stress, maternal depressive symptoms, and maternal exposure to cumulative lifetime IPV, and child stress biomarkers (urinary F2-isoprostanes, salivary alpha-amylase (sAA) and cortisol, blood pressure, heart rate, and NR3C1 methylation status) in rural Bangladesh. We estimated associations using generalized additive models, adjusting for potential confounders. We measured 686 mother-child dyads at Year 1 and 1,494 dyads at Year 2, overlapping with replacement. F2-isoprostanes outcomes yielded two opposing results: a negative relationship with maternal exposure to IPV and a positive relationship with maternal depression. In the sympathetic adrenomedullary axis, we observed a negative association between paternal stress and child mean arterial pressure (MAP), and positive associations between maternal perceived stress and post-stressor sAA; maternal depression and sAA reactivity and MAP; and paternal perceived stress and resting heart rate. In the hypothalamic pituitary adrenal axis, we observed a negative association between maternal depression and post-stressor cortisol and a positive association between paternal stress and pre-stressor cortisol. The negative relationship between maternal IPV and child F2-isomers, although counterintuitive, reveals possible alternative functional roles, which reactive oxygen species might play. Maternal depression was associated with the largest and most variable number of child stress biomarkers indicating the importance of this exposure in shaping stress responses. Future studies in different geographical contexts quantifying the multigenerational implications of such stressors to determine typical and atypical child stress responses during early life are critical.

**5840**

## UTILIZATION OF PANTOGRAPH AMONG NURSES AND MIDWIVES IN LABOUR WARD AT EDWARD FRANCIS SMALL TEACHING HOSPITAL

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Partograph is a graphic record of progress of labor, maternal and fetal condition plotted against time for intrapartum monitoring (Mathai 2009). Its aim is to provide a pictorial overview of labor, to alert obstetric care providers about deviations in maternal, fetal condition and progress of labour (Lavender et al 2008). The study aimed to describe the utilization of partogram among nurses and midwives, in the labour ward at Edward Francis Small Teaching Hospital (EFSTH). A descriptive quantitative research design to gather information on utilization of the partogram among nurses and midwives in the labour ward of Edward Francis Small Teaching Hospital was employed using a convenient sampling procedure. All the nurses and midwives in the labour ward were selected who were willing to participate in the study and consent was sought from both EFSTH and the participants. Questionnaires were used to collect data and analysis was done using spss version 21. The results indicate that 80% of all of respondents knew what a partogram was. The knowledge on the function of the action line and alert line was poor amongst nurses and midwives who participated in the current study. Only 40% (N=4) of the respondents could explain the function of action line on the partogram. There was poor utilization in labour monitoring. Only 40% (N=4) were found to properly use the partogram while 60% (N=6) were found to not properly use the partogram. The findings confirm the problem of shortage of nurses and midwives with only (N=10) covering the labour ward of EFSTH for all shifts. The recommendation includes deployment of nurses and midwives to EFSTH, training of nurses and midwives on the utilization of the partogram.

**5841**

## A NOVEL VIRULENCE MODIFYING EXOTOXIN SECRETED BY PATHOGENIC LEPTOSPIRA MEDIATES DISEASE PATHOGENESIS AND IS A PAN LEPTOSPIROSIS VACCINE CANDIDATE

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Leptospirosis, a globally neglected zoonotic disease caused by pathogenic Leptospira, affects >1 million people annually, with a 20% case fatality rate. Clinical pathogenesis mechanisms have not been known until the present work and there is no safe/effective vaccine for humans. We recently reported that Leptospira-secreted Virulence Modifying (VM) proteins, comprised of N-terminal ricin B domains and C-terminal DNase/toxin domains, mediate cellular cytotoxicity on HeLa and primary pulmonary endothelial cells; the latter cell type is likely to be involved in lung hemorrhage in severe leptospirosis. VM protein vaccination of two rodent models (mouse, hamster) that recapitulate human disease manifestations protects from lethal challenge infection. We hypothesized that anti-VM protein immunity is mediated by antibodies, given the premise that Leptospira, an extracellular pathogen, causes systemic disease via the circulation of secreted VM protein exotoxins. Polyclonal and monoclonal antibodies both neutralized VM cellular toxicity (HeLa cells). High-affinity anti-VM mAbs 5F8, 6A5, and 5G10 reacted with homologous VM proteins and cross-reacted with the VM protein family. Epitope mapping showed that mAbs and polyAbs recognized linear epitopes that are highly conserved among species and serovars. Serovar Copenhageni showed upregulation of multiple VM proteins, which are present in the serum of infected hamsters at nanogram/ml levels as determined by capture ELISA. This latter finding is

the first time, to our knowledge, that a secreted exotoxin can be detected and quantified in the blood of any infectious disease. Discovery of vaccine-induced, anti-VM antibody-mediated neutralization of VM activity will justify clinical development of a novel leptospirosis vaccine. Future experiments will test whether passive anti-VM protein antibody transfer will protect animals against lethal leptospirosis. Our findings are sufficiently compelling to put leptospirosis on the WHO's Neglected Tropical Disease list which is needed to enable the development of VM protein-based leptospirosis vaccines, diagnostics, and therapeutics.

**5842**

## VILLAGE COMMUNITY BANKING: POTENTIAL OF COMMUNITY-BASED FINANCING SYSTEM FOR HOUSE IMPROVEMENTS AND VECTOR CONTROL IN RURAL TANZANIA

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House improvement has consistently been associated with remarkable reductions in indoor bitingmosquitoes and disease incidences even in typical rural houses, however, its exploitation remainsextremely poor in Tanzania and other endemic countries due to limited financial resources. Nevertheless,the village community banks (VICOBA), practiced in Tanzania for nearly two decades, have proven toprovide financial services to rural communities who would otherwise not be able to get financial servicesfrom formal financial institutions. This study was conducted to explore the views, opinion andwillingness of VICOBA members on using VICOBA platforms as source of finance for improving localhouses and eventually control of mosquito borne diseases.A mixed method approach was used in this study, whereby a survey was administered to 150 participantsand twelve focus group discussion were done in three villages of Ulanga district rural Tanzania. TheFGDs comprised of 8 participants each with equal representation of males and females. The FGD guidewas used to probe the opinion of study participants on malaria transmission, housing conditionimprovements and financial resources.About 99% of all participants indicated the urgent need to improve their houses for preventing themselvesfrom mosquito bites and were willing to utilize VICOBA for improving their houses. In focus groupdiscussion majority of people who participated they were also in need of improving their houses. Allparticipants confirmed that they were at highest risk of getting mosquito borne diseases and they werewilling to use money that was either saved or borrowed from their VICOBA.Going forward with malaria elimination and economic growth agenda as stipulated in the SDG 3 and 8,self-sustaining financial system destined at house improvement and related interventions against malariaand other mosquito-borne diseases are crucial. The community members were willing to use VICOBA assource of finance for house improvement and disease control, however there were limited knowledge andsensitization on how they could utilize VICOBA for disease control.

**5843**

## INNOVATIVE FINANCE FOR NEGLECTED TROPICAL DISEASES

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Neglected tropical diseases (NTDs), impact more than a billion people globally and pose a significant barrier to economic and social development in low- and middle-income countries. Insufficient funding and limited research have caused critical gaps in prevention, diagnostics, and treatment. In the past decade, only ten drugs were developed to treat diseases affecting 2.5 billion people. Six out of 20 NTDs identified by WHO have no tests, while others are not fit-for-purpose or accessible in the necessary areas. These challenges hinder progress towards agreed targets, as evidenced by the WHO NTD Road Map gap assessment. Funding for NTD product development is inadequate to address the need of new and better tools that could make the elimination of several NTDs a reality –it is imperative to explore financing options that have not yet been applied to the NTD space. The post-COVID-19 global health architecture has provided an opportunity to assess new financing models for health initiatives and disease programs. In 2022, GLIDE led a six-month technical exercise with a core group of 20 experts to identify innovative finance mechanisms (IFM) for NTDs through i) condensing existing evidence, ii) stimulating cross-sectoral discussions, and iii) gathering expert input. Through literature and desk reviews, several IFM were assessed across the NTD value chain for scope and applicability to specific diseases. Financing mechanisms were qualified as innovative if they met the four criteria of catalytic, additive, complementary, and sustainable. Shortlisted mechanisms were then prioritised based on their perceived impact versus ease of implementation profiles and mapped into quadrants. This exercise resulted in the identification of four financing instruments to consider for NTDs: (i)debt swaps, (ii) milestone-based funding, (iii) impact bonds, (iv) and pooled procurement. The next steps will be to identify disease-country-mechanism opportunities for investment by public and private stakeholders. By leveraging IFM, innovative finance offers a new and dynamic approach to mobilize resources and drive greater impact in NTD elimination efforts.

**5844**

## MICROBIOME ANALYSIS OF PREGNANT WOMEN AND CHILDREN FROM AMANHI FECAL COHORT

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Characterization of the human microbiome can provide valuable insights to the status of human health and disposition to disease. There is an interest in understanding the role of the human microbiome in maternal, newborn and child health (MNCH). Gut microbiome shifts physiologically during pregnancy and appear to differ in women with pregnancy-associated complications, such as, Adverse Pregnancy Outcomes (APOs). For example, dysbiosis of microbial communities is implicated in a variety of adverse MNCH outcomes including preterm birth (PTB), cardiometabolic complications of pregnancy and childhood, and neonatal complications like necrotizing enterocolitis. Furthermore, the maternal microbiome is a key contributor in the initial microbial colonization and development of the infant microbiome which may have long-term implications for the child's physical growth, nutrition, and neurocognitive development. In this proof-of-concept study, we sequenced by shotgun metagenomiucs 750 trios (250 maternal and 250 infant fecal samples of two time points, postnatal 42 days, and 12-18 months of age) to generate the microbiome profiles of pregnant women in Pakistan collected as part of AMANHI cohort. This study will guide our university to plan further as lead analytical lab for clinical intervention studies focusing to observe the effects on gut microbiome, and to provide insights into complex health problems in maternal, newborn and child health, along with their nutritional status.

5845

## UNDERSTANDING HEALTH WORKER AND COMMUNITY ANTIBIOTICS PRESCRIPTION ADHERENCE PRACTICES FOR ACUTE FEBRILE ILLNESS: A NESTED QUALITATIVE STUDY IN THE SHAI-OSUDOKU DISTRICT OF GHANA, AND THE DEVELOPMENT OF A TRAINING AND COMMUNICATION INTERVENTION

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Most health facilities in sub-Saharan Africa, including Ghana, lack the diagnostic capacity to identify the cause of acute febrile illnesses. This leads to presumptive diagnosis and contributes to the inappropriate prescription of antibiotics. Hence, the evolving agenda towards implementing interventions to ameliorate inappropriate antibiotic prescription and consumption. We explored the social and contextual behavior determinants of adherence to prescription and communication of prescription adherence messages for patients with acute febrile illness. The outcome was used to develop a Training and Communication intervention which was delivered as part clinical trial. This was a qualitative study of a randomized controlled trial with the objective to assess the impact of an intervention package consisting of diagnostic tools, clinical algorithms, training, and communication on antibiotic prescriptions, compared with routine practices for patients presenting with acute febrile illness. Data was collected from 39 healthcare prescribers and 66 caregivers in primary healthcare facilities and communities in the Shai-Osudoku district in Ghana. We undertook a content analysis of primary, qualitative data collection using in-depth interviews and focus group discussions, informed by the Capability, Opportunity, Motivation theory of behavior, the Theoretical Domains Framework, and the Behavior Change Wheel approach. Health workers perceived factors that influence what and how prescribers and dispensers communicate include: patients' education level; existing disease condition; health worker's workload; patient's religion; language barrier between health worker and patient; the outcome of laboratory results, and medicine availability. Community members' adherence to prescription was influenced by: the availability of money and affordability of medicine, the severity of the condition, work schedule, and forgetfulness. Our study contributes to knowledge in qualitative methods nested in a clinical trial and reveals factors that affect antibiotic prescription with the communication process.

5846

## SEVERE ACUTE MALNUTRITION IN CHILDREN UNDER FIVE

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Acute malnutrition is a rapid onset condition characterized by bilateral pitting edema, sudden weight loss caused by a decrease in food consumption or illness. Severe Acute malnutrition is when weight for height is less than -3 z scores with MUAC less than 11.5cm, bilateral pitting edema and marasmic -kwashiorkor (both wasting and edema). The study was carried out to identify factors that contribute to prevalence and effects associated with Severe Acute Malnutrition(SAM) in children below five years in a Nutritional Unit in Mbale Regional Hospital, Eastern Uganda. This was a prospective study that involved use of different questionnaires that were answered by health workers on duty, mothers and care takers of children with SAM on nutrition unit respectively. Anthropometric measures at two weeks and on monthly basis were taken and compared to mean difference in weight, height, Mid upper arm circumference (MUAC.) The study was carried out from 1st of December 2022 to 14th of March 2023.A total of 1000 respondents were interviewed. Children between 3-4 years (46.7%) had the most effects due SAM and 2-3 years (13.3%) had least effects due SAM. Poverty at(33.3%) was the major contributing factor to SAM, building its roots from family level. The study further showed us that (50%) were fortune to get discharged and (6.7%) died. My findings indicate that, hypoglycemia, impaired growth and development were the commonest complications and effects observed. SAM is still a neglected tropical disease in Uganda. Recruitment of more health workers for easier identification of effects due to SAM among children and multisector intervention that can provide various support in management and prevention of SAM under-fives are some of the recommendations laid out. Also, retaining of health workers that work in lower health facilities was highly emphasized as this would enable easy identification of these children with this type of malnutrition, girl child education should be paramount in communities as way to halt early marriages. As it is said that when you educate a girl child you educate a nation.

5847

## PARTICIPANT ACCEPTABILITY OF AN ANCILLARY CARE POLICY DURING AN EBOLA VACCINE TRIAL IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Providing ancillary care (AC) for participants' medical conditions during study participation is an ethical responsibility of clinical researchers. During an Ebola vaccine trial (clinicaltrials.gov: NCT04186000) conducted in the remote area of Boende, DRC, the Universities of Antwerp (Belgium) and Kinshasa (DRC) developed a policy to medically and/or financially support concomitant medical events. By means of two short surveys, we assessed participants' acceptability of this approach. First, 152 participants with a self-reported medical event filled in a short quantitative questionnaire (6 questions; grading 1-5) regarding their experience with the AC policy. Second, at the end of the trial, 307 participants with and without self-reported medical events participated in a short telephone survey (3 questions; grading 1-3 and option for comments) that explored their endorsement of the policy, their experience on how the policy addressed their medical needs, and their opinion on the importance of the policy. Descriptive statistics were used to analyze the surveys and recurring themes from the comments were identified. The majority (89%) of participants with self-reported medical conditions gave a positive to very positive evaluation. All respondents of the telephone survey, with or without self-reported medical conditions, were in full support of the policy and emphasize its importance; 91% indicated that the support addressed all medical needs. Some limitations or constraints were mentioned, e.g. pharmacy stock-outs, conditionalities for financial coverage, the lack of specialized care facilities, etc. Despite limitations and contextual challenges, most participants assessed the policy to be beneficial on a personal and medical level. As clinical trials are increasingly conducted in resource-constrained settings, clinical research sponsors and teams should consider to systematically implement a AC package that is co-designed with representatives of the trial community and Ethical Committee, and tailored to the local health care system, research budget and duration.

5848

## EVALUATION OF THE PANBIO™ COVID-19 RAPID TEST DEVICE (ABBOTT) AT THE VIROLOGY LABORATORY OF THE ARISTIDE LE DANTEC UNIVERSITY HOSPITAL IN SENEGAL

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The management of the COVID-19 pandemic requires the widest possible access to diagnosis. To make this diagnosis available to populations, especially in countries with limited resources, it is important to have fast but reliable technologies that are less expensive than RT-PCR, which is the reference technique. For this reason, we evaluated the detection

performance of Panbio™ COVID-19 Ag Rapid Test Device (Abbott Molecular) antigen tests on nasopharyngeal and nasal swabs compared to the Abbott Real Time SARS COV-2 Assay (Abbott Molecular) RT PCR technique. After obtaining free and informed consent, 2 nasopharyngeal swabs (NP) and one nasal swab (NS) were taken from each participant. The NP and NS samples were tested with Panbio Ag and the results were compared with those of RT PCR obtained on the nasopharyngeal swab of the patient considered. Performance analysis included calculation of sensitivity (Se), specificity (Sp), positive (VPP) and negative (VPN) predictive values, and degree of agreement by Cohen's Kappa coefficient. A total of 179 patients were included, including 150 participants recruited from travellers attending the Bacteriology Virology Laboratory of the CHNU Aristide le Dantec and 29 patients from the Hospital Treatment Center. The mean age was 33 years and 40 years and the sex ratio was 0.85 and 1.2 respectively for travellers and COVID-19 patients.Of the travellers, 21 (20%), 13 (8%) and 11 (7.3%) tested positive by RT-PCR, Panbio NP and NS respectively. For CT values <31, Se and Sp were 91% and 100% for NP and 86% and 100% for NS. The VPP was 100% and the VPN was over 96% for the NP and NS. Similarly, the degree of agreement was over 90% for NP and NS. In conclusion, this study showed excellent agreement between the Panbio test and RT PCR on both NP and NS samples. This good performance and ease of use of the Panbio test confirm the interest of its use in the diagnosis of COVID.

**5849**

## APPLICATION OF THE THREE DELAYS MODEL TO UNDERSTAND HOW THE INTERACTION OF COMMUNITY, FAMILY AND HEALTH SYSTEMS CONTRIBUTE TO CHILD MORTALITY IN CHAMPS-KENYA; MAY 2017 - JUNE 2022

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Sub-Saharan African countries continue to bear the greatest burden of mortality in children under the age of 5 (<5's). The 3-delays model provides a useful framework for examination and interpretation of the complexity in the intersection of community, family and health systems factors and their contribution to <5 mortality. These delays include (1) a delay in deciding to seek care; (2) a delay in identifying and arriving at a health facility; and (3) a delay in receiving quality care at the health facility (i.e., suboptimal care).The Child Health and Mortality Prevention Surveillance (CHAMPS) in Western Kenya investigates <5 deaths by conducting extensive data collection and laboratory investigation using minimally invasive tissue sampling, verbal autopsy and data abstraction. Qualitative data routinely collected includes clinician narratives and verbal autopsy and was used to identify the delays. We reviewed cases from 2017-2022 to categorize and describe deaths using the 3-delays model. Nearly all <5's experienced one or more delays prior to death: whereas only 7.5% of those aged 0-1 day-olds had no delays, only 2.3% of neonates aged 1-28 days old had no delays (Figure 1).Delayed decision-making to seek care (Delay 1) co-occurred with suboptimal care received (Delay 3) in over one-third of all deaths, with this overlap greater among infants and children (64.4-68.9%) than stillbirths and neonates (32.7-44.4%). Cases with delays between the decision to seek care and arrival at a facility (Delay 2) co-occurred with Delays 1 and/or 3 for nearly all deaths. Suboptimal care (Delay 3) was observed in 83.6% of all deaths: Delay 3 was observed with no other delays in over half of stillbirths and neonates, and less than one-third of infants and children. Poor clinical care contributes to nearly 9 of 10 <5 deaths, and mothers of older infants and children are more likely to delay in seeking health care than are mothers of neonates. The 3 delays framework is useful. Further reduction in <5 mortality will require multi-level interventions especially earlier health seeking behavior by parents, and adherence to clinical guidelines by healthcare providers.

**5850**

## IMPACT OF MESSAGES ON MATERNAL CONDITION LEADING TO CHILD DEATH AND ON ANC SEEKING PRACTICES AMONG PREGNANT WOMEN IN RURAL BANGLADESH

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Child Health and Mortality Prevention Surveillance (CHAMPS) Program in Bangladesh identified intrauterine hypoxia resulting from maternal health issues as one of the major causes of stillbirth and early neonatal deaths. Quality antenatal care (ANC) is needed to prevent these maternal conditions. This information was communicated and explained to pregnant women and their female family members at the community level through courtyard feedback sessions in Baliakandi, a rural sub-district of Bangladesh. The messages emphasized the benefits of recommended ANC visits to qualified physician on healthy development of the fetus and to identify maternal conditions, major danger signs during pregnancy and after delivery. From December 2021 - November 2022, female team members facilitated 138 courtyard sessions. We assessed the impact of conveyed messages by comparing ANC seeking behavior among 632 pregnant women (aged 14-45 years). In the 60 days following the session, 247 pregnant women (39%; 247/632) received ANC. The proportion of pregnant women who received ANC in the 60 days after the session (100%; 247/247) was 38% higher than in the 60 days before the session (62%; 153/247). When pregnant women's in-laws or maternal family members joined them at the sessions, the number of pregnant women who received ANC increased by 60% (148/247). Besides, the majority of the pregnant women (85%; 126/148) received ANC within 60 days following the session when their in-law's family members learned about the causes of child deaths and prevention strategies from the same session with them as opposed to (15%; 22/148) when they did not attend the session. The messages on main causes of death and understanding about the maternal conditions during pregnancy, likely facilitates in-law's family members to decide on ANC seeking of pregnant women. To improve maternal and child health, it is important to educate pregnant women along with their in-law's families, about the specific causes of maternal conditions that can lead to child death. Additionally, explaining the importance of antenatal care linking with potential risk as a preventive measure can increase its uptake.

**5851**

## A SYSTEMATIC REVIEW OF PREVALENCE AND RISK FACTORS OF TRANSFUSION TRANSMISSIBLE INFECTIONS AMONG BLOOD DONORS, AND BLOOD SAFETY IMPROVEMENTS IN SOUTHERN AFRICA

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Blood and blood products are listed as one of the essential medicines by the World Health Organization (WHO). In addition to inadequate supply, most sub-Saharan Africa (SSA) nations fail to meet blood needs because many donated units are discarded due to contamination with transfusion-transmitted infections (TTIs). We sought to estimate the prevalence of TTIs, identify the risk factors for TTIs among blood donors, and identify

interventions to improve blood safety in southern African nations, particularly the nations of the South African Development Community (SADC).
We investigated prevalence and risk factors for TTIs, and blood safety improvements in the SADC region from PubMed/MEDLINE, Cochrane Library, and HINARI databases from January 1, 2011 to April 31, 2021. All investigations followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). In meta-analysis, we estimated the pooled TTIs prevalence and summarized using forest plots. A total of 180 articles published from the sub-Saharan Africa region were identified covering our three targeted themes. Of these 180 articles, only 27 (15%) focused on the SADC region. The overall pooled TTI prevalence estimate was 2.0% (95% CI: 1.0-3.0) and hepatitis B was the most prevalent TTI in the region (prevalence = 3.0; 95% CI: 2.0-5.0). The prevalence of HIV, HCV, and syphilis was 2.0%, (95%CI:1.0-4.0) 1.0% (95%CI:0.0-2.0), 2.0% (95%CI: 0.0-8.0) respectively. In general, replacement donors and first-time donors were more likely to be infected with TTIs than repeat donors. 12 articles explored blood safety in the region however, they vary greatly highlighting the need for more comprehensive research. Few publications were identified that were from the SADC region, indicating lack of research or resources towards improving quantity and quality of blood donation. TTI prevalence remains one of the highest in the world and blood safety recommendations vary across the region. More effort should be directed towards developing a cohesive regional blood transfusion policy and effective blood monitoring and evaluation strategies.

5852

## THE REMOTE EMERGING DISEASE INTELLIGENCE NETWORK (REDI-NET): PREPARING FOR ZOONOTIC SPILLOVER THREATS

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Spillover and zoonotic disease outbreaks of known and novel emerging infectious pathogens are becoming more frequent worldwide due to climate change, globalization and increased interaction among humans, domestic animals and wildlife within a shifting landscape. These outbreaks will inevitably continue into the foreseeable future. The Remote Emerging Disease Intelligence NETwork (REDI-NET) is a multisectoral, multidisciplinary Consortium with the overarching goal to quicken the timeliness in collecting and processing data from across a wide range of ecologies to guide appropriate response options. The REDI-NET is founded on a multi-year, phased approach to leverage pre-existing and real-time long read shotgun metagenomic sequencing surveillance outputs in a flexible, scalable data repository and computing platform to support detection of pathogens, estimate risk of exposure and guide policy-decisions on animal, environmental and public health. Our core objectives focus on leveraging partner expertise to fill gaps in existing surveillance efforts to include 1) strengthening infrastructure where capacity may need to be built and/or enhanced (remote research stations); 2) standardizing sample collection, storage, and testing processes for assurances in data rigor and big data management; 3) leveraging existing networks and health data to widen the global surveillance footprint; and 4) transferring knowledge on surveillance system activities and providing actionable data. Here we will present program successes to date from early phases and describe next steps in expanding the operational framework with broad applicability across international regions.

5853

## KNOWLEDGE OF COVID-19 SYMPTOMS, TRANSMISSION, AND PREVENTION: EVIDENCE FROM HEALTH AND DEMOGRAPHIC SURVEILLANCE IN SOUTHERN MOZAMBIQUE, SEPTEMBER 2021-JANUARY 2022

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Over 230,000 COVID-19 cases and 2,200 deaths have been reported in Mozambique though March 2023. Understanding community members' knowledge and perception of SARS-CoV-2 transmission and prevention is essential for directing public health interventions to reduce disease spread and improve vaccination coverage. We describe knowledge of COVID-19 transmission, prevention, and symptoms among community residents in Mozambique. We conducted a cross-sectional survey among all households (n=33,087) in a Health and Demographic Surveillance System in Manhiça, Mozambique, at the tail end of the Delta variant wave in September 2021 to the peak of Omicron cases in January 2022. Principal components analysis was used to create scores representing knowledge of COVID-19 symptoms, transmission, and prevention. Multiple imputation and quasi-Poisson regression were used to examine associations between demographic characteristics and sources of COVID-19 information, and knowledge of COVID-19 symptoms, transmission, and prevention. Across this rural community, 98.2%, 97.0%, and 85.1% of household respondents reported knowing how COVID-19 could be prevented, that SARS-CoV-2 can cause disease, and how SARS-CoV-2 is transmitted, respectively. Most recognized symptoms were cough (51.2%), headaches (44.9%), and fever (44.5%). Most cited transmission mechanisms were droplets (50.5%) or aerosol (<5 μm diameter) (46.9%) from an infected person. Most cited prevention measures were handwashing (91.9%) and mask-wearing (91.8%). Characteristics associated with greater knowledge of symptoms, transmission, and prevention included having at least primary education, older age, employment, greater wealth, and Christian religion. Respondents who had had COVID-19 symptoms were also more likely to have knowledge of symptoms, transmission, and prevention. Community public health measures to reduce infectious disease transmission are contingent upon perceptions of risk and knowledge. These findings support the need for outreach and for community-engaged messaging to promote prevention measures, particularly among people with low education.

5854

## DEVELOPMENT OF SINGLE DOMAIN ANTIBODY-BASED LUMINEX ASSAY FOR THE DETECTION OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS-2 IN CLINICAL SAMPLES

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Viruses from the genus Betacoronavirus have emerged in the 21st century. These viruses have high morbidity and mortality, starting with Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) in 2002, Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in 2012, and SARS-CoV-2, which is the causative agent of coronavirus disease-2019 (COVID-19) in 2019. Thus, there is a critical need for rapid, low cost, sensitive, and reliable diagnostic assays for these emerging viral diseases. The ideal assay format would be rapid and multiplexed, enabling the processing of many samples and simultaneously providing information on the presence

of known diseases, while also giving a warning for the possibility of a new emerging disease. High-throughput MagPlex assays have the advantage of being relatively fast and simple in comparison to PCR based formats, while providing the additional capability to be multiplexed. Single domain antibodies (sdAbs) provide an economical means to perform multiplexed assays which can be expanded to include additional assays as desired or tested in a serial manner. Here, we developed a bead-based assay using sdAbs against SARS-CoV-2 nucleocapsid (N) protein. Additionally, we also used SpyTag/SpyCatcher system to improve the sensitivity of the assay. The sdAb-coated beads were able to detect N protein down to 10 ng/mL, however by using SpyTag/SpyCatcher system to orient the sdAbs on the beads, we were able to increase the sensitivity 10-fold to 1 ng/mL. We further assessed the sensitivity and specificity of the assay by using COVID-19 and seasonal coronavirus clinical samples. Both regular sdAb-coated and SpyTag/SpyCatcher sdAb-coated beads were able to specifically detect SARS-CoV-2 in the samples with SpyTag/SpyCatcher sdAb-coated beads performing better. In summary, we provide a proof-of-concept that bead-based assay to detect SARS-CoV-2 is feasible and future research by combining it with other sdAb-coated beads that can detect other coronaviruses may be a useful tool in responding to future pandemics.

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## COVID-19 SELF-TESTING: A PROMISING OPPORTUNITY FOR LOW AND MIDDLE INCOME COUNTRIES, YET A REALITY CHECK OF GLOBAL INEQUALITIES

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The widespread use of antigen-detection rapid diagnostic tests has revolutionized the decentralized testing and self-testing of COVID 19. However, the full extent of AgRDT utilization for self-testing remains largely unexplored. To provide vital information for the development of the recently released global guidelines on COVID 19 self-testing, we conducted a cross-sectional survey targeting policy makers, researchers, and implementers worldwide. The survey was shared through professional networks via email and social media. We used questions related to policy and program information concerning the regulation, availability, target population, indications, implementation, benefits, and challenges of COVID 19 self-testing. Descriptive summaries, cross-tabulations, and proportions were used to calculate outcomes at the global level and by World Bank region and income classifications. Between 01 and 11 February 2022, 844 individuals from 139 countries responded to the survey . 504 respondents from 101 countries reported policies supporting COVID 19 self-testing for a range of use cases, including symptomatic and asymptomatic populations. Significantly, more respondents from low-and-middle-income countries than high-income countries reported a lack of C19ST policy (61 vs 11 countries) and low population-level reach of C19ST. Respondents with COVID 19 self-testing experience reported that the tests were mostly acceptable to target populations, provided significant programmatic benefits, and highlighted several key challenges to be addressed for increased success. While the widespread global interest, policy support, and implementation of COVID 19 self-testing demonstrate an opportunity for bridging the testing gap experienced by LMIC populations, the unequal access exposes the realities of global inequalities. There is urgent need for Global Health actors to sustainably address unequal access to lifesaving interventions especially during high consequence epidemics. Benefits, challenges and opportunities shared by respondents should inform development of national and global policy.

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## ENVIRONMENTAL HYGIENE FOR HOSPITAL INFECTION PREVENTION AND CONTROL MANAGEMENT IN BANGLADESH: EDUCATING HOSPITAL CLEANING STAFF REQUIRES PRIORITY

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Cleaning staff is a critical part of the hospital infection prevention and control (IPC) program. Despite involvement in environmental hygiene management including patient care, cleaning staff are often overlooked in education and training, resulting in a lack of optimal IPC and hygiene knowledge. This study assessed the existing knowledge and practices regarding IPC and environmental hygiene among cleaning staff at tertiary hospitals in Bangladesh. Between July and December 2022, a cross-sectional study was conducted at six tertiary hospitals among randomly selected 259 cleaning staff involved in hospital environmental cleaning and waste management. Data was collected through face-to-face interviews using a semi-structured questionnaire, and descriptive statistics were used for analysis. The mean age of participants was 36.7 years (SD 10.1) and the majority (65.6%, 170/259) were outsourced workers. None of the cleaners had obtained any formal orientation or training on hygiene from the hospital. Four-fifths (78.4%) of those polled had not received any basic IPC training in the previous two years. Only 7% (18/259) of respondents were aware of the standard cleaning agent-to-water ratio for environmental cleaning. Approximately 15% of respondents correctly identified specific color-coded waste disposal bins. As precautionary measures for IPC management, one-third (35.9%, 93/259) mentioned hospital waste management and environmental cleanliness. Less than half (46.3%, 120/259) of cleaning personnel considered performing hand hygiene (HH) required after contact with patients and surroundings, but 17% (44/259) reported practicing HH in hospitals for these purposes. Only about one-third (29%) of cleaners weekly disinfected high-touch areas, but once a month for low-touch areas of the hospital. The cleaning staff lacked basic knowledge and standard practices on IPC and environmental hygiene in Bangladesh. Establishing environmental hygiene guidelines, hands-on orientation, and refresher training including routine monitoring can enhance the level of skilled cleaning staff for hospital IPC management.

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## ONE HEALTH BIOSECURITY: DEVELOPING RECOMMENDATIONS TO ADDRESS LEGISLATIVE GAPS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Strong whole-of-government biosecurity systems are critical for supporting biological threat reduction efforts. Legislative and regulatory frameworks ensure successful implementation, sustainability, and alignment of these systems with international agreements, including the International Health Regulations and the Biological and Toxin Weapons Convention. The extent to which legislative support for biosecurity systems is successful can vary based on national or regional governance structures, stakeholder capacity and available resources, which itself requires a robust understanding of a country's existing legislative landscape prior to any capacity-strengthening efforts. In support of the Global Partnership's Signature Initiative to Mitigate Deliberate Biological Threats in Africa, we developed a methodology for assessing national legislative landscapes with respect to a broad definition of biosecurity, through a One Health lens, which we then piloted in the Democratic Republic of the Congo (DRC). Through implementation of the methodology, we mapped and analyzed existing national and sub-national

legislation, regulations, and other applicable policy documents, and also engaged key informants to provide additional contextual information relating to awareness and implementation of key legislation. We convened national stakeholders, together with regional and international experts, to validate the analysis findings, and to develop recommendations and an action plan for strengthening DRC's biosecurity legislative structures. We demonstrate that our methodology is an effective approach to biosecurity legislative assessment, which can be adapted for use in other countries, thus making it a useful contribution to global health security scholarship and practice.

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## BURIAL SITE SURVEILLANCE TO MONITOR EXCESS MORTALITY DURING THE COVID-19 PANDEMIC IN KARACHI, PAKISTAN

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Population death rates are an important measure of health status and Pakistan is among those countries which has a poor functioning national system of registering deaths and determining their causes. In the context of the COVID-19 pandemic, accurate documentation of fatalities became even more critical. Without accurate data on the number of fatalities and their distribution, it becomes a challenge to track the progression of the pandemic and determine the effectiveness of interventions such as lockdowns, social distancing, and vaccination programs. In this regard, our study aims to identify and monitor disparities in COVID-19 fatalities and their causes by using burial site surveillance in Karachi, Pakistan, which involves collecting both retrospective (2016-2021) and prospective (2022 onwards) data which will be collected till June 2023 from graveyards and morgues. Surveillance process includes data from graveyard registries and verbal autopsy using the WHO verbal autopsy questionnaire. The study's results indicated that among the total deaths (N=161,142), mortalities observed in the year 2021 were 37,778 followed by 2022 (36,719), 2020 (35,858), 2019 (27,018) and data observed between 2016-2018 were 24,119 with work in progress. Majority of fatalities occurred in individuals aged 65-74 (age adjusted mortality ratio = 0.1960) and that among the seven districts of Karachi, Karachi Central had the highest mortality proportion (1922/100,000). Further we found 1612 fatalities attributed to COVID-19, and males exhibited a greater prevalence of mortality than females (64% vs. 36%). Also, 469 cases were recorded in individuals aged between 65-74 indicating elderly population was more vulnerable to COVID-19 related deaths. As compared to pre-surveillance mortality data, there was an increase of 2-4 standard deviation in mortalities during COVID-19 pandemic. In conclusion, most of the deceased in Pakistan are buried as part of religious and cultural norm. Hence mortality data can be a good indicator to understand increase in mortality rates during COVID-19 pandemic and can be used to measure the burden of mortalities during pandemic.

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## AN ETHNOGRAPHIC APPROACH TO UNDERSTAND THE FEASIBILITY OF GRAVEYARD SITE SURVEILLANCE TO ASSESS EXCESS MORTALITY IN A RESOURCE CONSTRAINT SETTING

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Population mortality is a crucial statistic that combines data from several risk factors to provide a single measure of public health. This study provides insights related to ethnographic data collection of major graveyards in Karachi, Pakistan from November 2021 to April 2022. The main objective of this study was to understand the process of death to burial in graveyards belonging to various religious and ethnic groups. Qualitative interview guides were developed to interview various stakeholders, including graveyard caretakers (121), ethnic groups (46), Karachi Metropolitan Corporation officials (15), funeral bus/ambulance services staff (9), family members of deceased individuals (4) and NGOs (2). We found that burial of the deceased was the most commonly practices and culturally accepted method in different religions e.g., Muslim, Christianity and Hinduism. We found that obtaining death certificates and registration involved providing a copy of the deceased individual's National Identity Card. However, the process was faster for deceased children which led to incomplete documentation. For obtaining death registration and certificate, union council facilitate urban areas and union committee for peri-urban areas. We found different perspectives related to the cause of death (COD) e.g., parents expressed their curiosity to know the COD of their children, and hospitals shared this information as well. However, it was deemed unethical to disclose the COD when it occurred at community setting. The data explored the concept of doubling graveyards and reasons were lack of space and desire to be buried near ancestors and ease in visit. Data identified the potential graveyards, stakeholders, and information about death records. Further, confidentiality of death record was found as major obstacles among few communities and civilians. In conclusion, this is a unique ethnographic information related to graveyard and burial process in a resource constraint setting. Further the study will also help in exploring social/cultural context related to a mortality surveillance in a community setting.

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## THE RELATIONSHIP BETWEEN PRE-EXISTING COMORBIDITIES AND IN-HOSPITAL CARDIOVASCULAR EVENTS AMONG COVID-19 PATIENTS IN BANGLADESH: A PROSPECTIVE COHORT STUDY

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The first case of SARS-CoV-2 was detected on 8 March 2020 in Bangladesh just before 03 days of the announcement of coronavirus global pandemic by World Health Organization. As of March 16, 2023, a total of 2,037,947 cases have been confirmed, resulting in 29,445 deaths. Cardiovascular disease (CVD) and diabetes are scientifically recognized as major risk factors for poor prognosis and fatalities. Bangladesh has the highest prevalence of CVD risk factors among South Asian countries. Majority of the studies on Covid-19 conducted in Bangladesh were related to clinical symptoms, mental health and impact on health systems and scarcity in data exists on the CVD risk factors and outcome of the hospitalized Covid-19 patients. Therefore, a prospective cohort study was conducted as a part of multi-country project to explore the relationships between cardiovascular risk factors and preexisting comorbidities to the cardiovascular in-hospital events of covid-19 patients. Data were collected from 897 adult Covid-19 PCR positive patients of 3 hospitals at the time of hospital admission and at 30 days, discharge or death. Statistical analysis was done using epicalc R package, Descriptive analyses and multivariable log-binomial regression models, adjusted for age, sex, risk behavior and clinical events were performed. Mean & maximum age was 18 and 96 respectively, 52.1% female, 54.8% patients with cardiac comorbidities and the most common preexisting comorbidities were hypertension (54.1%) and diabetes (44.1%). Almost one fifth of the patients developed cardiac events including Myocarditis (0.7%), Myocardial Infarction (3.5%), Pericarditis (0.6%), Acute Heart Failure (6.1%), Endocarditis (0.4%), Atrial Fibrillation (0.2%), Cardiac Arrest (8.0%), Shock (1.0%) and Cardiac Blocks

(0.8%). The predictors of overall in-hospital cardiac events included male sex, overweight, lower education level (<college/university), former smoker, heart rates and low SPO2 ($p < 0.05$ for each). This study provides robust evidence on COVID-19 in-hospital cardiac events which guides future health care preparedness on cardiac emergency for the pandemic globally.

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## PATTERNS OF DATE PALM SAP HARVESTING AND TRADING PRACTICES AND RISK OF NIPAH VIRUS TRANSMISSION AT COMMUNITIES IN BANGLADESH

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Bangladesh has been experiencing a nearly annual Nipah virus (NiV) outbreak through drinking contaminated raw or fermented date palm sap (RDPS) with bat excreta since 2001. RDPS harvesting practices and trading patterns have not been explored extensively. Hence, we conducted a study to understand RDPS collection, consumption, and selling practices and the risk of NiV spillover at the community level. We performed an explorative qualitative study in two NiV outbreak districts, Rajbari and Naogaon, between 2021 and 2022. We recorded participant observations (n=14) and conducted ethnographic interviews (n=31) with RDPS collectors (Gacchi) on collecting and selling practices and using diversity of protective apparatus. The interview data were analysed using coding and thematic analysis based on the grounded theory approach. Gachi prefers selling RDPS more than making molasses due to time consumption, fuel costs of preparing it, and the high demand for consuming RDPS. They informed RDPS selling is not limited to their local community and sale distends location of non-harvesting areas based on customer demand through a middleman, which increases the transmission risk of NiV and other bat-borne diseases in wider geographical areas. We observed, and participants reported that Pteropus and non-Pteropus bats and rodents visited the trees and drank RDPS. They are replacing clay pots with discarded plastic pots, due to the free cost. They also prefer to use non-conventional protective apparatuses like jute bags, plastic bags, and nylon nets due to the time and resources to prepare bamboo skirts. Moreover, they reported that bats scratched out bamboo skirts and trunks to lick sap. We recommend adopting a culture-sensitive intervention, including efficacy tests of bat access protection on several apparatuses with economic outcomes of the date palm sap harvesting practices to prevent spillover of NiV and other bat-borne emerging viruses in Bangladesh.

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## UNDERSTANDING VACCINE HESITANCY IN BOENDE, WESTERN DR CONGO: A MIXED-METHODS STUDY

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In 2019, WHO identified vaccine hesitancy as one of the top 10 global health threats. Reports following the SARS-COV-2 pandemic indicate a general decline in trust in vaccines, including routine childhood vaccination. The Democratic Republic of Congo (DRC) has a low childhood vaccine coverage (around 50%) and one of the world's highest mortality rates of children under 5 at 79 deaths per 1000 live birth. While previous studies showed a good level of confidence in the safety and importance of vaccines in the country, they relied on data that was mainly quantitative and/or focused on urban areas. To address this gap, we conducted a mixed-method study in Boende, Tshuapa province (western DRC), and surrounding villages in the Boende health zone. The region was hit by an Ebola outbreak in 2014 and witnessed COVID-19 restrictions between 2020 and 2022. Boende was the site of the EBL2007 Ebola vaccine trial between 2019 and 2022. Alongside this vaccine trial, we aimed to explore perceptions and attitudes towards childhood and adult vaccination through individual interviews and focus groups with people living in Boende (mostly non-participants of the trial), as well as a 30-cluster survey covering the entire health zone of Boende. Our analysis revealed that adult vaccines appear to be less trusted than childhood vaccines, with Covid-19 vaccines being the least trusted, most likely due to the low number of related hospitalizations and deaths in the region. The majority of interviewees in Boende stated that their children are vaccinated. Nonetheless, over half of the interviewees expressed concerns about the common side effects of childhood vaccines or had doubts about the storage conditions of these vaccines, others believed that vaccines were given only to people living in Africa and not White people. These reasons were found to deter some study participants and their acquaintances from vaccinating their children. These findings could be used to inform future vaccination and sensitization campaigns in the region by addressing common concerns and misconceptions about vaccines, particularly for adults, to increase trust in and uptake of vaccines.

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## PERCEPTIONS ON ACCEPTANCE AND BARRIERS RELATED TO MORTALITY SURVEILLANCE FOR DRY NASAL SWAB PROCEDURE RELATED TO COVID-19 IN PERI-URBAN SETTINGS, PAKISTAN

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COVID-19 is a major public health threat, declared a pandemic by WHO in January 2020. Understanding community perceptions of the disease and its associated mortalities is crucial for protection and planning in LMICs. The objective of the study was to investigate the community perceptions about dry nasal swab procedure for COVID related mortality surveillance in peri-urban settings of Pakistan. This exploratory study design was used to conducted eleven focus group discussions (FGD)with various Muslim ethnicities residing in Ali Akbar Shah, an urban slum area on the outskirts of Karachi, including parents, religious leaders, community leaders, graveyard caretakers, traditional birth attendants, bathers, and community health workers. Three qualitative main themes were identified, namely the community's health-seeking behavior towards COVID-19, the understanding of death rituals in Muslim ethnicities and feasibility of obtaining dry nasal swab samples. Various perceptions were reported on COVID-19 spread e.g. getting vaccination and follow COVID-19 SOPs, while others remained unsure and stated "Covid-19 is there in the entire world, there is no doubt in this, but it is not here in our area .We have never seen it in two years, so we consider it all a lie" (Graveyard caretakers). Further, we explored that in Muslim communities before burial, a body is ritually washed which involves using cotton to clean the nostrils of the deceased and taking a dry nasal swab sample at the time of burial bath would be religiously and contextually acceptable. The findings revealed that the approach to the family elder and parents prior to the sample collection would be a better strategy to implement mortality surveillance, to overcome perceived barriers such as pain to the body, unethical and disrespectful to deceased and grief amongst families. In conclusion, Mortality surveillance studies within community setting is difficult to execute however, community buy-in and liaison with key stakeholders are essential. In addition, community grief support and counselling after death would be beneficial.

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## METAGENOMIC DETECTION OF PATHOGENIC BACTERIA IN TICKS FROM ISIOLO AND KWALE COUNTIES IN KENYA

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Ticks are arachnid ectoparasites that are second only to mosquitoes in the transmission of human diseases including bacteria responsible for anaplasmosis, ehrlichiosis, spotted fevers, and Lyme disease among other febrile illnesses. Due to paucity of data on bacteria transmitted by ticks in the Kenya, the present study undertook a bacterial metagenomic-based characterization of ticks collected from Isiolo, a semi-arid pastoralist county in Eastern Kenya and Kwale a county in coastal Kenya. Bacterial 16S rDNA PCR amplicons obtained from the above samples were sequenced using the MinION (Oxford Nanopore Technologies) platform yielding 547,780 reads with a median length of 1.54kb. The resulting reads were demultiplexed in Porechop, followed by trimming and filtering in Trimmomatic before clustering into OTUs using Qiime2-VSearch. A SILVA database pretrained naïve Bayes classifier was used to taxonomically classify the OTUs. A total of 2,918 ticks belonging to 3 genera and 10 species were screened in the present study. The pathogenic bacteria detected in pooled tick assays were as follows: Rickettsia spp. 59.43% of pools, Coxiella burnetii 37.88%, Proteus mirabilis 5.08%, Cutibacterium acnes 6.08% and Corynebacterium ulcerans 2.43%. These bacteria are responsible for spotted fevers, query fever, urinary tract infections, eye infections and diphtheria-like infections respectively. P. mirabilis, C. acnes and C. ulcerans were detected only in Isiolo. Metabarcoding was carried out on the tick species from which bacteria were detected showing that from Isiolo Hyalomma truncatum had the highest number of bacterial species at 12 while from Kwale Rhipicephalus boophilus decoloratus had the highest number of bacterial species at 6. The detection of Cutibacterium acnes, commonly associated with human skin flora suggests that the ticks may have contact with humans thus exposing them to infections caused by the identified bacteria. The findings in this study highlight the need for increased surveillance of tick-borne bacteria to discern their public health burden.

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## ENGAGING ANTHROPOLOGY IN NIPAH OUTBREAK: FACTS BEHIND THE HUMMING

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Anthropological approaches play a pivotal role in outbreak investigation by bringing forth sensitive, tabooed, or stigmatized practices, behaviors, and contexts interconnected with the disease paradigm. Unanticipated situations such as the spread of rumors, public panic, uncontrolled prevalence of obscure and misinterpreted information in the community, cause psychological and emotional tensions during an outbreak. In the 2023 Nipah Virus (NiV) outbreak in Bangladesh, a combined team of anthropologists from the Institute of Epidemiology Disease Control and Research (IEDCR) and icddr,b embedded the rumor management method concurrently to tackle unforeseen situations. Alongside functioning anthropological approaches, we especially focused on documenting the 'rumors' spread from social media and communities. We included both human experts and human-computer methods. This year the situation started off with 2-3 Nipah cases similar to other years, and no one was alarmed considering the situation as 'normal'. However, with the increasing number of cases, people's reactions and perceptions changed. Old-aged people were prone to dismiss the linkage between NiV and drinking raw date palm sap (DPS). The young generation apprehended drinking raw DPS as a challenge and started representing this as a trend on social media. Some business groups capitalized on the idea by promoting people through advertising on various social platforms. In some communities, people barely perceived the connection between drinking raw DPS with bats and disease transmission, as they are more used to seeing the abundance of bats in the lychee season. Besides, there was diversion and reluctance among community people to talk about this due to the presence of the media. In this context, along with documenting the then-prevailing rumors, beliefs, and perceptions, we followed the 'slow science' method and invested quality time to observe, listen, build rapport, and then collected data. Sharing the experiences gained through anthropological methods during outbreak investigation in the global forum can generate new risk communication and prevention initiatives.

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## SURVEILLANCE OF ACUTE FEBRILE ILLNESS IN JORDAN DURING THE TIME OF PANDEMIC OF COVID-19

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Surveillance of Acute Febrile Illness (AFI) during the pandemic is important to understand the burden of the infectious diseases presenting with fever, which is also a common presentation of COVID-19 cases. In Jordan, AFI surveillance started in June 2020, in 2 tertiary care hospitals in the north, and south of the Kingdom. It was expanded to a third hospital in the middle in September 2021 and to 2 other hospitals in the northwest in June 2022. Cases that require hospitalization presenting with current or history of fever (≥ 38Co) within 10 days before admission with no identified origin, were enrolled, and leftovers of their clinical samples of blood were tested at the Central Public Health laboratory using a multiplex qrt-Polymerase Chain Reaction of 15 pathogens, in addition to PCR testing for SARS-CoV2. Blood culture results were recorded if requested. Through January 2023, a total of 890 patients were enrolled. Male gender constituted 55.2 % (491/890) of cases and median age was 3 years old. Median duration of hospitalization was 4 days. Eight cases died during hospital admission with 0.9 % mortality rate. Out of 890 samples, a total of 157 pathogens were detected in only 15.8% (141/890) of the samples. Of the 157 pathogens detected, Epstein Barr Virus was most frequently detected (42 %) followed by Cytomegalovirus (21.7%) and Human Adenovirus (12.1%). Arboviruses were detected in 8 samples (West Nile virus 6 samples, and one sample of Dengue and Rift Valley virus each). None of the samples tested positive for Zika virus. SARS-CoV2 was detected in only four samples. Other pathogens including Salmonella (6 samples), Leptospira and Brucella (2 cases each), Rickettsia Spp. and Coxiella burnetii (one sample each) were also identified. Expanding the surveillance to mild to moderate cases (e.g., outpatients) is planned to comprehensively describe the epidemiology of AFI in Jordan. Integration of the results with other surveillance programs such as vector surveillance is implemented to provide enhanced data to improve public health and accompanying health risk mitigation knowledge, as well as augment national control measures in Jordan and the region.

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## IMPLEMENTING A "TEST AND TREAT" STRATEGY FOR COVID-19 IN BOLIVIA AND PARAGUAY: LESSONS AND CHALLENGES

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Prompt diagnosis of COVID-19 is a key intervention for COVID-19 clinical care and control. The ECO project was designed to enhance the coverage of diagnosis of COVID-19 in Bolivia and Paraguay. We carried out a formative assessment of the national capacities to implement, scale-up and track the use of Rapid Diagnostic Tests (RDTs). When oral treatments became available, this was further built into a 'test and treat' strategy for implementation in selected districts in each country, in collaboration with district leaders, clinics, and the Ministry of Health. The formative assessment identified factors associated with community hesitancy to access COVID-19 diagnostics, but also others that could facilitate diagnosis, including the impact of availability of effective treatment to prevent progression in high risk patients. Catalytic implementation in each country with distribution of 20,000 RDTs to health facilities and health posts afforded the opportunity to track how the supply chain was managed, distribution prioritized, obtaining timely data between first symptom, testing and results to the patient, status of training, and patient follow-up. The findings were built into an implementation strategy, along with enhanced data reporting and visualisation, and carried out in parallel with a communication campaign co-created with the community and the health centres. Despite the delays in access of COVID-19 treatments such as Tocilizumab and subsequently oral drugs, these have begun to reach both countries. The systems to diagnose, identify high risk groups, and deliver drugs have been put in place. We will present the evaluation data on planned endpoints for tracking acceptability and uptake of diagnosis and medicines, including time from diagnosis, which is critical for use of the new oral drugs. In addition, significant progress was made on systems for data capture and visualization, community engagement and communication strategies, and pandemic preparedness, which might extend beyond the duration of the project. Finally, we will summarize lessons learned in the midst of the COVID-19 pandemic in two LMIC Latin American countries.

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## COVID 19 KNOWLEDGE, ATTITUDES, PRACTICES (KAP) AND MENTAL HEALTH BEHAVIORS IN LIBERIA: FINDINGS, IMPLICATIONS AND FUTURE DIRECTIONS

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Evidence from the WHO revealed that age, comorbid medical conditions, gender, and occupations are risk factors for CoVID 19. Limited information exist in Liberia on population-based risks. The CoVID 19 outbreak led to the implementation of public health guidelines by the Ministry of Health to protect the health and welfare of the general population. However, risk reduction messaging and risk communication strategies were inconsistent, unclear and non-compliance. Also, there existed significant community distrust in CoVID 19 public health information dissemination channels, limited integration of community structures in the national mitigation strategies and significant increase in public perceptions about conspiracy theories in the country. We conducted a cross-sectional study with multi-stage sampling frame among 250 adult males and females from primary CoVID 19 hotspots in Liberia based on the WHO's Conceptual Framework for Action on Social Determinants of Health. The specific aims were to determine the prevalence of (1) CoVID 19 related knowledge, attitudes, practices and behaviors, (2) CoVID 19 risk factors, and (3) mild-to-moderate mental health related disorders, and (4) the involvement and integration of urban and rural community structures in the primary epicenter in the mitigation of public health outbreaks in Liberia. The study findings revealed a mean age of 34 years, 60% urban and 40% rural, equal proportion of males and females, and majority single and employed, with evidence of formal education. The findings clearly demonstrated that CoVID 19 negatively affected health and social services, employment and lost wages, especially in rural settings; that there was no correlation between CoVID 19 Knowledge and Practices; that rural residence and women were better predictors of CoVID 19 Knowledge, and that depression and anxiety were associated with lower adherence to CoVID 19 prevention practices. We recommend that future research studies may be warranted in post-conflict Liberia to better understand the social determinants and effects of disease outbreaks, and its related impacts, on health equity.

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## COMMUNITY ENGAGEMENT IN EPIDEMIC MANAGEMENT: AN ANALYSIS OF THE EBOLA VIRUS DISEASE AND COVID-19 RESPONSES IN BOENDE, WESTERN DR CONGO

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In 2020, the WHO identified several challenges to the simultaneous management of Ebola virus disease (EVD) and COVID-19 in low and middle-income countries, including health systems' overload, international coordination, and communication between response teams and affected communities. Between 2020-2022, the Democratic Republic of Congo (DRC) faced 6 EVD outbreaks while also battling the COVID=19 pandemic. Recent publications highlight that the experience gained during EVD outbreaks has facilitated the implementation of prevention and control measures against COVID19. These publications primarily focus on national-level management and are often narrative reviews. To better understand how DCR's response to COVID-19 built on previous experiences of EVD management, we conducted a qualitative study in Boende (Tshuapa province, western DRC), which faced an EVD outbreak in 2014, hosted the EBL2007 Ebola vaccine trial between 2019-2022, and is currently dealing with COVID-19. We reviewed relevant literature, including official documents, and interviewed healthcare providers, health authorities, political authorities who were actively involved in managing both diseases in Boende, as well as members of the affected community. We found that community leaders (local authorities, religious leaders, teachers, and the relais communautaires, community members trained in health surveillance and communication) played a central role in community-based surveillance, both during the EVD management and in the fight against COVID-19. However, during the EVD response, community leaders were extensively trained on EVD and risk communication and provided with bicycles, communication material, and a financial motivation. During the COVID-19 response, their involvement was rather limited to informing communities of the existence of the disease. This may partially explain the low level of community engagement in the fight against COVID-19. These findings suggest that the role of community leaders should be reconsidered in order to gain community engagement in community-based surveillance and thus improve future outbreak management.

5870

## PERCEPTIONS OF PREVALENCE, IMPACT, AND MANAGEMENT OF POST-ACUTE SEQUELAE OF SARS-COV-2 INFECTION AMONG HEALTHCARE WORKERS IN KWENENG DISTRICT, BOTSWANA

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Since March 2020, over 760 million confirmed cases of COVID-19 infection have been identified, with 9.5 million recorded in Africa. Post-acute sequelae of SARS-CoV-2 infection (PASC) affects an estimated 32% to 87% of COVID patients globally. Data regarding the current prevalence and impact of PASC in Botswana are limited. Utilizing a cross-sectional survey design, we surveyed healthcare workers about the perceived prevalence of PASC, the duration of and characterization of common symptoms, the impact of PASC symptoms on patients' daily lives, and current management strategies. The survey was disseminated to healthcare workers via pre-existing WhatsApp groups and on paper. A total of 79 respondents completed the survey, from an estimated 650 staff meeting eligibility criteria (12% participation). Of these, 91% provided informed consent; 45% were female and 26% were male. The majority (90%) were nurses, with doctors and "other" accounting for 6% and 4% of respondents, respectively; no administrators responded. Over half (70%) worked at primary care facilities, and over a quarter (28%) worked in hospitals. Most (93%) indicated seeing patients with PASC on a weekly basis, though the majority (61%) identified these patients as <10% of total patients. Persistent cough was the most common PASC symptom (64%), followed by fatigue and muscle/body aches (4% each). A substantial proportion of respondents were unsure how to respond to questions regarding the management of common PASC symptoms, with 29% and 36% indicating uncertainty regarding the management of persistent cough and fatigue, respectively. These data indicate that health care healthcare workers frequently encounter patients with PASC at various health system levels across Kweneng, coupled with high levels of uncertainty regarding the best management for this syndrome. Key informant interviews will be conducted to further explore these themes and identify existing PASC management strategies. Data will be used to develop standardized PASC evaluation and management algorithms for use in Kweneng District.

5871

## VERTICAL TRANSFER OF HUMORAL IMMUNITY AGAINST NIPAH VIRUS: A NOVEL EVIDENCE FROM BANGLADESH

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Human Nipah virus (NiV) infection is rapidly progressive and highly fatal, which is one of the major barriers to in-depth exploration of the immune response. Bangladesh nurtures the largest cohort of Nipah infection survivors, and through their contribution, significant discoveries have been made that have substantially helped the scientific community understand the interaction between the virus and the human immune system. One such novel finding is described in this abstract regarding the vertical transfer of immune properties against NiV. In January 2020, a mother and her four-year-old daughter were infected with NiV. Both had a raw date palm sap consumption history and were diagnosed as confirmed NiV-positive cases. Unfortunately, the child succumbed during the course of the infection, while the mother survived with significant residual neurological impairment. Struggling through post-infection physical challenges and psychological trauma, the couple conceived a year and a half later. Per the mandate of national Nipah surveillance, thorough antenatal follow-up and routine Nipah survivor follow-up were done. The pregnancy was uneventful, and a healthy male baby was born. Being the baby of a NiV infection survivor, to exclude the possibility of vertical transmission of NiV infection, specimens were collected from the newborn and tested at the reference laboratory of the Institute of Epidemiology, Disease Control and Research (IEDCR) and icddr,b. While anti-Nipah IgM and PCR tests for NiV were negative, a high titre of anti-Nipah IgG was detected. From mother to neonate, the transfer of humoral immunity against the Nipah virus was confirmed for the first time. This finding will serve as a reference for further research on the transfer of NiV-specific antibodies and warrants further exploration of its effectiveness in virus neutralization and its potential to protect newborns. This will also serve as a resource for future research on vaccine recommendations for pregnant or young women against NiV.

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## SURVEILLANCE OF OPERATIONALLY RELEVANT VIRAL HEMORRHAGIC FEVER AND RICKETTSIAL VECTOR BORNE INFECTIOUS DISEASE THREATS, INSECTICIDE RESISTANCE, AND ASSESSMENT OF VACCINE EFFICACY TO PREDICTED T CELL EPITOPES AND B CELL ANTIGENS IN AFRICOM

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Crimean Congo hemorrhagic fever (CCHV), Yellow Fever Virus (YFV), and rickettsial diseases are important emerging or reemerging vector borne infectious diseases of public health concern in Africa and Europe. Expanded vector surveillance, increased insecticide resistance studies, and novel vaccine development efforts for CCHV, YFV, and rickettsial infections in AFRICOM and EUCOM are needed to better detect and prevent disease. While an inactivated vaccine for YFV has been administered prophylactically for decades and is required for AFRICOM entry, there is no licensed vaccine currently available for CCHV or rickettsial species despite recent progress in vaccine research and development for these pathogens. Multiplex PCR panels now provide convenient mobile forward deployable surveillance tools to rapidly detect host, YFV, CCHV, and rickettsial genes in real time and can streamline identification of new strains, variants of concern, or resistance genes. Naval Medical Research Unit-Three (NAMRU-3) sites in Ghana, Egypt, and Djibouti routinely conduct or manage vector-borne infectious disease surveillance in support of AFRICOM. Expansion of vector surveillance sites and sample sequencing to better characterize insecticide resistance, viral hemorrhagic fever, and rickettsial risk within the NAMRU-3 area of responsibility is needed. We expect that broadened surveillance will identify conserved coding sequences in the bunyavirales families, flavivirus families, and rickettsial species that align with immunodominant epitopes and antigens of novel vaccine candidates. Monitoring vector borne pathogen strains will help assess existing vaccine efficacy against operationally relevant infectious diseases in the NAMRU-3 area of responsibility and predict strain-dependent variation in immunological recognition of linear epitopes and surface antigens of vaccines currently in development. These collective activities and network of analyses will have implications for immunization and insecticide spray policies.

5873

## THE REMOTE EMERGING DISEASE INTELLIGENCE-NETWORK: ENHANCING BIOSURVEILLANCE USING WATER AND SEDIMENT SENTINEL SAMPLES FROM BELIZE, CENTRAL AMERICA

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The past decades have seen a dramatic increase of emerging and/or re-emerging infectious diseases worldwide. More outbreaks are foreseen for the future, yet proactive surveillance remains limited due to many challenges including lack of technical expertise and characterization of reliable sentinel sample types for accurate detection of circulating pathogens reflecting potential zoonotic spillover threats. The Remote Emerging Disease Intelligence-NETwork (REDI-NET) is a phased initiative project which aims to enhance current surveillance efforts to detect, predict and contain potential emerging infectious diseases in an efficient and timely manner. Partners have established robust standard operating procedures, including those for standardized field sample collection, storage and metagenomic next-generation sequencing (mNGS) to capture a broad spectrum of pathogens circulating in REDI-NET early phase surveillance sites of Belize, Kenya, and Florida. In Belize, active biosurveillance sampling was performed monthly from ten permanent water bodies, in the Corozal, Orange Walk, Stann Creek and Toledo Districts from November 2021-March 2022 and incorporating two additional permanent water bodies in the Cayo and Belize Districts in November 2022-March 2023 to determine effectiveness of water and sediment samples to serve as sentinels for circulating pathogens. Here we report mNGS outputs on viral and non-viral (e.g., bacterial, parasitic) pathogens using MinION/GridION sequencers (Oxford Nanopore Technologies) to inform on whether both water and sediment sampling are needed, or if one sample type could be used to inform pathogen presence effectively. Findings are meant to provide guidance to field collection efforts in order to streamline resource allocation which is often a limitation in surveillance efforts.

5874

## CAUSE SPECIFIC MORTALITY FROM VERBAL AUTOPSY FOR UNDER FIVES IN WESTERN KENYA, 2019 TO 2022

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Verbal autopsy remains the most widely used and feasible method for measuring cause of death, especially in low and middle in-come countries, which still lack reliable information on pediatric causes of death. Child Health and Mortality Prevention Surveillance (CHAMPS) network that includes Health and Demographic Surveillance Systems (HDSS) in urban (Manyatta) and rural (Karemo) settings in Western Kenya, conducts verbal autopsy (VA) to help investigate and monitor causes of death. We examined U5 cause-specific mortality rates and fractions using VA data from the two CHAMPS HDSS sites for the period 2019 - 2022. VA data were collected using the 2016 WHO form and processed using the InterVA5 probabilistic model in R software. A total of 3642 U5 deaths were recorded, of which 3273 (90%) had VAs conducted. Overall under-five mortality rate in the rural site 14.2(13.3, 15.2) per 1000 live births was higher than the urban site 10.1(9.4, 10.7) per 1000 live births. Neonatal mortality rate was 21.8 per 1000 live births for rural and 23.7 per 1000 live births for urban. Infant mortality rate in the rural site of 24.5(19.6, 29.4) per 1000 live births was higher than the urban site of 16.7(14.5, 17.5) per 1000 live births. Child mortality rate 6.3 (6.0, 6.6) per 1000 live births for rural and 2.3 (2.2, 2.4) per 1000 live births for urban. The leading specific causes of death according to VA were birth asphyxia (141, 13.0%), prematurity (142, 13.1%), stillbirth (136, 12.6%), diarrheal diseases (104, 9.6%), meningitis & encephalitis (80, 7.4%), and malaria (58, 5.4%). The under-five cause-specific mortality rates generated using the InterVA-5 model reflects prevailing knowledge on the under-five disease burden in western Kenya, but better correlation is needed with CHAMPS findings generated using minimally invasive tissue sampling and specific diagnostics. Though U5 survival has increased in recent years, a lot more needs to be done to alleviate under-five deaths in the two sites.

5875

## PUBLIC HEALTH DECISION-MAKING DURING COVID-19 PANDEMIC: A DETERMINANTS FRAMEWORK

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Public health governance and decision-making processes during the Coronavirus disease 2019 (COVID-19) pandemic were constrained by ambiguity about the rapidly evolving epidemiological situation, urgency of response and paucity of robust scientific evidence. Among recent analyses on governance during the pandemic, few studies have focused on the process of decision-making. Comprehensive frameworks for understanding the process and determinants of public health decision-making at different levels are needed for effective future pandemic preparedness and response. Through a series of key informant interviews and focus group discussions with national officials, academic experts and civil society representatives from Singapore, we developed a framework describing the determinants of public health decision-making during the pandemic in Singapore. In this framework, we draw from crisis management theories and present three key determinants of decision-making: centralization, agility and adaptability. Centralization refers to concentration of decisional power with a limited number of executives. Agility refers to timely action and quick redirection of resources towards priority issues. Adaptability refers to modifying existing structures and processes or establishing new structures and processes to respond to the existing crisis. We highlight that these determinants are further shaped by three factors. First is the availability of human, material and information resources. Second by is by political, social and economic context. Third by the governance values of accountability, transparency, equity and trust. We present five key characteristics for each determinant, which we suggest should be strengthened for future pandemic preparedness efforts. Further work is needed to assess generalizability of our results. However, we hypothesize that this framework can be applied across different contexts and governance systems.

5876

## INVESTIGATION AND MANAGEMENT OF A STREPTOCOCCUS PNEUMONIAE MENINGITIS EPIDEMIC IN DJADOUBANGO, IVORY COAST

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On January 21, 2023, the Tanda health district, in the East of the country, was alerted by the general hospital following the death of 2 schoolchildren from Djadoubango, who presented with headaches & a stiff neck. A team from the health district immediately went to the village. The objective of this work is to describe the phenomenon, identify the cause & propose control measures. This multidisciplinary team, made up of doctors, nurses & biologists, reviewed the consultation registers of the health facilities,

interviewed the populations, examined the patients, looked for other patients in the community & collected blood & cerebrospinal fluid samples. From this investigation, the following points emerged: (i) 14 registered patients whose age varies between 6 & 16 years old. They were all from Djadoubango, with family ties & all from the primary school of the village but with no notion of travel during the previous month, (ii) 05 deaths including 03 during evacuation to hospital & 02 during hospitalization, (iii) No information was found on the vaccination status of the children, (iv) cerebrospinal fluid samples were collected from 6 children & sent to Pasteur institute laboratory in Abidjan, (v) 9 patients were hospitalized & treated free of charge at Tanda General Hospital, (vi) the results of the analysis of the samples showed cases of malaria & 2 cases of Streptococcus pneumoniae meningitis. One of the 2 cases died while the other recovered after treatment with antibiotics. These pneumococcal meningitis cases show the change in the ecosystem of the cerebrospinal meningitis germ following vaccination campaigns against meningococcus A & C. Indeed, meningitis epidemics were until then due to Neisseria meningitidis. Vaccination of contact subjects with pneumococcal vaccine was carried out to contain the epidemic. Promiscuity in the classrooms & climatic factors such as the harmattan marked by drought & dust may have favored the occurrence of the disease. Reinforcing pneumococcal vaccination in the Expanded Immunization Program is necessary.

**5877**

## UNDERSTANDING THE USE OF UTEROTONICS BY COMMUNITY HEALTHCARE PROVIDERS DURING HOME DELIVERY IN RURAL BANGLADESH

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In Bangladesh, uterotonics, e.g. oxytocin, are widely used to augment uterine contractions during home delivery, which may increase the risk of asphyxia leading to stillbirth or neonatal death. A pregnancy surveillance system in rural Baliakandi, Bangladesh systematically identified pregnant women and followed them to identify the care received through their birth outcome. We conducted a mixed-method study to identify the frequency and extent of use of uterotonics during home delivery. Among the 5337 completed pregnancies during September 2021 to December 2022, 23% (1186/5337) delivered at home with a birth attendant. In 47% (563/1186) of deliveries a uterotonic was given to increase contractions. We conducted 14 interviews with birth attendants and six mothers, recently delivered at home. All the birth attendants reported that almost in every case they provided an uterotonic, mainly to increase uterine contractions for normal delivery and usually give the first ampule (5 IU/ml) oxytocin when the water breaks or/and baby's head moves into the vaginal canal and commonly continue to give up to three ampules. Most mothers also shared the same experience. If a mother does not deliver within 30 minutes of the third ampule, birth attendant referred mother to hospital. They also give this drug in some critical conditions, such as when the baby's head is in the birth canal but the mother does not have contraction. A few skilled attendants reported using oxytocin to decrease postpartum bleeding, to discharge placenta and uterus involution. Birth attendants noted, families often pressured them to provide this drug for a quicker delivery. Half of them remarked that if the cervix was not opened but the injection was given, it creates pressure in the uterus and could lead to baby's death, though they rarely experienced it. Among the 563 deliveries in which oxytocin was given, 98% reported to a livebirth outcome compared to 97% livebirth when it was not given. Although the results of our study found a livebirth outcome after frequent use of oxytocin during labor, further follow-up of those livebirths may provide more definitive evidence of adverse outcome of such use.

**5878**

## ROUTINE CHILDHOOD IMMUNIZATION IN BURKINA FASO: IDENTIFYING AND REACHING ZERO-DOSE AND UNDER-VACCINATED CHILDREN IN A SECURITY CHALLENGED COUNTRY

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WHO-UNICEF estimates and coverage survey data show about 10% of children miss vaccination in Burkina Faso yearly. These children, often from marginalized groups, are susceptible to vaccine-preventable diseases and maintain avoidable outbreaks in the country. Defining Zero-dose (ZD) children as those who have not received a first dose of the combined Diphtheria, tetanus, pertussis, hepatitis B and Haemophilus influenzae type b (DTP-Hep-Hib) vaccine at 8 weeks, we conducted a secondary analysis of five years of administrative data (2017 - 2021 DHIS2) to map ZD children and prioritize strategies to reach them. We triangulated administrative data with survey data (2020 Demographic health survey, 2020 vaccination coverage survey). To reduce the limitation of coverage survey data at the district level, we created a correction factor (f) using the ratio of regional coverage and applied it to the district level. The determining factors of ZD children and successful immunization strategies were assessed using the Immunization Equity Survey, the Gender Action Plan, the 2021-2025 National Immunization Strategy, the Immunization Strategy for Security Challenge Areas and post-vaccine introduction assessments. Overall, there were an estimated 45,193 (5.6%) ZD children in 2020 and 32,023 (3.8%) ZD children in 2021. From 2020 to 2021, fourteen (14) health districts accounted for 85.4% of the ZD children. The highest number of ZD children were found in Djibo, Dori, Gorom Gorom and Barsalgo districts in 2020, all areas affected by insecurity, while urban districts (Boulmiougou, Bogodogo Sigh Nonghin) and districts dealing with significant security challenges (Diapaga, Barsalgo Djibo et Titao) accounted for the largest number in 2021. The key determining factors for ZD and under-vaccinated children included insecurity, availability and accessibility of health services and poverty, yet there was an upward trend of ZD children in the richest wealth quintiles (from 2.8% in 2010 to 4.4% In 2020).

**5879**

## ASSESSING KNOCK DOWN RESISTANCE MUTATIONS IN THE DENGUE VECTOR, AEDES AEGYPTI, IN POSADAS, ARGENTINA

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The control of adult populations of the arboviral vector, Aedes aegypti, is mainly based on the application of insecticides, and the widespread use of pyrethroids led to the selection of genetic resistance to insecticides on a global scale. The increased frequency and distribution of non-synonymous knock down resistance (kdr) mutations in the voltage gate sodium channel gene (Nav), impose a threat to the success of mosquito control programs. In this work we investigated the presence of two kdr mutations (V1016I and F1534C) in the Nav gene across four neighbourhoods in Posadas, Argentina. Alleles at both loci were interrogated using TaqMan SNP genotyping assays in DNA extracted from 100 adult females. We report the presence of pyrethroid resistance alleles, kdr 1016I=29.08% and kdr 1534C=70.70%, among adult females. The combined kdr genotypic frequency reveals that approximately 67% of local adult females have an enhanced resistance to pyrethroids, carrying at least one kdr allele at each locus. Genotyping is an invaluable tool for kdr resistance monitoring in vector control campaigns. This is the first report of kdr mutations in Ae. Aegypti in the Northeast of Argentina, a region with recurrent dengue

epidemics. Our results emphasise the need to extend the kdr frequency assessment to other cities alongside with arboviral and entomological surveillance.

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## USING TRANSCRIPTOMIC DATA TO IDENTIFY POTENTIAL MARKERS OF TRANSFLUTHRIN INSENSITIVITY IN ANOPHELES GAMBIAE SS

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The emergence of resistance to pyrethroids, threatens the efficacy of insecticide treated nets (ITNs) and necessitates the development of complementary tools. Spatial repellents (SRs), are a promising new intervention that reduce human vector contact, thereby breaking transmission by creating vector-free spaces. Although they are structurally different from insecticides used on ITNs, insecticides used in current SRs such as transfluthrin are pyrethroid insecticides raising the possibility of SR insensitivity among vector populations with high levels of resistance to pyrethroids. We used a high throughput screening system to determine the spatial activity index(SAI) of three strains of An. gambiae s.s. (Kisumu, susceptible; Bungoma, local resistant; Pimperena, resistant) to transfluthrin-treated surfaces. A whole transcriptome analysis approach was used to determine differentially expressed genes and identify potential markers for transfluthrin insensitivity. The SAI analysis showed a heterogeneous response based on mosquito population. Bungoma (12.515 ug/ml : SAI - 0.069), Kisumu (0.0025 ug/ml : SAI - 0.117) and Pimperena (125.15 ug/ml : SAI - 0.111). The differential expression analysis in non-responders relative to responders showed an over-expression of primarily members of the cytochrome P450 monooxygenases. CYP12F12 was the most overexpressed with a fold change (FC) of 36.64 in Bungoma and 43.80 in Pimperena relative to responder Kisumu strain. Olfactory-related genes were mostly globally down-expressed in all test populations. This study provides pertinent background for understanding the effects of transfluthrin pressure on Anopheles gambiae ss and the first evidence of differential gene expression linked to behavioral insecticide resistance in malaria vectors. The roles of these genes in transfluthrin insensitivity need to be validated to further substantiate their involvement in behavioral responses to transfluthrin, in order to provide a basis for the development of molecular surveillance tools for early detection of transfluthrin failure.

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## IMPACT OF SUGAR DIET ON THE SENSITIVITY OF INSECTICIDES-RESISTANT MOSQUITOES

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The resistance of mosquitoes to insecticides is a rapidly growing problem. While resistance has a strong genetic basis, the environment also affects the extent of resistance. However, we know little of how, for example, the mosquitoes' diet affects their resistance. Our aim was to see how different types of sugar found in nectar influence the sensitivity of strongly insecticide-resistant mosquitoes. To do so field-collected larvae of Anopheles gambiae s.l. from Tiassalé were reared to adults then, their offspring (F1 generation) reared were used for the experiment. We provided adult female sugar meals consisting of sucrose, glucose, fructose or trehalose dissolved in distilled water at concentrations yielding 1.97 or 19.7 kcal/100ml. After five days we measured their knockdown rate and their mortality within 24 hours of exposure to 0.5% deltamethrin with WHO tube tests. We found that there was a positive correlation between the rate of knockdown and mortality for all sugar meals. Mosquitoes fed on the lower concentration were 1.4 to 2 times more likely to die than the better fed mosquitoes, but that the type of sugar had no effect on resistance. Results indicate that the amount of calories provided by sugar is a potential determinant of mosquito susceptibility to insecticides. Sugar meal containing fewer calories may provided less energy to mosquitoes, making them less vigorous and more sensitive to insecticide. Further studies will consider other components of nectar in an attempt at using plants in an integrated approach to manage infection resistance of mosquitoes.

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## EFFECTS OF AGRICULTURAL PESTICIDES ON THE SUSCEPTIBILITY AND FITNESS OF MALARIA VECTORS IN RURAL SOUTH-EASTERN TANZANIA

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Agricultural pesticides may exert strong selection pressures on malaria vectors during the aquatic life stages and may contribute to resistance in adult mosquitoes. This could reduce the performance of key vector control interventions such as indoor-residual spraying and insecticide-treated nets. The aim of this study was to investigate effects of agrochemicals on susceptibility and fitness of the malaria vectors, across farming areas in Tanzania. An exploratory mixed-methods study was conducted to assess pesticide use in four villages (V1-V4) in Tanzania. Larvae were collected from agricultural fields in the same villages and their emergent adults examined for insecticide susceptibility, egg-laying, and wing lengths. These tests were repeated using laboratory-reared An. arabiensis, one of which was pre-exposed for 48hrs to sub-lethal aquatic doses of agricultural pesticides found in the villages. Farmers lacked awareness on the links between public health and agriculture sectors but were interested in being more informed. Agrochemicals usage was reported as extensive in V1, V2 & V3 but minimal in V4. Similarly, mosquitoes from V1-V3 but not V4 were resistant to pyrethroids, and either pirimiphos-methyl, bendiocarb or both. Adding the synergist, piperonyl butoxide, restored potency of the pyrethroids. Pre-exposure of laboratory-reared mosquitoes to pesticides during aquatic stages did not affect insecticide susceptibility and fecundity (except in organophosphates) in emergent adults of the same filial generation. Wild mosquitoes were smaller than laboratory-reared ones, but fecundity was similar. In conclusion, in this study, susceptibility of mosquitoes to public health insecticides was lower in villages reporting frequent use of pesticides compared to villages with little or no pesticide use. Variations in the fitness parameters, fecundity and wing length, marginally reflected the differences in exposure to agrochemicals. Pesticide use may exert additional life-cycle constraints on mosquito vectors, but this likely occurs after multi-generational exposures.

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## SUSCEPTIBILITY OF ANOPHELES GAMBIAE SENSU LATO TO FOUR CLASSES OF INSECTICIDES AND THE ALLELIC FREQUENCIES OF GENES KDR L1014F AND ACE 1 G119S IN TWO VILLAGES OF THE CIRCLE OF KATI IN MALI

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The multiple resistance situation in Anopheles gambiae sensu lato found in Mali with the concomitant presence of the kdr L1014F and ace-1 G119S mutations constitutes a major threat to the success of current malaria control strategies (LLIN and IRS). While it is obvious that with the different

uses of insecticides (controlled or not) it is difficult if not impossible to avoid the installation of resistance at any time, it is crucial to accompany malaria vector control strategies with an efficient insecticide resistance monitoring system. The present study aims to evaluate the susceptibility of Anopheles gambiae sensu lato to the four classes of insecticides commonly used in public health for vector control and the allelic frequencies of the kdr L1014F and ace-1 G119S genes. The study took place from June to October 2021 in Ouassorola and Sogolombougou, two villages in the Kati circle, Koulikoro region. Bioassays were performed according to WHO standard procedures, identification of Anopheles gambiae sensu lato species and detection of kdr L1014F and ace-1 G119S mutations were done by PCR. The mortality rates were respectively in Ouassorola and Sogolombougou of : 100% and 91.25% for fenitrothion; 97.5% and 92.5% for bendiocarb; 67.5% and 50% for DDT; 8.75% and 0% for deltamethrin 3.75% and 0% for permethrin and 10% and 0% for lambda-cyalomethrin. The allelic frequencies of the kdr L1014F mutation were 72.93% in Ouassorola and 79.67% in Sogolombougou and those of ace-1 G119S were 25% in Ouassorola and 46.15% in Sogolombougou. Anopheles coluzzii and Anopheles gambiae were the only members of Anopheles gambiae sensu lato identified in both study sites. This study showed strong resistance of An. coluzzii and An. gambiae to deltamethrin 0.05%, lambda-cyhalothrin 0.05%, permethrin 0.75%, DDT 4% and bendiocarb 0.1%. Anopheles gambiae sensu lato was susceptible to fenitrothion 1% in Ouassorola. A high frequency of genes kdr L1014F and ace-1 G119S was observed in both villages. The species of the Anopheles gambiae sensu lato complex found were Anopheles gambiae and Anopheles coluzzii.

**5884**

## ASSESSING INSECTICIDE RESISTANCE PROFILE OF ANOPHELES GAMBIAE S.L. FOR STRATEGIC VECTOR CONTROL DECISION MAKING IN GUINEA

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Mass and continuous distributions of insecticide-treated nets (ITNs) are the main malaria vector control strategy implemented in Guinea. To support the strategic selection of the most appropriate ITN to deploy across Guinea, vector susceptibility to insecticides used in WHO-recommended ITNs was assessed between July and December 2022 in seven prefectures located in the four natural regions of the country. Wild Anopheles gambiae s.l. larvae were collected from development sites, reared into adults, and exposed to the diagnostic dose of pyrethroids (deltamethrin 0.05%, alpha-cypermethrin 0.05%, and permethrin 0.75%) and chlorfenapyr (100 µg/bottle) using the WHO tube test and bottle bioassay respectively. When pyrethroid resistance was confirmed at the diagnostic dose, intensity test (i.e., exposure to 5× and 10× the diagnostic dose) and piperonyl-butoxide (PBO) synergism tests (i.e., PBO pre-exposure followed by exposure to 1× pyrethroid) were conducted. Pyrethroids resistance was observed at all tested sites with mean mosquito mortalities ranging from 3% to 22% at the diagnostic dose, and the intensity was generally high with less than 90% mortality at the 10× diagnostic dose. Pre-exposure to PBO increased the mortality of all three pyrethroids (between 10% to 50%) but did not result in absolute mortality greater than 70%. Mortality of An. gambiae s.l. after exposure to chlorfenapyr was 100% at all seven prefectures. Hence, there is a high frequency and intensity of pyrethroid resistance in An. gambiae s.l. in Guinea, PBO partially increases susceptibility to pyrethroids, and, at the time of this study, there was complete susceptibility to chlorfenapyr. Based on these results, the scale-up of dual active ingredients or PBO ITNs could help mitigate pyrethroid resistance and improve the impact on malaria vectors in the country. Insecticide resistance monitoring should continue in Guinea, including the identification of mechanistic markers of insecticide resistance.

**5885**

## IDENTIFICATION OF INSECTICIDE RESISTANCE MARKERS IN ANOPHELES ARABIENSIS AND AN. GAMBIAE FROM KENYA AND BENIN USING WEIGHTED GENE CORRELATION NETWORK ANALYSIS

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Indoor Residual Spraying (IRS) and Insecticide-Treated Nets (ITN) are the two main methods used to control mosquito populations for malaria prevention. Currently, efficacy of these strategies is threatened by the appearance and the spread of insecticide resistance (IR), limiting success of malaria control. Studies of the genetic evolution leading to insecticide resistance could enable identification of molecular markers that can be used for IR surveillance and an improved understanding of the molecular mechanisms associated with IR. This study aimed to use a Weighted Gene Co-Expression Network Analysis (WGCNA) algorithm, a systems biology method, to identify genes with similar co-expression patterns and hub genes that can potentially be used as molecular markers for insecticide resistance surveillance in Kenya and Benin. Anopheles arabiensis and An. gambiae from Kenya and Benin were phenotyped for resistance to alphacypermethrin, permethrin and deltamethrin insecticides. RNA was extracted from unexposed, susceptible and resistant samples followed by Illumina sequencing. WGCNA was conducted to evaluate co-expression patterns of genes to identify modules, hub genes and generate a gene co-expression network. A total of 20 and 26 gene co-expression modules (sft:20,18) were identified via the average linkage hierarchical clustering from An. arabiensis (Kenya) and An. gambiae (Benin), respectively. The top modules based on the number of genes in An. arabiensis and An. gambiae were identified to be salmon (n=3197) and blue (n=3839) modules. The genes with the strongest connection (hub genes) were found in all modules. Serine protease, E3 ubiquitin-protein ligase, cuticular protein RR2 and leucine-rich immune protein were identified as hub genes in both species. This was the first study to conduct WGCNA based on IR transcriptomic data. Four biologically relevant hub genes shared between the two species were identified as potential markers for insecticide resistance. The next phase will be to undertake in vitro and in vivo studies to functionally validate these genes as IR markers.

**5886**

## POPULATION GENOMICS OF THE INVASIVE MALARIA VECTOR ANOPHELES STEPHENSI IN ETHIOPIA

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Anopheles stephensi has been identified as a major threat to malaria control and elimination efforts in the Horn of Africa (HOA). The species, native to South Asia, is a highly competent vector in urban areas and can transmit both Plasmodium falciparum and vivax. Since An. stephensi

was first identified in Dijbouti in 2012, the country has seen malaria cases skyrocket. Integrated vector management is often the primary strategy used to interrupt disease transmission, but levels of insecticide resistance are growing and could severely impact malaria control. Very little is known about the population genetics of this invasive species spreading in the HOA. In this study we aim to better understand the population genetics of this vector and help better inform vector management programmes. Twenty-eight An. stephensi samples collected from a central Ethiopian town, were whole genome sequenced. Average coverage was ~30-fold per sample, with 14930416 SNPs identified across the genome. We found one known mutation associated with insecticide resistance: A296S, or the rdl mutation. The SNP was found in 46% of the Ethiopian samples analysed, and results in resistance to the insecticide dieldrin. Other putatively novel non-synonymous SNPs were found in genes associated with insecticide resistance: ace-1 and GSTe2.In addition, we examined the population structure of the Ethiopian isolates in the context of publicly available WGS data from Colony Pakistani and wild-type Indian An. stephensi mosquitoes. Pre-liminary ancestry analysis using admixture indicates genetic distinctness between Ethiopian and Indian populations of An. stephensi. Significant Fst was observed between pairwise analysis of these two populations. Phylogenetic analysis suggests a closer relationship between SDA500 Pakistani strains and Ethiopian An. stephensi isolates.Here we have identified an insecticide resistance marker and given insight into the population genomics of Ethiopian isolates of An. stephensi, in the context of native South Asian populations of this invasive vector.

**5887**

## CHARACTERIZATION OF A NEW LABORATORY COLONY OF ANOPHELES FUNESTUS MOSQUITOES ESTABLISHED IN IFAKARA, TANZANIA

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Anopheles funestus carries most malaria in east and southern Africa. However, it has been challenging to study this species because it is difficult to colonize inside laboratories. Our team was able to successfully colonize a strain of this species from Tanzania (FUTAZ) for >20 generations. We compared the FUTAZ strain to two other strains of mosquitoes: a strain of An. funestus from Mozambique (FUMOZ) and a strain of Anopheles arabiensis. We examined the differences in fitness between these strains through measures like body size and mating success. We also looked at the genetic makeup of the mosquitoes using PCR analysis of mitochondrial clades and restriction fragment length polymorphisms (RFLP) on the 28S ribosomal DNA. We found that the FUTAZ strain had a decline in mating success and body size in the first six generations, but then they adapted, and these measures improved. By the ninth generation, the FUTAZ strain had similar fitness measures to the FUMOZ strain. Fecundity was similar across all strains tested. It took twice as long for the FUTAZ and FUMOZ strains to mate compared to the An. arabiensis strain. The genetic analysis showed that the FUTAZ strain was similar to the wild-caught Tanzanian An. funestus strain, but it was different from the FUMOZ strain. Our study shows that it's important to have a large founder population when starting a new colony to ensure that the mosquitoes can adapt to laboratory conditions.

**5888**

## CO-OCCURRENCE OF MULTIPLE KDR MUTATIONS (F1534C, V1016I, V410L) IN AEDES AEGYPTI FROM COASTAL AREAS IN GHANA AND ASSESSMENT OF THE ROLE OF MOSQUITO COIL IN CAUSING PYRETHROID RESISTANCE

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The rapid spread of knockdown-resistance kdr mutations in Africa calls for monitoring and investigation into the cause of pyrethroid resistance to inform management strategies. This study investigated the pyrethroid resistance profile of Ae. aegypti from coastal towns in Ghana and the impact of mosquito coils, a popular household pyrethroid-based anti-mosquito tool, on the development of pyrethroid resistance. Susceptibility to deltamethrin and the presence of kdrmutations were determined in adult female mosquitoes reared from larvae. Furthermore, a laboratory colony was exposed to a sub-lethal dose of a mosquito coil once per generation for six generations (F6). The susceptibility of the exposed colony to deltamethrin (0.05%) was determined using WHO protocols. The Ae. aegyptipopulations from the coastal towns were resistant to deltamethrin with co-occurrence of F1534C, V1016I and V410L kdrmutations. In the experimental study, the LT50 (95% CI) of the exposed colony against the coil rose from 8 minutes (95% CI; 6-9) at F0 to 28 minutes (95% CI; 23-34) at F6. Nonetheless, deltamethrin caused similar mortalities in the exposed and control colonies. The resistant allele frequencies of 1534C and 410L were identical, but 1016I was higher in the exposed colony than in the control. However, the increased tolerance to the coil and high resistant allele frequency of 1016I in the exposed colony did not affect the mosquito's resistance to deltamethrin insecticide. Further study is needed to elucidate the role of pyrethroid-based mosquito coils in developing insecticide resistance in mosquito vectors.

**5889**

## TEMPORAL RESISTANCE ESCALATION AND N1575Y MARKED DETECTION IN ANOPHELES GAMBIAE S.L POPULATION IN ATATAM, AN EXPERIMENTAL HUT STATION SITE IN SOUTHERN GHANA

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Major malaria vectors in Africa are increasingly developing high intensity of resistance to approved used in vector control. This threatens to impact efficacy of insecticides-based tools necessitating the need to monitor resistance in vector populations to support insecticide resistance management. This study reports the temporal aggravation of resistance and significant detection of N1575Y-Kdr marker in the primary malaria vector Anopheles gambiae s.l at Atatam, a rural Ghanaian community. Indoor resting blood-fed Anopheles mosquitoes were collected twice each year coinciding with the peaks of the major and minor raining season between 2021-2022 were forced to lay eggs to generate F1 adult mosquitoes. Insecticides susceptibility bioassays, PBO synergist assay and cone assays with pyrethroid-only nets and PBO-based nets were performed on the F1 adults. Furthermore, molecular basis of resistance were characterized in F0 populations using TaqMan genotyping. An. gambiae s.l consisted of An co.luzzii and An. gambiae s.s. These displayed high levels of resistance to Permethrin, Deltamethrin, Alpha-cypermethrin and DDT with moderate resistance recorded against Bendiocarb in contrast to full susceptibility

recorded for Pirimiphos-methyl. PBO synergist assays with Permethrin and Deltamethrin induced only a marginal recovery of susceptibility in An.gambiae population (6.2% to 43% and 2.2% to 37.1% mortality, respectively). The high pyrethroid/DDT resistance in An. gambiae correlated with high frequency of 1014F, N1575Y knockdown resistance allele (91% and 50%) and GSTe-I114T resistant allele (59.6%). The G119S allele was detected at low frequency (1.7%) indicative of the increased susceptibility to Bendiocarb and full susceptibility to Pirimiphos methyl. Cone assays reveal loss of efficacy against pyrethriod-only based nets and a reduced efficiency against PBO based nets against these resistant An. gambiae s.l population. These results highlight the escalation of insecticide resistance and the challenges that control programmes face to maintain the continued effectiveness of existing insecticide-based interventions.

**5890**

## INSECTICIDE SUSCEPTIBILITY OF ANOPHELES ALBIMANUS IN THE TWO MAIN ACTIVE MALARIA FOCI OF HONDURAS

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Malaria cases in Honduras have increased over the previous four years, rising from 386 in 2019 to 3,601 in 2022. Around 90% of cases are reported in Puerto Lempira and Villeda Morales municipalities, in the department of Gracias a Dios. Vector control in Gracias a Dios employed the use of pyrethroids-impregnated bednets, with occasional indoor spraying with bendiocarb. The Ministry of Health in collaboration with other partners has conducted insecticide resistance surveillance over the last 5 years in one sentinel site at Gracias a Dios, however, no fine scale evaluations per foci have been carried out thus far. The aim of this work was to evaluate insecticide susceptibility status in the two main malaria transmission areas using both a phenotypic and genotypic approach. Adult entomological collections were carried out at Kaukira, Puerto Lempira and Raya, Villeda Morales in Gracias a Dios. Deltamethrin and Bendiocarb phenotypic status were assessed using CDC bottle bioassays with diagnostic doses. Sequencing analysis was used on a selection of 50 Anopheles albimanus individuals (30 from Kaukira and 20 from Raya) for target-site mutations detection at 995 and 280 positions in the Voltage-Gated Sodium Channel (VGSC) and the Acetylcholinesterase (Ace-1) gene, respectively. Between September and December of 2022, a total of 701 mosquitoes were collected, with An. albimanus (87%) as the most common specie across the sites, however, secondary species as An. vestitipennis and An. crucians were also identified. Deltamethrin and Bendiocarb show 100% mortality in susceptibility assays. Sequencing analysis revealed that all populations had the wild-type genotype, TTG at 995 (VGSC) and GGC (Ace-1) at 280 positions, respectively, supporting the phenotypic results. Altogether, this data provides current evidence of An. albimanus susceptibility to the insecticides employed by the MoH in vector control and supports their use in routine interventions. Further studies are required in other foci as well with secondary species on a routine basis to drive future vector control operations

**5891**

## ENTOMOLOGICAL STUDY OF MALARIA TRANSMISSION PARAMETERS AS A PRELUDE TO A PHASE III CLINICAL TRIAL OF ATTRACTIVE TOXIC SUGAR BAIT STATIONS IN THE KOULIKORO REGION, MALI

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Despite the use of prevention and control tools, malaria remains a major public health problem in sub-Saharan African countries, including Mali. Parasite resistance to anti malaria drugs and vector resistance to insecticides are undermining prevention and control efforts. In addition, mosquito biting behavior is shifting from predominantly indoor biting to more outdoor biting. New control methods are therefore urgently needed. It is in this context that the attractive targeted sugar baits or Attractive Toxic Sugar Bait (ATSB) based on the “attract-kill” principle have emerged, trapping mosquitoes with a sugar bait containing an oral toxin. ATSB have had many successes after their use in numerous experimental studies in the control of Anopheles vectors, Aedes and other insect vectors. We therefore conducted this study with the aim of evaluating the entomological parameters of malaria transmission before an epidemiological phase III trial. It took place in five villages in the district of Kangaba and two villages in the district of Ouélésséboungou; all in Koulikoro region of Mali. It was a longitudinal study with monthly mosquito collection from July to December 2021. We used three trapping methods: human landing catch (HLC), daytime capture by pyrethroid spray catch and CDC UV light trap. We are here reporting only the data for HLC outdoor where we caught 3289 An. gambiae s.l. They had completed 1 or 2 gonotrophic cycles. Their longevity was greater than or equal to 50 days except for Solonkorein and Balala. The maximum infection, 0.17 infectious bites/person/night was observed in Balala. Our findings will inform the randomization of clusters for epi trial aiming at demonstrating an effect of ATSB on malaria incidence.

**5892**

## PHENOTYPIC INSECTICIDE RESISTANCE STATUS AND MOLECULAR DETECTION OF RESISTANCE MUTATIONS IN ANOPHELES GAMBIAE SENSU LATO IN THE GAMBIA

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The use of long-lasting insecticide treated bed nets (LLINs) and indoor residual spraying (IRS) in high burden areas has played a major role in the reduction of malaria cases and deaths in The Gambia. Widespread resistance of Anopheles gambiae sensu lato to pyrethroids, scale-back of IRS, high costs, and suboptimal compliance to vector control interventions threaten these gains and have led to the adoption of the WHO insecticide rotation plan by the National Malaria Control Programe (NMCP). This study aimed to describe phenotypic and molecular resistance of An. gambiae s.l. to pyrethroids to guide the NMCP’s selection of insecticide for IRS in The Gambia. From July-October 2021, An. gambiae s.l. larvae were collected and reared to adults from 7 sentinel sites: Brikama, Essau, Farafenni, Njabakunda, Georgetown, Basse, and Gambisarra. Using the WHO tube bioassay, a total of 3,237 An. gambiae s.l. were exposed to Deltamethrin 0.05%, Deltamethrin 0.25%, or Pirimiphos-methyl 0.25%. A total of 195 mosquitoes were genotyped at 7 loci for 11 SNP mutations associated with insecticide resistance. Resistance to Deltamethrin 0.05% was confirmed in all sites, with mortality ranging from 30% in Bakau to 73% in Georgetown. Moderate to high resistance to Deltamethrin 0.25% was observed in 6 sites (81%-97% mortality) and low resistance in one site (Georgetown; 99% mortality). An. gambiae s.l. from all sites were susceptible to

Pirimiphos-methyl. Sequencing detected 8 SNPs: kdr-1014S, kdr-1014F, Coeae1d, A296S rdl, T345S rdl, Cyp6jS, Gste2-119V, and Gste2-114T with allele frequencies varying between 0.03 and 0.54. One to 6 SNPs per mosquito were identified with over 80% with 1-3 SNPs. An. gambiae s.l. in The Gambia are susceptible to Pirimiphos-methyl 0.25%, resistant to Deltamethrin 0.05%, and exhibited moderate to high resistance to Deltamethrin 0.25%. The lowest and highest frequencies were observed for Cyp6jS and Coeae1d, respectively. Since no resistance was confirmed for Pirimiphos-methyl 0.25%, the NMCP will continue using this insecticide for IRS in The Gambia, while also adhering to the insecticide rotation plan to delay expansion of resistance.

**5893**

## OVEREXPRESSING IMMUNE SIGNALING PROTEIN VAGO RESTRICTS DENGUE VIRUS INFECTION IN AEDES AEGYPTI MOSQUITOES

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Aedes aegypti is the principal vector for pathogens like, Dengue virus, Zika Virus, and Chikungunya virus. The lack of effective vaccines and drugs against these pathogens has led to severe economic and healthcare burdens in developing countries, leading to the need for new control measures. The four major pathways through which Aedes mosquitoes mount an immune response are, the JAK-STAT, RNAi, IMD, and Toll pathways. Recent publications have indicated that there is a crosstalk between these pathways during viral infections. The Vago gene which encodes for a cysteine-rich 18Kd polypeptide has been shown to be involved in antiviral activity. In Culex quinquefasciatus mosquitoes, Vago has been shown to be involved in crosstalk between the JAK-STAT, RNAi, and IMD pathways upon West Nile Virus infection. The potential of the Aedes orthologue of Vago to connect immune pathways has not yet been studied and its mode of action is still unknown. In the present study, we have created two transgenic lines that overexpress Vago in the fat body and the midgut, using the PiggybacTM transposon system. A significantly lower titer of Dengue virus was observed 14 days post-infection in transgenic lines overexpressing Vago in the midgut. We also aim to study the role of Vago in connecting immune pathways and its potential anti-viral activity when overexpressed in the fat body.

**5894**

## DEFINING THE ROLE OF JUVENILE HORMONE AND ITS RECEPTOR, METHOPRENE-TOLERANT, IN ANOPHELES GAMBIAE REPRODUCTION AND PLASMODIUM TRANSMISSION

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Controlling the reproductive cycle in Anopheles mosquitoes can serve as a critical tool for vector control and contribute towards the overall goal of malaria elimination. Our previous studies have demonstrated a functional role for the insect steroid hormone 20-hydroxyecdysone (20E) in Plasmodium falciparum development in its main vector Anopheles gambiae, where blocking 20E activation resulted in reduced parasite survival but faster parasite growth through the insect stages. In the adult female mosquito, the vitellogenic phase that is governed by 20E regulation temporally follows the post-eclosion (PE) development phase (day 1-3 PE) that is regulated by another insect hormone, Juvenile Hormone (JH). JH is responsible for initiating the cascades responsible for tissue maturation that are required to prime the female reproductive machinery prior to the acquisition of a blood meal. Here, we analyzed the role of the JH binding receptor, Methoprene-tolerant receptor (Met), in An. gambiae reproduction and P. falciparum development. After characterizing the gene expression pattern of Met and two down-stream transcription factors (Kruppel homolog-1 and Hairy), we demonstrate that JH has an important functional role in regulating gene expression during the PE period by artificially inducing the JH-mediated pathway through the topical application of Methoprene. Next, we administrated dsRNA targeting the Met receptor to block the JH response and subsequently measured vector reproductive fitness and parasite transmissibility. Our results indicate that blocking JH activation results in both reduced mosquito egg development and parasite survival, but also leads to faster parasite growth. It is likely that blocking JH activity results in alterations in mosquito immunity and nutritional status that need to be further explored to understand its full impact on Plasmodium transmission. These data unveil a key role of JH in regulating Anopheles-Plasmodium interactions, and suggest a critical cross-talk between 20E and JH in regulating reproductive processes that also impact the survival and growth of the deadliest human malaria parasites.

**5895**

## COMPOSITIONAL DIVERSITY IN THE EARLY-DEVELOPMENTAL MICROBIOME OF AEDES ALBOPICTUS LEADS TO HETEROGENOUS IMMUNE EXPRESSION OF ADULT MOSQUITOES

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Mosquitoes rely on a community of symbiotic microorganisms to sustain biological processes.Variation in the diversity of the microbiome has profound impacts on mosquito physiology thatscale up to host phenotypes. Of special interest are phenotypes permissive to sustained diseasetransmission. We examined the immunological responses of Aedes albopictus mosquitoes to assess how exposure to compositionally different microbial communities during earlydevelopment impacts adult responses to infection. We sourced water from three differentenvironments—bromeliad axils, artificial containers, and purified laboratory water—to createlarval habitats. The water sourced from bromeliads and artificial containers was filtered using30-50 µm, 10 µm, and 0.2 µm filters. Upon eclosion, adults were provided a 10% sucrosesolution and allowed to feed ad libitum for 72 hours. After 72 hours, adults were injected withapproximately 500 live Escherichia coli cells suspended in Luria-Bertani (LB) broth or injectedwith sterile LB broth. At 24 hours post-injection, adults were processed for immune expressionanalyses using quantitative reverse transcriptase PCR. We assayed five gene markers in total:relish 1 (REL1), relish 2 (REL2), STAT, cecropin A (CEC-A) , and defensin C (DEF-C). REL1, REL2,and STAT are transcription factors unique to the three innate immune pathways; CEC-A andDEF-C are antimicrobial peptides. Using generalized linear mixed models, we found thatexposure to compositionally different microbial communities during early mosquitodevelopment impacted the immune response of adult mosquitoes when infected with E. coli.Immune expression patterns varied across treatments and by gene; however, the general trendshowed that mosquitoes from lower filtration habitats (highest microbial diversity) hadincreased upregulation of immune activity. Our results suggest that exposure to diversemicrobial communities in early development may be a significant predictor for the vectorial capacity of Ae. albopictus and that microbial “hotspots” have an important ecological role insustaining mosquito-borne disease transmission.

5896

## ALTERNATING CURRENT ELECTROPENETROGRAPHY REVEALS IN SITU BEHAVIORAL CHANGES OF AEDES AEGYPTI BITES ASSOCIATED WITH DENGUE VIRUS INFECTION

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Human infection with dengue virus (DENV) results in significant morbidity and mortality around the world. Observations of infectious blood feeding events of the primary DENV vector Aedes aegypti have identified DENV-associated behavioral changes that may promote DENV transmission. Current methods are largely restricted to video analysis of feeding events outside of the host or microscopic video capture of biting events on thin tissues. To supplement these methods, AC-DC electropenetrography (EPG) was investigated to assess the method's potential to record infectious mosquito bites and quantify the amount of time a mosquito spends in different stages of the feeding process. IFN-αβ receptor-deficient mice and DENV-2 (S-14635) were used to determine the feasibility and utility of EPG for arbovirus research. EPG recordings were made of Ae. aegypti feeding on anesthetized mice under three conditions: mosquito/mouse uninfected, DENV-infected mosquito/uninfected mouse, and uninfected mosquito/DENV-infected mouse. Successful and interpretable EPG recordings were obtained for seven feeding events under each condition. Ae. aegypti with disseminated DENV-2 had significantly shorter probe durations and more probes per feeding than uninfected controls. This group also spent significantly more time in the low-voltage initial skin penetration stage of feeding and switched more frequently between two different vessel locating behaviors within the skin than controls. During the ingestion phase, uninfected mosquitoes fed on DENV-infected mice spent significantly less time in the classic M1 ingestion phase and much longer in the more irregular M2 phase, though overall ingestion lengths were not significantly different. These data suggest that there are quantifiable in situ behavioral changes associated with DENV infection, especially with respect to behaviors between initial skin penetration and the beginning of ingestion. Moreover, these data demonstrate that EPG is a suitable tool for interrogating arbovirus associated behavioral changes during blood feeding events.

5897

## TEMPERATURE DEPENDENCE OF ANOPHELES IMMUNE RESPONSE KINETICS AND VECTOR COMPETENCE

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Most studies on mosquito innate immunity responses to malaria parasite infection have relied on rodent Plasmodium berghei laboratory models. However, we and others have shown that the Anopheles immune factors and mechanisms involved in eliminating the clinically relevant human P. falciparum may differ from those against murine parasites. A significant difference between the two malaria species is the fact that P. berghei achieves unnaturally high infection intensities in the vector mosquito, frequently exceeding 200 oocysts per midgut, while median infection intensities for P. falciparum of lower than 3 oocysts per mosquito midgut are usual. Using CRISPR/Cas9-mediated gene knockout and RNAi-mediated silencing of A. gambiae immune factors, we demonstrate that the immune regulation of Plasmodium is dependent on the intensity of infection. Here, we show that the differences in intensity are in part due to the parasite's optimal infection temperature: P. berghei sexual sporogonic development occurs at ~19 °C whilst P. falciparum's is optimal at ~27 °C. We hypothesize that this rather large temperature difference results in a slower rate of development for the rodent parasite than for the human malaria parasite within its vector. Temperature also influences mosquito immune response kinetics and the length of exposure of the parasites to these immune responses. In this study, we predict that temperature variations and climate change may significantly affect mosquito transmission of malaria parasites and other pathogens, by altering the infection kinetics. Our study stresses the importance of conducting malaria laboratory-based transmission studies using clinically relevant species.

5898

## DISHEVELLED ACTIVITY DIFFERS IN AEDES AEGYPTI AND CULEX TARSALIS INFECTED WITH RIFT VALLEY FEVER VIRUS

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Aedes and Culex mosquitoes are vectors of Rift Valley Fever Virus (RVFV), a zoonotic virus endemic to Africa that causes episodic outbreaks and has emerged on the Arabian Peninsula. RVFV outbreaks are characterized by abortion storms and occasional death of livestock. The specific mechanisms that underpin differences in efficiency of virus transmission are still not understood. To address this, we sought to better understand mosquito host signaling responses late in RVFV MP-12 infection through analysis of differentially expressed genes (DEGs) in two mosquito strains with marked differences in vector competence, Aedes aegypti (Aae, low competence) and Culex tarsalis (Cxt, higher competence). Mosquito-host transcripts related to three different signaling pathways were investigated. The Wingless (WG, WNT-beta-catenin) pathway is a conserved regulator of cell proliferation and differentiation; changes to FRIZZELED2 (FZ2), DISHEVELLED (DSH), and ARMADILLO (ARM, beta-catenin) were assessed. Importantly, DSH differentially regulates progression/inhibition of the WG and JNK (c-Jun N-terminal Kinase) pathways through interaction with NAKED CUTICLE. A negative regulator of the JNK signaling pathway, PUCKERED, was also assessed. Lastly, Janus Kinase/signal transducers and activators of transcription (JAK-STAT) is a multi-functional signaling pathway important for innate immunity; in this context, we tested DOMELESS levels. Here, individual Aae and Cxt were exposed to RVFV MP-12 in oral bloodmeals and then held for 14 days at 28oC. Robust decreases in expression of signaling transcripts in both Aae and Cxt were observed. In particular, Aae DSH expression, but not Cxt DSH, was correlated to the presence/absence of virus at 14 days post-infection (dpi). This effect was not seen in Culex, perhaps due to its high susceptibility to RVFV. Moreover, there was an inverse relationship between viral copy number and aaeDSH expression that did not occur for any other transcript. Silencing of DSH by dsRNA injection resulted in increases in viral copy numbers compared to controls at 7 dpi, consistent with a role for aaeDSH in antiviral immunity.

5899

## MAMMALIAN HEMOPEXIN REGULATES OXIDATIVE STATE IN ANOPHELES MOSQUITOS DURING PLASMODIUM INFECTION

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During mosquito midgut invasion, Plasmodium ookinetes cross the peritrophic matrix barrier bringing pro-oxidant molecules in contact with the midgut epithelial cells. Our previous data show that during midgut invasion hemopexin from mouse blood is enriched in the mosquito midgut epithelium and hemolymph. We hypothesize that the accumulation of hemopexin, an antioxidant molecule that binds free heme, protects the parasite and the mosquito from heme and iron-induced cellular damage. To further investigate the impact of hemopexin on oxidative state in Anopheles mosquitos we measured the concentration of hydrogen peroxide in the hemolymph from mosquitos that fed on uninfected or infected wild type (WT) or hemopexin-null mice. Hydrogen peroxide was increased in the hemolymph in the absence of host hemopexin. Additionally, lipid peroxidation, protein carbonylation and nitration, markers of oxidative damage, were increased in the hemolymph of mosquitos that fed in hemopexin-null mice. We also compared the impact of hemopexin in

Aedes aegypti mosquitos which are known to have a robust system to control reactive oxygen species (ROS) after ingesting blood. In contrast to Anopheles, the absence of host hemopexin didn't increased oxidative damage markers in A. aegypti suggesting an important role for hemopexin in controlling ROS triggered by blood digestion in Anopheles mosquitos. Mosquitoes feeding on hemopexin-null mice had a significant decrease in survival, egg oviposition and egg hatching, which points that human hemopexin is a key molecule for the mosquito adaptation and survival to feeding on blood. Moreover, Plasmodium infection was significantly decreased in mosquitos that fed in hemopexin-null mice and was restored in mosquitos that fed in hemopexin-null mice that had been injected intravenously with hemopexin. Together, these results show that hemopexin is an essential molecule for the survival of Anopheles mosquitos and malaria parasite transmission.

**5900**

## TNF-A SIGNALING MEDIATES MOSQUITO CELLULAR IMMUNITY TO PROMOTE PLASMODIUM KILLING

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Malaria is a devastating vector-borne disease caused by Plasmodium parasites and transmitted to humans through the bite of Anopheline mosquitoes. Therefore, understanding mosquito innate immunity and the mechanisms that influence vector competence are crucial to efforts to block malaria transmission. In vertebrates Tumor Necrosis Factor- α (TNF-α) is a well-defined proinflammatory cytokine with essential roles in regulating immune cells, yet our understanding of TNF-α signaling in invertebrate systems is limited. Here, we characterize a functional TNF-TNFR-like system in the mosquito Anopheles gambiae, comprised of the TNF-α ortholog Eiger and its two cognate receptors, Wengen and Grindelwald. We demonstrate that the direct injection of recombinant TNF-α limits malaria parasite survival and provide evidence that these killing responses are immune cell-mediated. The injection of TNF-α increases granulocyte numbers via Wengen and leads to the reduced expression of oenocytoid-specific genes, implying the role of TNF-α in oenocytoid rupture, with evidence supporting both immune cell phenotypes in malaria parasite killing. Additional gene-silencing experiments confirm the involvement of Eiger, Wengen, and Grindelwald in anti-Plasmodium immunity, with initial experiments suggesting that Wengen and Grindelwald may act interchangeably to promote TNF-α signaling to suppress Plasmodium development. Together, our data support the role of a conserved TNF-α signaling pathway in influencing the cellular immune system of mosquitoes, providing new insight into the mechanisms of malaria parasite killing and mosquito vector competence.

**5901**

## EFFECT OF LOW RELATIVE HUMIDITY OVER MORTALITY AND VIRAL VECTOR COMPETENCE IN AEDES AEGYPTI

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Vector-borne diseases (VBDs) cause over 700,000 deaths every year. The mosquito species Aedes aegypti is a competent vector of multiple viral pathogens including dengue, Zika, chikungunya, and Mayaro viruses. Ae. aegypti was originally distributed in Africa, however, it is currently present in the Americas, Oceania, Asia, and Europe. Because of climate change, it is expected that the distribution of this mosquito species and the pathogens they transmit will change even more. Relative humidity is an environmental variable that affects the mosquito biology and distribution. This variable can differ between indoors and outdoors, and it oscillates over the course of the day and year, thus it is expected that mosquitoes face variations in relative humidity during their lifespan. Low relative humidity can induce dehydration in mosquitoes, leading to alterations in physiological and behavioral responses such as bloodfeeding and host-seeking behavior, which are relevant for pathogen transmission. The aim of our research was to evaluate the short and long-term effects of low relative humidity over mortality and viral vector competence in Ae. Aegypti, using two different experimental designs. Briefly, we tested mosquitoes under three different conditions of relative humidity to induce dehydration, and measured mortality and bloodfeeding rates. Then, we used a cell culture and immunofluorescence-based assay to quantify the viral load in different parts of the mosquitoes at 7 and 14 days post infection. Our results show that under our experimental designs, low relative humidity does not impact the viral loads, nor the infection, dissemination and transmission rates, in mosquitoes infected with Mayaro-L virus. However, we detected a significant difference in mosquito mortality between treatments regardless of whether the mosquitoes were previously exposed to viral infection or not. These findings allow us to further understand the role of relative humidity in vector-borne disease dynamics.

**5902**

## PLAYING SMART: HOW MALE AEDES AEGYPTI MOSQUITOES USE JUVENILE HORMONE TO MAKE FEMALES FITTER FOR REPRODUCTION BY SUPPRESSING THEIR IMMUNITY AND PROMOTING GUT MICROBIOTA EXPANSION

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Aedes aegypti mosquitoes are notorious vectors of diseases, and understanding their reproductive biology is essential for developing effective control strategies. Our research reveals how mating induces midgut growth in female mosquitoes, a response mediated by the transfer of Juvenile Hormone (JH) from males during copulation. In addition, we demonstrate how mating and JH modulate the mosquito's immune response in the gut, leading to the establishment of a core microbiota population that enhances reproductive output. Specifically, we have observed a JH-dependent suppression of Anti-Microbial Peptides (AMPs) in the female gut, resulting in an increase in bacterial load within the midgut. Our findings demonstrate for the first time how mating and Juvenile Hormones influence organ size and immune responses in the mosquito gut, and how this modulation leads to improved fitness in the form of increased egg counts and lifespan. Our results provide new insights into the molecular mechanisms underlying the interplay between nutrition, immunity, and reproduction in the mosquito gut, highlighting opportunities for further study and potential strategies for mosquito-borne disease control.

**5903**

## WHOLE BODY VOLATILOMICS TO COMBAT VECTOR-BORNE DISEASE

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Human scent is a complex blend of hundreds of volatile organic compounds (VOCs) released via breath and skin emissions that guides arthropod disease vectors towards us. To improve our understanding of human scent signatures, we have developed a field-deployable booth-style collection chamber which enables characterization of inter-individual variability in the chemical composition of human body odor. To validate this sampling chamber for whole body volatilomics, we first performed a screen of 20 humans in a laboratory setting to characterize and quantify the most frequent and abundant compounds present in whole body odor headspace across participants using thermal desorption-gas chromatography/mass spectrometry (TD-GC/MS). Application of this method facilitated detection of a range of VOCs including ketones, aldehydes, carboxylic acids,

alcohols, and hydrocarbons, and quantification of their emission rates in whole body headspace across large numbers of humans for the first time. Leveraging this information, we next developed ratio-specific slow-release lure formulations, mimicking the emission rates of the major components of whole body scent signatures, for use as mosquito attractants. In the laboratory, adhesive trapping assays with a synthetic human scent mimic that we developed revealed that An. gambiae are equally attracted to this novel lure relative to human foot odor. We next performed semi-field assays in Macha, Zambia to test the attractiveness of this lure within the context of the CDC-miniature light trap broadly used for surveillance of malaria vectors. The use of our human scent mimic lure supplemented with yeast-generated CO2 significantly boosted the performance of the CDC light trap, capturing almost four times as many mosquitoes as traps baited with yeast-produced CO2 alone. We propose the use of whole body volatilomics has the potential to instruct development of highly attractive synthetic lure blends for enhanced surveillance and control of varied arthropod disease vectors including major malaria vectors such as Anopheles gambiae and An. stephensi which is currently invading Africa.

**5904**

## A MICROSCALE PLATFORM FOR IMAGING NEURAL CIRCUITS IN THE AFRICAN MALARIA MOSQUITO

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The African malaria mosquito Anopheles gambiae is a deadly vector of malaria that detects humans using its sense of smell. We have recently applied CRISPR/Cas9-mediated homologous recombination and transposon-mediated transgenesis to generate transgenic strains of An. gambiae that express neural activity sensors in defined subsets of mosquito olfactory sensory neurons. These include olfactory sensory neuron populations expressing the olfactory co-receptors Ir76b, Ir25a, Orco and Gr22 - each responsible for detecting varied components of human scent. To overcome previous technical barriers for generating viable surgical preparations to image neural activity in the olfactory center of the An. gambiae brain, we have engineered a laser microsurgery apparatus to create precise micron-scale excision windows in mosquito head cuticle to reveal underlying brain tissue. This microscale platform for imaging neural circuits stands to provide significant insights into the molecular and cellular basis of An. gambiae attraction to human scent. By extension this open-source laser microsurgery device may also be used to study other facets of mosquito biology via intravital imaging of previously hidden mosquito tissues occluded by cuticle.

**5905**

## WARBURG METABOLISM IS CRITICAL FOR ANOPHELES MOSQUITOES ANTI-PLASMODIUM IMMUNE DEFENSE

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Warburg metabolism is an inefficient metabolic shift characterized by increased glycolysis and lactate production. This phenomenon has been observed in mammalian rapidly proliferating cancer and immune cells. Anopheles mosquitoes, which are the vectors for malaria, rely on their metabolic system to provide energy and intermediates for their innate immune system, known as immunometabolism. We posited a disruption of Warburg metabolism would impair the immune response of Anopheles mosquitoes to Plasmodium parasites. To test this hypothesis, we treated Anopheles mosquitoes with dimethyl fumarate (DMF), a GAPDH inhibitor, or used CRISPRi to silence the genes coding for GAPDH and LDH, key enzymes of Warburg metabolism. We then challenged the mosquitoes with either P. berghei (Pb, rodent malaria) or P. falciparum (Pf, human malaria). Our results showed that both DMF treatment and CRISPRi knockdowns increased parasite load in both Pb and Pf infections, and DMF-treated mosquitoes had a significant mortality in both infections. These findings suggest that Warburg metabolism is essential for mosquito anti-Plasmodium immunity.

**5906**

## SAMPLING EFFICIENCY AND MOLECULAR SCREENING OF YELLOW FEVER VIRUS IN AEDES MOSQUITOES IN NIGER DELTA REGION OF NIGERIA

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The vector potential of Aedes mosquitoes in the transmission of the arbovirus responsible for the transmission of yellow fever around the world is well documented. Although Nigeria is a high risk country for yellow fever, there is paucity of information in the Niger Delta region on the distribution of Aedes mosquito vectors and molecular detection of the virus in infected mosquitoes. This study was carried out to breach the gap. The mosquitoes were sampled in four communities (Otolokpo, Ute-Okpu, Umunede and Ute Alohen) in Ika North-East Local Government Area, Delta State, Nigeria. The efficacy of various methods of sampling the mosquitoes (Odour baited traps (BG sentinel), CDC light trap with attractant, CDC light traps without attractant and modified human landing catch (mHLC) were assessed for 12 weeks. Collected mosquitoes were transferred into a holding cages, killed by freezing at -4oC for 20 minutes. They were morphologically identified as Ae. albopictus using standard identification keys. A total of Seven hundred and Twenty-five (725) mosquitoes were obtained from the various traps. They were then preserved in RNAlater by pooling 10 mosquitoes per Eppendorf tube. The preserved mosquitoes were transported to National Arbovirus and Vector Research Centre Institute, Enugu Nigeria for screening of the virus strain using yellow fever primers and probes. Two samples (made up of 10 mosquitoes each) for every sample location were analyzed. It was observed that mean abundance of the mosquitoes was highest in mHLC (42.9 and the difference was highly significant ($p< 0.0001$). The mean abundance of mosquitoes was lowest in CDC light traps without attractant (0.29) compared to other means in other sampling techniques. It was also observed that no yellow fever virus strain was detected in all the mosquitoes sampled at the four locations. The possibilities of not encountering viral strains in mosquitoes may be due to the mass vaccination exercise that was carried out the previous year in the study area. Conclusively, adequate monitoring using the mHLC and continuous research are required to avoid resurgence of these virus in these locations.

**5907**

## SURVEILLANCE OF ARTHROPOD-BORNE VIRUSES IN BENIN, WEST AFRICA 2020-2021: DETECTION OF DENGUE VIRUS 3 IN AEDES AEGYPTI (DIPTERA: CULICIDAE)

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The resurgence of arboviruses in recent decades is a major public health issue facing the international community due to the domestication of insect vectors. Aedes aegypti is the most important vector of arboviruses. In West Africa, it is known to transmit dengue virus (DENV), yellow fever virus (YFV), chikungunya virus (CHIKV), and Zika virus (ZIKV). To determine the abundance of arboviruses a longitudinal surveillance study was conducted in three consecutive years in Benin, West Africa. Adult mosquitoes were captured on human bait, Biogents Gravid Trap and BG-Sentinel traps at five ecological different locations in Benin from June 2019 to September 2021. A total of 3749 mosquitoes were collected and tested by RT-PCR

for arboviruses. One pool of Ae. aegypti captured on 7 July 2021 in Porto Novo tested positive for dengue virus. PCR results were confirmed by sequencing and showed the occurrence of dengue virus serotype 3. Our study highlights that there is a need to implement further investigations and surveillance strategies to prevent and control future outbreaks of mosquito-borne viruses in Western Africa.

**5908**

## COMMON PREDATORS AND FACTORS INFLUENCING THEIR ABUNDANCES IN ANOPHELES FUNESTUS AQUATIC HABITATS IN RURAL SOUTHERN TANZANIA

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The role that larval predators play in regulating the population of malaria vectors remains relatively unknown. This study aimed to investigate the common predators that with Anopheles funestus group larvae and evaluate factors that influence their abundance in rural south-eastern Tanzania. Mosquito larvae and predators were sampled concurrently using standard dipper (350ml) or 10L bucket in An. funestus habitats in south eastern Tanzania. Predators were identified using standard identification keys. Habitats were characterized, geo-located and water physicochemical parameters were recorded. Generalised linear mixed effects models (GLMM) using template model builder (TMB) with zero-inflated negative binomial implemented under the glmmTMB package. Result presented as risk ratios at 95% CI and statistical significance considered when P<0.05. A total of 85 identified An. funestus habitats were sampled for larvae and potential predators. A total of 8,295 predators were sampled, with Coenagrionidae 57.7% (n=4785), Corixidae 12.8% (n=1,060), Notonectidae 9.9% (n=822), Aeshnidae 4.9% (n=405), Amphibian 4.5% (n=370), Dytiscidae 3.8% (n=313) being common. There were 5,260 mosquito larvae sampled, consisting of An. funestus group 60.3% (n= 3,170), Culex spp. 24.3% (n=1,279), An. gambie s.l. 8.3% (n=438) and other anophelines 7.1% (n=373). Permanent and larger than 100m2 habitats were positively associated with An. funestus group and predator abundance (P<0.05). Habitats with submerged vegetation were negatively associated with An. funestus group (P<0.05). Only dissolved oxygen positively affected the abundance of An. funestus group (P<0.05). Predators' abundance was not impacted by any physicochemical parameters. The study highlighted six common predators and factors influencing their abundances in An. funestus aquatic habitats. Further studies are needed to demonstrate the efficacy of predators on larval density and adult fitness traits. Interventions leveraging the interaction between mosquitoes and predators can be established to disrupt malaria transmission and survival of the An. funestus mosquitoes.

**5909**

## FINE-SCALE SPATIAL AND TEMPORAL DYNAMICS OF ANOPHELES GAMBIAE SWARMS IN SOUTH CENTRAL UGANDA

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In Africa, and especially in Uganda, sibling mosquito species belonging to the Anopheles gambiae species complex are some of the most widespread and important malaria vectors. New tools to complement existing ones are needed to control and eliminate malaria as progress has stagnated over the last few years, and even reversed in 2020. A number of new approaches for malaria vector control are being explored including population suppression through swarm reductions and genetic modification involving gene drives. These new interventions will involve understanding of the biology and mating behaviour of target vectors in order to be successful. However, Anopheles mosquito swarms have historically been hard to locate in Uganda and only one study documenting swarm collection has been published in the last two decades. This study located, characterised and collected An. gambiae s.l. swarms in study sites in south central Uganda during 2017 and 2018, filling an important gap in knowledge on the mating behaviour of An. gambiae in Uganda. A majority of swarms solely composed of An. gambiae s.s. were collected during this study, however some mixed An. gambiae s.s. and Culex spp. mosquito swarms were also observed. Swarms were larger in the wet season than in the dry season. Mean swarm height ranged from 2.16 metres to 3.13m off the ground and only varied between villages but not by season. An. gambiae mosquitoes were present in all three villages, preferred to swarm over bare ground markers, and could be effectively collected by field collectors. This study demonstrated that An. gambiae s.l swarms could be effectively located and collected in South Central Uganda and contributed to bridging the knowledge gap regarding the mating behaviour of An. gambiae mosquito species in Uganda in terms of swarm species composition, height above ground, swarm sizes, swarm marker preference and swarm distribution. While mixed species swarms have been reported before, this is the first documented instance of mixed genera swarms found in Uganda, and this warrants further study to fully understand the species mating dynamics.

**5910**

## ANOPHELES STEPHENSI: THE EMERGING VECTOR OF MALARIA IN THE REPUBLIC OF DJIBOUTI, HORN OF AFRICA

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The present study investigated mosquito species composition and phenotypic insecticide resistance profile to support decision-making in malaria vector control in the Republic of Djibouti at the Horn of Africa. Adult mosquitoes were collected between December 2016 and December 2017 across 20 sentinel sites established in the 6 regions of the country using both Centers for Disease Control (CDC) miniature light traps and pyrethrum spray catches (PSC). Female mosquitoes were kept aside, for morphological identification to species by an expert entomologist using appropriate taxonomic keys by Gillies & Coetzee and Glick. WHO tube bioassays were also conducted in An. stephensi from Djibouti-ville against nine insecticides used in public health. A total number of 12,538 host-seeking mosquitoes belonging to four genera (Anopheles, Culex, Aedes, Uranotaenia) comprising 12 species were collected. Among these, An. gambiae s.l. and An. stephensi were the two major malaria vectors identified while secondary malaria vectors such as An. nili somalicus, An. dthali and An. azaniae were also collected. Cx. quinquefasciatus was the most abundant mosquito species in the 6 regions. WHO susceptibility tests performed on An. stephensi population from Djibouti-ville showed resistance to pyrethroids, organophosphates, carbamates and DDT. The resistance intensity bioassays indicated low to moderate intensity of resistance with pyrethroid insecticides and the organophosphate pirimiphos methyl. Meanwhile pre-exposure to PBO suggested involvement of P450 detoxification enzymes in pyrethroid resistance.These findings revealed the urgent need to develop and implement a programme for monitoring and managing insecticide resistance in local vector populations with efficient control strategies in Djibouti.

5911

## VIRAL INFECTION PROFILE OF AEDES MOSQUITOES IN SOME FORESTED AREAS IN GHANA

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Aedes-borne arboviral infections including Yellow fever (YF), Zika (ZIK), Dengue (DEN) and Chikungunya (CHK) have recently become a major public health concern worldwide and in Africa. Most arboviruses originate from the forest and circulated among non-human primates (NHPs) by arthropods including mosquitoes. Several outbreaks of arboviral diseases such as Dengue have been reported in many West African countries including those that share borders with Ghana. Recently, Yellow fever outbreak was reported in Ghana which resulted in 35 deaths. Although Ghana has not recorded other arboviruses, previous studies have indicated the presence of Dengue serotype-2 antibodies in some febrile patients. This study investigated the viral infection profile of Aedes mosquitoes collected from two forested areas in Ghana. A cross-sectional study was conducted in two forested areas, Achimota and Kakum National Park. The different stages of Aedes mosquitoes (eggs, larvae, pupae and adults) were collected using standard collection methods, identified and analysed. A total of 1,080 adult mosquitoes were morphologically identified. Aedes eggs collected from ovitraps in Kakum National Park yielded 17052 (87.2%) and in Achimota forest yielded 2498 (12.8%). The most prevalent Aedes subspecies collected from both sites were Ae. aegypti formosus, 156 (19.3%) in the Kakum National Park and 164 (60.1%) in the Achimota Forest which had diverse species. Culex fuscocephala were the most abundant Culex species identified in Kakum National Park (648). A total of 80 Anopheles gambiae (29.3%) were identified in the Achimota forest and 3 in the Kakum National Park (0.37%). Using RT-PCR, all 109 pools of Ae. aegypti mosquitoes were negative for YFV, DENV, ZIKV and CHIKV. The Positive Ovitrap Index (POI), a measure the risk of transmission was significantly higher (P= 0.03) in Kakum National Park: POI = 81.5% than Achimota forest :POI = 55.3%. The two forested sites are high risk areas for transmission of Aedes-borne arboviruses and sustained surveillance is needed to prevent future outbreaks.

5912

## THE CHANGING ECOLOGY OF LARVAL MALARIA VECTORS IN THE CITY OF ACCRA, GHANA

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There is increasing evidence of malaria vectors adapting to breeding in polluted and other unexpected habitats in Sub-Saharan Africa. Historically, Anopheles mosquitoes breed in clean, unpolluted waters, and have shunned breeding in effluents from households and industries in cities. However, this seems to be changing in urban settings. This study investigated the Anopheles mosquito habitat types, their species composition, and their physicochemical parameters in the city of Accra, Ghana. Larval surveys and collections were undertaken in fifteen sites within the city of Accra (5° 36' 53.3448" N, 0° 12' 21.1464" W), Ghana, using the WHO standard dipping technique. These sites were selected and categorized into five sectors (three sites per category) based on the following: Irrigated Urban Farming (IUF), Lower (LS), Middle (MS) and High (HS) socioeconomic status, and Peri-urban (PU) sites. Physicochemical parameters were measured, and species identification was done using morphological and molecular methods. A total of 727 breeding habitats were found, of which [65.34%, n = 475/727] were positive for Anopheles larvae. Drainage ditches were the most abundant [48.21%, n = 229/475] habitat type. Overall, the abundance of An. gambiae s.l. was highest in IUF sites [6,244/22,919], and in the rainy season (77.01%; 17,650/22,919), (R2 = 3.46, P = 0.000). The highest larval densities of 19.22 and 13.22 larvae/dip were recorded in a swamp and tire track respectively in the rainy season. Malaria vectors were found to breed in much polluted waters including effluents from households. Polluted waters had on average dissolved oxygen of 17.6% compared to unpolluted (26.1%). Other parameters that distinguished polluted breeding habitats from unpolluted were conductivity (7332.5 uS/cm vs 2932.0 uS/cm); total dissolved solids (4567.0mg/L vs 1780.2mg/L); NH4-N (11.9 mg/L vs 4.9 mg/L). An. coluzzii 54.4% (368/677) was the most abundant species. The invasive An. stephensi, which has not yet been reported in Ghana was detected. The findings in this study provide evidence for the consideration of environmental management for malaria control in urban Accra.

5913

## "FIGHTING AGAINST MALARIA IS EVERYONE'S CONCERN": A RANDOMISED CONTROL TRIAL ASSESSING THE ROLE OF INCENTIVES FOR ENCOURAGING LOCAL COMMUNITIES TO RECORDING AND UPLOAD MOSQUITO SOUND USING MOZZIWEAR APPLICATION

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Current malaria surveillance methodologies are considered too expensive to scale within resource limited settings, hence new technologies and approaches are necessary to maximize data collection and ultimately design new malaria control tools. Effective mosquito surveillance can be enhanced through the utilization of digital technologies and engagement of citizen to real time data collection. This study was a follow-up research on the use of mobile phone application (mozziewear) for detecting and identifying wild host-seeking mosquitoes using their flight tones. In this particular study, citizens were provided with airtime incentives to encourage them to participate in recording and uploading mosquito sound. This study was randomised controlled trial (RCT) conducted in four villages in rural Tanzania between April and August 2022. Participants were randomised into two groups; 1) control group: nothing was provided to participants and 2) Incentive group: airtime was provided to participants. Both groups were then asked to record and upload mosquito flight tone data once per week for a period of four months. At the end of the study, experience survey was administered to participants in both groups to assess their experience of participating in this study. The results indicate that the participants were willing to record and uploaded mosquito flight tone data even without being paid incentives. They expressed that fighting against malaria is everyone's concern in rural Tanzania. In addition, the participants expressed their interest in being involved in future research efforts related to mosquito surveillance and the fight against malaria. In conclusion, citizens can still play a valuable role in scientific research, even without the promise of incentives. By participating in mosquito surveillance and malaria prevention studies, community members have significant contribution in addressing mosquito borne diseases and improvement of health outcomes

5914

## MOLECULAR SURVEILLANCE LEADS TO THE FIRST DETECTION OF ANOPHELES STEPHENSI IN KENYA

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Anopheles stephensi is an invasive malaria vector that is endemic to south Asia and the Arabian Peninsula. Recently reported in the Horn of Africa countries including Djibouti (2012), Ethiopia, Sudan (2019), Somalia (2019) and Nigeria (2020). This mosquito is a competent vector for both Plasmodium falciparum and P. vivax. It is characterized by a high degree of behavioral plasticity and the ability to reproduce in various types of breeding sites including containers and therefore has the potential to propagate malaria transmission in rapidly urbanizing settings with poor drainage and waste disposal containers. The World Health Organization (WHO) has called on all countries to scale up surveillance efforts to report invasion by this vector and institute appropriate and effective control mechanisms. In Kenya, the Division for National Malaria Program (DNMP) and its partners have been conducting entomological surveillance in coastal and northern counties that are suspected to be at risk of An. stephensi invasion as well as counties at risk of malaria. These efforts were supported by molecular surveillance of Anopheles mosquitoes by the Kenya Medical Research Institute (KEMRI) to try and identify An. stephensi. We report the first detection of An. stephensi in Kenya in three sub-counties of Marsabit County in December 2022 and February 2023. Polymerase Chain Reaction (PCR) was used as the primary method of identification in addition to morphological identification with further confirmation of results by amplicon sequencing of the ITS2 region. Out of 566 samples analyzed by PCR 44 were confirmed to be An. stephensi. Sequencing of the ITS2 region was performed on 4 samples, 3 of which clustered closely with isolates from India, Yemen, Iraq, and Nigeria based on phylogenetic analysis. The detection of this vector in Kenya presents an urgent need to re-examine and expand the vector surveillance and control effort to include An. stephensi which is likely to increase transmission in Northern Kenya and spread further to highly populated areas and existing malaria-endemic counties further compounding the problem of malaria control in the country.

**5915**

## ARBOVIRUS SURVEILLANCE AND BLOOD-MEAL ANALYSIS OF MOSQUITOES IN JAMAICA

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Annually, mosquitoes are responsible for over 1 million deaths and 700 million infections. Arboviruses transmitted by mosquitoes are of global public health and veterinary significance, causing disease syndromes such as encephalitis, viral hemorrhagic disease, and sometimes death. Despite identifying several arboviruses across Jamaica, little is known about the non-human hosts that may be involved in the maintenance of these viruses in the different ecological habitats, as well as the contributions of various mosquito species in transmission cycles. Therefore, we aimed to identify blood-feeding patterns of mosquitoes in different ecological niches in addition to characterizing novel or re-emerging arboviruses and their hotspot localities before future outbreaks. Mosquitoes were collected from four ecological habitats (mangroves, forested, rural and peri-urban) using CO2-baited BG-sentinel and CDC light traps. Samples were transported to the laboratory on dry ice, where mosquitoes were identified and pooled into groups of 25 based on species, sex, date and location and stored at -80℃ until used for NGS. All blood-fed specimens were processed and stored on FTA cards for cytochrome c oxidase subunit I (COI) gene analysis. A total of 2,150 mosquitoes were collected belonging to 6 genera Aedes, Culex, Anopheles, Mansoni, Psorophora and Wyeomia. Culex quinquefasciatus, a competent vector for several arboviruses, was the dominant species identified. The information from this study can be used for informed and targeted vector control strategies and also as an early warning system to predict potential future outbreaks.

**5916**

## PHENOTYPIC AND MOLECULAR INSECTICIDE RESISTANCE MONITORING OF ANOPHELES FUNESTUS MOSQUITOES TO GUIDE MALARIA CONTROL EFFORTS IN TANZANIA

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Insecticide-based vector control approaches remain the mainstay of malaria control in Africa. The effectiveness of these tools is however threatened by the increasing vector resistance to the insecticides. In Tanzania, Anopheles funestus is an increasingly important malaria vector mediating most of the transmission in some settings compared to other vectors. Consequently, its routine resistance monitoring is crucial to ensure the continued efficacy of control tools against it. Between November 2021 - December 2022, we used WHO tube bioassays to assess the response of field-collected adult An. funestus to pre-determined doses of insecticides in nine regions in Tanzania representative of geographical variations and malaria transmission intensity. We further genotype the mosquitoes to monitor allele frequencies of three metabolic resistance genes - CYP6P9a, L114F-Gste2, and CYP6P9b; additionally, we analyzed the association between surviving the standard insecticide dose (phenotypic resistance) and these resistant alleles (genotypic resistance) using generalized linear models. We found high resistance to pyrethroids (deltamethrin and permethrin) across the country (mortality range 24-55%); however, susceptibility was fully restored following pre-exposure of the mosquitoes to piperonyl butoxide. Resistance to bendiocarb was mostly observed in the South (Mtwara, Lindi, & Ruvuma) and North (Kagera) of the country (mortality range 57-77%) with full susceptibility to DDT and pirimiphos-methyl. Moderate resistance intensity to deltamethrin was observed in coastal regions (Mtwara, Lindi, & Pwani) with high intensity confirmed in Pwani. Similarly, we detected moderate intensity for permethrin in Katavi and Mtwara. We observed a positive association between CYP6P9b and L114F-Gste2-resistant alleles and surviving lethal insecticide doses. However, carrying CYP6P9a-resistant alleles did not increase the chances of insecticide survival. These findings provide a basis for implementing resistance management strategies to limit the further exacerbation of resistance and its impact on malaria control efforts.

**5917**

## DEVELOPMENT, PILOTING, AND EVALUATION OF AN ENTOMOLOGICAL ADAPTIVE SAMPLING FRAMEWORK (EASF) IN MOZAMBIQUE AND GHANA

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Routine entomological surveillance is important for monitoring and evaluating vector control interventions, however there is no operational guidance for optimizing entomological sampling in programmatic settings. An adaptive sampling framework responds to existing data and can adjust the sampling strategy to maximize representativeness and accuracy of the data across time and space. Applied as part of a malaria control program, this may be able to better capture changes in disease transmission dynamics to produce better data that can guide programmatic and strategic decisions.A spatiotemporal EASF model was developed for Mozambique and Ghana and used to inform adaptive entomological sampling designs across space and time for detecting changes in the three priority indicators: 1) vector species compositions, 2) vector behaviour, and 3) insecticide resistance (IR). The optimal sampling approach suggested by the spatiotemporal model is running in parallel with each country's routine surveillance framework. The EASF will be compared with routine surveillance in terms of the primary outcomes of representativeness, cost-effectiveness, and acceptability. In Mozambique the EASF will run for 2 years while Ghana 1 year. Here, we will present the year one EASF preliminary results from Mozambique and Ghana pilots.The EASF may help programs establish a cost-effective adaptive entomological surveillance strategy that is responsive to changing transmission dynamics and optimizes the use of available resources by obtaining more robust and informative data.

**5918**

## INVESTIGATING THE SIBLING SPECIES DIVERSITY AND BREEDING BEHAVIOR OF THE MAJOR MALARIA VECTOR ANOPHELES GAMBIAE SENSU LATO IN SOUTHERN NIGERIA

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Mosquitoes in the Anopheles gambiae complex are major malaria vectors in Nigeria. However, studies that characterize the diversity and breeding behaviors of sibling species of the group in southern Nigeria are limited. To address this knowledge gap, we inspected water bodies for mosquito larvae in southern Nigeria. Results revealed the presence of Anopheles larvae in 53.49% (23/43) of mosquito breeding sites inspected between September and November 2022 in three southern Nigeria states (Edo, Delta, and Anambra). Molecular analysis identified wild-caught Anopheles larvae as An. coluzzii (89.44%, 95% CI: 84.91, 93.98) and An. gambiae sensu stricto (10.56%, 95% CI: 6.02, 15.09). An. coluzzii occurred in 95.45% (95% CI: 86.00, 100) of surveyed locations, whereas An. gambiae ss occurred in 31.82% (95% CI: 10.68, 42.50) of the same area. The larvae of both species were present in urban and periurban areas, and in puddles, stream margins, and open drains. Anopheles gambiae ss were further discovered in a concrete underground well. The overall average abundance of malaria larvae in water bodies was 1.82 larvae per dip (95% CI: 0.76, 2.89). Logistic regression analyses indicated higher odds of Anopheles larval presence in lowlands, and natural and shallow water bodies. Future work to be completed prior to the meeting include assessing population genetic structure of An. gambiae s.l. across diverse ecological habitats for understanding genetic diversity, vector dispersal, and insecticide resistance spread of the species in southern Nigeria. So far, we show that An. coluzzii are major malaria mosquitoes infesting extensive urban and periurban areas in southern Nigeria.

**5919**

## MALARIA TRANSMISSION RISK INDICES OF SECONDARY VECTORS FROM COASTAL AND FOREST AXES OF NIGERIA

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We sampled 14,332 female anophelines belonging to seven different species from coastal and forest axes in Nigeria between 2019 and 2022 using both morphological and molecular identification methods (using species specific PCR) to specific level. Major vectors including Anopheles gambiae Giles, An. arabiensis Patton, An. funestus Giles and An. melas represented ~98% of the total anopheline fauna identified. The remaining 2% was composed of An. moucheti Evans, An. coustani Laveran complex, An. leesoni Evans and An. nili (Theobald)(only species habiting both the coastal and forest areas). Enzyme Linked Immunosorbent Assay (ELISA) was used to determine the Circumsporozoite (CSP) infection status of the species of interest. Circumsporozoite (CSP) infection status showed that the mean infection rate of minor vectors (0.82%) was significantly (P=0.7652) lower than that of major vectors (2.36%). Plasmodium falciparum infection was high and repeatedly found in An. moucheti, indicating its contributory role to the total malaria transmission especially in the forest area. EIR indicates a mean of 146 infective bites/year: 51.8 from An. gambiae s.s., 29.2 from An. funestus, 14.6 from An. arabiensis, 29.2 from An. moucheti, 13.7 from An. melas, 7.3 from An. leesoni, 0.2 from An. coustani and 0.00 from An. nili respectively.

**5920**

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

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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 eight species: Ae. aegypti formosus (n=5), Anopheles rhodesiensis, and six additional Culex and Aedes species. Species identifications will be confirmed using molecular techniques 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.

**5921**

## ROLE OF MALARIA VECTORS BLOOD-MEAL PREFERENCES ON MALARIA TRANSMISSION RISK IN MASENO AND KOMBEWA, WESTERN KENYA

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Mosquito feeding behavior provides a baseline in understanding malaria transmission risk. Accurate identification of relative proportion of bloodmeals taken from humans and alternative hosts is important in calculating

entomological indexes such as Human Biting Rate (HBR) an important component in calculation of vectorial capacity- a measure of malaria transmission that increases with HBR. A longitudinal study involving collection of Anopheles mosquitoes was carried out from July 2015 to August 2016 in Kombewa and Maseno study sites, Western Kenya using Prokopac aspirator for indoor collections. Microscopy was used to identify An. gambiae sensu l ato (s.l)and An. funestus species. Polymerase chain reaction (PCR) was used to further characterize mosquito species within the Anopheles gambiae s.l into An. gambiae sensu stricto (s.s) and An. arabiensis. Enzyme-linked immunosorbent assay (ELISA) was used in blood meal identification. A total of 573/923(62.1%) Anopheles mosquitoes had blood meals of which An. funestus and An. gambiae s.s were 373/573 (65.1%) and 200/573 (34.9%) respectively. An.arabiensis was rather transient with only 4/309 testing positive of An.gambiae s.l identified . Bloodmeals were inclusive of human 485/573 (84.6%), cow 40/573 (7.0%), dog 7/573 (1.2%), chicken 3/573 (0.5%), goat 1/573 (0.2%), while cat and donkey appeared in mixed sources. Blood meals containing human and alternative sources were 13/200 (6.5%) and 20/373 (0.5%) in An. gambiae s.s and An. funestus respectively. Surprisingly, mixed non-human blood meal sources were observed in An. gambiae s.s at 4/200 (2.0%) contrary to some of the previous studies. An. funestus was majorly anthrophilic as opposed to An. gambiae s.s that showed opportunistic behavior. This marginal shift from normal feeding patterns exhibited by An. gambiae s.s appears to suggest increased risk of malaria transmission in study populations. Additional survey on host feeding preferences is necessary in order to support reciprocal changes in malaria control strategies to contain this enhanced risk.

**5922**

## BITING PATTERN OF ANOPHELES ARABIENSIS, HUMAN BEHAVIOUR, AND SOCIO-ECONOMIC MALARIA RISK FACTORS IN AN IRRIGATED AGROECOSYSTEM IN WESTERN KENYA

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Knowledge on the biting patterns of malaria vectors, human behavior, and malaria risk factors is important in understanding malaria transmission dynamics, evaluating the effectiveness of vector control interventions, and informing the development of appropriate interventions. This study assessed vector biting patterns, human behavior, and socio-economic malaria risk factors in an irrigated agro-ecosystem in western Kenya. Adult mosquitoes were sampled using human landing catches. The mosquito samples were identified to species using polymerase chain reaction (PCR). Malaria parasites were detected by quantitative PCR. Human behavior data was collected using a questionnaire to understand human night activities and sleeping patterns and how they overlap with vector behavior. In order to investigate the association between malaria prevalence and socioeconomic risk factors, these parameters were also included in the questionnaire. The mean indoor and outdoor host-seeking densities of An. arabiensis varied significantly, with the highest density collected indoors. During the first half of the night (6 p.m.-12 a.m.), indoor biting activity of An. arabiensis showed a peaked plateau between 8 p.m. and 10 p.m. before the local community went to sleep. Data on human behavior and indoor mosquito biting activity showed that there is a high risk of transmission at dusk and dawn when people are not under the protection of bed nets. Outdoor biting activity of An. arabiensis occurred throughout the night; nonetheless, the greater proportion of the human population was indoors from dusk to dawn; therefore, there was a low risk of outdoor malaria transmission. These findings demonstrate that there was a higher risk of malaria transmission due to factors such as sleeping indoors, sleeping late, a lack of bed nets, and low utilization. The study underscores the necessity of complementing the core malaria vector control strategies by integrating larval source management and novel techniques such as transgenic mosquitoes.

**5923**

## PREVALENCE OF MICROSPORIDIA MB AMONG ANOPHELES MOSQUITOES MAY BE ASSOCIATED WITH MICRO-ECOLOGICAL FACTORS OF BREEDING NICHES

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Microsporidia MB is found associated with Anopheles mosquito vectors and shown to interfere with the development of Plasmodium. In Kenya where this was first reported, Microsporidia MB was widespread in An. arabiensis collected from rice fields. Since then, the symbiont has also been reported in An. gambiae and An. funestus. As a potentially useful tool for symbiont-based disease control, its ecological distribution is still not clearly understood especially since Anopheles mosquitoes have different niches. This study was aimed at determining the prevalence of Microsporidia MB in Anopheles mosquitoes in selected sites across Ghana, and measuring the physicochemical parameters of breeding sites to determine the associated micro-ecological factors. Anopheles larvae and pupae were collected from study sites in northern and southern Ghana between Aug-October of 2021 and 2022. A total of 4195 immature mosquitoes were raised to adults and DNA was extracted from the abdomen. Microsporidia MB was detected by qPCR and mosquito species molecular identification using a SINE and RFLP methods. The overall prevalence of Microsporidia MB was 1.9% (78/4195) with prevalence similar among An. gambiae (41.0%; 78/4195) and An. coluzzii (30.8%; 24/78) ($\chi$;2, P= 0.28), reiterating previous findings from archived samples. Microsporidia MB was observed in only one An. arabiensis (1.3%; 1/78). Male mosquitoes showed predominance in Microsporidia MB infections ($\chi$;2, P= 0.001). Sites in the Savannah ecological zone of Ghana, which had no associations with rice fields, had the highest prevalence of Microsporidia MB (55.1%; 43/78) compared to other sites ($\chi$;2, P= 0.0009). Overall, the study suggests the distribution of Microsporidia MB is most widespread among predominant malaria vectors in a geographical region and their sustainability in the aquatic stages of the host may be influenced by microecological factors. Further analyses on the physiochemical parameters collected will help in our understanding of the distribution of this symbiont among Anopheles mosquitoes in their different breeding niches.

**5924**

## THE CHANGING LANDSCAPE OF DENGUE AND CHIKUNGUNYA VECTORS IN KENYA – A THREAT TO PUBLIC HEALTH.

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Dengue and chikungunya are a major problem in Africa, causing outbreaks annually. In Kenya, dengue (DENV), chikungunya (CHIKV), primarily transmitted by Aedes aegypti, frequently cause outbreaks mainly in urban coastal (Mombasa, Lamu) and northern (Mandera and Wajir) counties resulting in significant morbidity, associated lost man-hours, enormous socio-economic impact among communities, and burden on health systems. Entomological Surveillance was conducted during dengue

and chikungunya outbreaks in Mombasa to i) determine the presence and densities of Ae. aegypti vectors, ii) identify breeding habitats and iii) identify other potential vector species previously not associated with these viruses. Sampling involved collection of adult mosquitoes using BG sentinel and CDC light traps; and larval collection from affected areas. During the 2013/2014 dengue outbreak, 2,069 adult Aedes mosquitoes were collected: Ae. aegypti (n=2,069) and Ae. vittatus (n=4); while larvae comprised of n=2,510 Ae. aegypti outdoor (n=1,515), indoor(n=995) and Ae. vittatus (n=0). In 2018 chikungunya outbreak, 2,086 adults: Ae. aegypti (n=911), Ae vittatus (n=1,175); and n=528 larvae: n=526 Ae. agypti (indoor, n=280; outdoor, n=246), and Ae. vittatus, (outdoor, n=2) were collected. N=2,511 adult female Culex quinquefasciatus mosquitoes were also collected, and two CHIKV isolates obtained. Ae. vittatus occur in abundance, voraciously bite humans despite being majorly zoophilic. CHIKV has also been repeatedly isolated from them and have been shown to efficiently transmit CHIKV. Therefore, it plays a critical role in Key in sylvatic maintenance; and urban transmission of DENV, CHIKV, YFV, ZIKV whenever it invades urban areas. Although predominantly a rock-pool, tree-hole breeder; it can breed in diverse macro-, micro-habitats. A combination of Ae. aegypti, Ae. vittatus and Cx. quinquefasciatus poses a major challenge in vector control. Therefore, intensive surveillance to identify breeding habitats for Ae. vittatus in Mombasa is instrumental in devising appropriate vector control tools specific to this species.

**5925**

## THE DISTANCE-DENSITY RELATION TO INFORM LARVAL SOURCE MANAGEMENT: HOW FAR IN SUGAR IRRIGATION SCHEMES DO MALARIA MOSQUITOES BREED

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While agricultural production is expanding in low-and mid-income countries, the vast majority of plough able land is too dry to depend only on rain-fed agriculture. Irrigation is the most effective and supplementary system to increase crop production and yields as well as to ensure reduced risk of crop failure. While these large-scale agriculture may enhance local economies and consequently boost livelihoods, some crop systems create and sustain suitable mosquito breeding habitat and hence affect malaria transmission especially where drainage system is poor. We conducted a study in Manhica district in 2020, south of Mozambique to characterize mosquito-breeding habitats along an irrigated sugarcane plantation. Additionally this study aimed at assessing how mosquito immatures change in different environments as well as which breeding types contribute and sustain malaria mosquitoes throughout the year. Here we will present the outcome of this survey in Manhiça district. This study will provide useful information to the programme and significantly enhance understanding of use of focal control of larvae in Mozambique either through aerial spray or manual where applicable.

**5926**

## DETECTION OF INSECT-SPECIFIC VIRUSES IN MOSQUITOES COLLECTED IN URBAN AND FOREST FRAGMENT AREAS OF NORTHWEST OF SAO PAULO STATE, BRAZIL

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Insect-specific viruses (ISVs) can affect arboviruses cycle transmission, mainly impacting vector competence, and have a great potential to be used as biological control agents or vaccine platforms. From March 2022 to March 2023, we collected 463 mosquitoes (allocated in 273 pools) from urban and forest fragments of Sao Jose do Rio Preto (SJdRP), Sao Paulo, Brazil. The mosquito species belong to different genera, including Culex, Aedes, Psorophora, Sabethes, and Limathus. So far, 136 pools have been submitted to viral isolation and molecular investigation for medically important flaviviruses, alphaviruses, and for insect-specific viruses such as PCLV, HTV, GUAPV, CxFV, and AeFV. We detect HTV in 22 pools (16.17%) of mosquitoes from Aedes, Culex, and Sabethes genera. PCLV was detected in 30 pools (22%) of mosquitoes from Aedes and Culex genera. CxFV was detected in 20 pools (14.70%) of Culex genus. We detect GUAPV in three pools (2.2%) of mosquitoes from Limathus, Culex, and Psorophora genera. PCR products were sequenced by the dideoxy method confirming these results. In addition, we had 15 pools (11%) of mosquitoes from Aedes and Culex genera positive in a pan-Flavivirus PCR. The PCR products of these samples were sequenced by the dideoxy method, and the nucleotide sequences obtained showed similarity with other mosquito flaviviruses. In addition, we have some co-infections: 13 pools of Aedes aegypti and one pool of Culex sp. co-infected with HTV and PCLV; two pools of Aedes aegypti co-infected with FLAV-like and PCLV; one pool of Culex sp. co-infected with PCLV and CxFV; one pool of Culex sp. co-infected with GUAPV and CxFV; and one pool of Aedes aegypti co-infected with HTV, PCLV, and FLAV-like. All pools tested negative for the medically important arboviruses. Our next steps are genomic characterization/biological characterization and electron microscopy. Our findings demonstrate the viral diversity in mosquitoes from SJdRP and open perspectives for further studies of metagenomics, vector competence, and interactions between these ISVs and circulating arboviruses.

**5927**

## A METHODOLOGICAL FRAMEWORK TO UNDERSTAND THE DRIVERS OF DENGUE FOR DESIGNING OPERATIONALLY EFFICIENT AND SUSTAINABLE VECTOR CONTROL POLICIES IN ENDEMIC SETTINGS; A CASE STUDY FROM KALUTARA DISTRICT, SRI LANKA

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Dengue imposes a substantial public health and economic burden on affected populations in endemic countries, including Sri Lanka. Complex interactions among susceptible humans, viruses, and Aedes mosquitoes determine dengue's transmission and spread. Assessments of context-specific climate and non-climate drivers of dengue transmission and the analysis of effectiveness and cost-effectiveness of dengue control interventions are required to develop pragmatic and sustainable dengue control programs. To meet this demand, we developed a methodological framework which is capable of (1) identifying the most vulnerable populations by assessing delayed and non-linear associations between

climate and non-climate drivers of dengue across different geographical settings; (2) evaluating the effectiveness and cost-effectiveness of population-level vector control interventions implemented to address the identified drivers in the above settings. We found that early and targeted interventions, triggered by the evidence generated through the methodological framework, were effective and cost-effective, reducing the burden of dengue by 50% in a highly endemic area in Kalutara district, Sri Lanka. Given the capacity is established, program managers can use this methodological framework to develop a decision-support platform to set up short-, intermediate-, and long-term targets for designing and deploying equitable and efficient operational response to dengue. The inbuilt monitoring and evaluating framework would promote a more informed and transparent public health decision-making process towards attaining the World Health Organization's dengue and other vector-borne disease targets by 2030 in endemic and resource-constrained settings.

**5928**

## COMMUNITY PERCEPTIONS OF NUMBER OF MOSQUITOES AND MOSQUITO BITES AFTER USE OF WOLBACHIA SUPPRESSION AS A CONTROL METHOD FOR AEDES AEGYPTI MOSQUITOES IN PONCE, PUERTO RICO

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Wolbachia suppression (WS) can reduce Aedes aegypti mosquito populations, the main vector of the dengue virus, by decreasing the hatch rate of eggs from crosses of released Wolbachia-carrying male and wild-type female mosquitoes. Because WS involves releasing mosquitoes, understanding community members' perceptions of the number of mosquitoes and mosquito bites after releases are important to inform the acceptability of WS. During 2020-2021, WS was tested in a randomized cluster trial in 8 communities in Ponce, PR. We conducted phone and in-person surveys of adult residents of the 4 release (intervention) and 4 non-release (control) areas during August-October 2022. We compared post-release community perceptions of the number of mosquitoes and mosquito bites in intervention and control areas using chi-square tests of proportion. We also identified factors associated with reporting fewer mosquitoes and fewer or no change in mosquito bites in intervention areas using logistic regression. Of 258 total respondents, 70% were female, the median age was 51 years (range 19-93), 69% were aware of mosquito releases, of whom 75% had supported releases. Demographic characteristics and awareness of and support for WS were similar in release and control areas. While not statistically significant, a higher proportion of respondents in release areas reported perceiving fewer mosquitoes (19% vs. 12%, Chi-square p=0.4) and mosquito bites (27% vs. 16%, Chi-square p=0.06) compared to respondents in control areas. Among respondents in release areas, age, sex, awareness, and support of releases were not associated with reporting fewer mosquitoes; however, women were more likely to report perceiving less or no change in mosquito bites (adjusted OR 3.3 [95%CI 1.4-7.9]) compared to men. Community perceptions of mosquito numbers and mosquito bites were consistent with expected results from implementation of WS, where total mosquito numbers and mosquito bites decrease due to decreased egg hatch rates. Our findings suggest that WS may be associated with perceptions of fewer mosquitoes and mosquito bites, informing the acceptability of WS.

**5929**

## USING HIGH POWER ELECTRIC FIELDS TO REPEL MOSQUITOES

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To control and prevent existing and (re) emerging mosquito-borne diseases, insecticides are often our only option. Insecticides reduce mosquito population sizes and/or prevent human-vector contact, but their excessive use raises concerns for our environment, our health, and led to the rapid development and spread of insecticide resistance. As a result, the development of novel technologies for mosquito control is urgently needed. One of the new tools in development is the use of high power pulsed electrical fields (EFs) that create an invisible barrier and repel mosquitoes. I will provide an overview of the current knowledge of this first non-chemical insect repelling technology, and present novel data on how EFs generated with cheap over-the-counter insulated conductor wires prevent host-seeking Aedes aegypti mosquitoes from entering spaces, allowing us to protect typical mosquito entry points in houses (such as eaves, windows, and doors) as well as groups of people outdoors.

**5930**

## ASSOCIATION OF WATER AVAILABILITY AND AEDES AEGYPTI PUPAE AND ADULTS IN AN URBAN/RURAL MOSAIC IN NICARAGUA

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The increase in dengue virus (DENV) epidemics and the latitudinal expansion of Aedes mosquitoes globally has made it critical to understand the ecological and social factors that modulate its population abundance locally. We used multilevel models to evaluate the different life stage abundances of Ae. aegypti collected from October to December of 2022 (rainy season) in 500 households in 21 urban and peri-urban neighborhoods in District 3 of Managua, Nicaragua, as part of an arbovirus surveillance study (A2CARES). A total of 1,194 pupae and 201 adult female mosquitoes (indoor: 129, outdoor: 72) were collected. We used a GLMM and GAMM approach to estimate pupae and adult female indoor and outdoor abundance. The number of pupae found in a home was directly associated with number of containers present (exp= 1.41, SE= 0.09, p< 0.001) and outdoor female specimen abundance (exp= 2.73, SE= 0.47, p< 0.03). We observed that outdoor female abundance increased with frequency of water service interruptions per day (exp= 1.07, SE= 0.02, p< 0.01), total number of pupae found in the household (exp= 1.027, SE= 0.009, p< 0.01) and neighborhood of collection (exp= 0.501, SE= 0.027, p=0.01). The GAMM smooth for water interruptions was also significant, increasing with the number of female mosquitoes outdoors. Indoor female abundance was only associated with the number of pupae found in a home (exp= 1.021, SE= 0.008, p< 0.01). We are currently untangling the fine-scale spatial patterns for mosquito abundance, human density, access to water and services, and most productive containers to evaluate how these variables impact mosquito ecology using a urbanicity mosaic perspective within our study site. Our results suggest that for our geographic setting, pupae can be an adequate proxy for female abundance. More importantly, the inclusion of stakeholders involved in household water container management and municipal services are critical for future intervention projects in the region. Fine-tuning hotspot analysis of mosquito abundance to identify key factors that modulate their population is critical to improve vector control activities in limited-resource settings.

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## IMPACT OF STANDARD AND LONG-LASTING IVERMECTIN FORMULATIONS IN CATTLE AND BUFFALO ON WILD ANOPHELES SURVIVAL ON SUMBA ISLAND, INDONESIA

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Sumba Island has some of the highest diversity of Anopheles species in Indonesia, contributing to its high malaria incidence. Southwestern Sumba, the area with highest malaria burden, has small livestock holder systems where the animals (e.g. cattle, buffalo, horses, and pigs) are kept near the owners' house at nighttime, often underneath the house. Anopheles mosquitoes frequently blood-feed on livestock providing an opportunity for vector control by treating the animals with insecticides. Ivermectin is a systemic endectocide used to control helminth and ectoparasites in livestock, and ivermectin-treated hosts are lethal to Anopheles vectors. Both standard (Ivomec) and long-lasting (Ivergen Platinum) commercial formulations of ivermectin were investigated in cattle and buffalo in this study. Four villages were selected to maximize the diversity of Anopheles species. In each village, three cattle and three buffalo were treated with either standard (200 ug/kg), or long-lasting ivermectin (630 ug/kg), or kept untreated as controls. Study animals were placed in net traps, which allowed mosquitoes to enter and blood feed on the animals. Mosquitoes were collected by mouth aspiration. Animals were exposed to mosquitoes before treatment and up to 70 days post treatment. A venous blood sample was collected from the study animals the morning after each mosquito collection for ivermectin quantification. Blood-fed Anopheles were transported to a field insectary where their survival was monitored daily. Each day the dead mosquitoes removed, identified to species, and on day ten of monitoring the mosquitoes were frozen and counted as alive. Ten Anopheles species were frequently collected including: An. aconitus, An. annularis, An. barbirostris, An. flavirostris, An. kochi, An. maculatus, An. subpictus, An. sundaicus, An. tessellatus, and An. vagus. Cattle treated with long-lasting ivermectin were lethal to all Anopheles species through 46 days post treatment. Ivermectin concentrations will be quantified and linked to mosquito mortality to characterize the lethal concentration that kills 50% of the mosquitoes for each species captured.

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## AUTOMATING MOSQUITO STERILE INSECT TECHNIQUE (SIT)

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One of the best prevention methods for vector-borne diseases is control of the vector itself. For mosquitoes that transfer a number of viruses and parasites affecting millions, an integrated management approach has proven most effective. Integrated control brings together tools like source control, larviciding and adulticiding. For a subset of species coined as "container breeding" this approach has fallen short. The main difficulty in controlling container breeding mosquitoes is that source control and larviciding are near impossible. One important tool that could close the gap is the sterile insect technique (SIT). SIT works by overwhelming the vector population with sterile males causing the production of unfertilized eggs. SIT has been in use for decades against a couple of agricultural pests and numerous field tests for mosquito vector control have shown great success. As male mosquitoes will seek out females even when humans can't find all their hidden breeding sites, SIT can close the hole in integrated control of container breeders. Mosquito SIT requires accurate sex-sorting to ensure no females are released. Currently, state of the art technologies sort at the late developmental stages. However, as adult mosquitoes have a short lifespan and are fragile, they must be produced near the release site (usually manually). This makes mosquito SIT prohibitively expensive. Uniquely, Diptera.ai can sex-sort mosquitoes and numerous agricultural pests at the larval stage, previously considered impossible. Larvae can be shipped long-distance from centralized facilities, thus allowing for economies of scale and introduction of rearing automation. Our sex sorter utilizes proprietary optics coupled with machine learning algorithms to rapidly and precisely predict and separate larvae sexes. The system is comprised of an array of small autonomous units. This design allows for maximum flexibility and means that units can be serviced without shutting down production. Using the systems described above we successfully controlled the Aedes albopictus population in a small town over an entire season.

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## SPATIAL ANALYSIS OF ENVIRONMENTAL DRIVERS AND MOSQUITO SPECIES ABUNDANCE ON MALARIA PREVALENCE IN KENYA FROM JANUARY 2019 TO JUNE 2021

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Over 50% of the global population is at risk of contracting malaria, especially those living in 85 endemic countries- the majority of which are in Sub-Saharan Africa. Climate change is impacting malaria prevalence and mosquito species distribution and densities. Our objective was to estimate the effect of climate parameters (temperature, humidity, rainfall) and mosquito species on malaria prevalence in Kenya from January 2019 to June 2021 We compiled data across 31 counties in Kenya from previous surveillance work: a malaria indicator survey, secondary data from a monthly entomological survey and a household malaria indicator survey. We analyzed the data using linear regression, correlation and used least absolute shrinkage and selection (LASSO) for selection of coefficients. We found that malaria prevalence in Kenya is highest in the lake region with prevalence at 19%, coast endemic at 5%, semi-arid seasonal at 2%, and low risk at 0.9% for 2020. The mosquito species distribution varied across malaria epidemiological zones. In the coastal region, An. Funestus was more abundant, while in the lake region An. Gambiae was more abundant, although significant An. Funestus' presence was also detected. The regression results suggest that a combination of environmental factors (such as temperature, vegetation, and aridity) as well as public health interventions (such as ITN coverage) are important predictors of malaria prevalence. However, the relationship between some of these factors and malaria prevalence is complex and may vary across different regions and time periods.Overall, this study provides insights into the spatial-temporal dynamics of mosquito species and their distribution in relation to malaria epidemiological zones in Kenya and can inform control strategies within specific ecological zones. Further studies are needed to better disentangle the factors influencing the distribution and abundance of mosquito species in different malaria epidemiological zones in Kenya

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## STUDY OF THE DIVERSITY OF MACRO INVERTEBRATES ASSOCIATED WITH THE LARVAL HABITATS OF ANOPHELES GAMBIAE COMPLEX IN TWO VILLAGES OF THE KATI DISTRICT MALI

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Target Malaria is a non-profit research consortium, which aims to develop new vector control strategies against malaria using genetic technologies based on population reduction. The aim is to reduce the malaria-carrying mosquito population in the Anopheles gambiae s.l. complex in the long term. Before that, it is important to understand the biodiversity and

abundance of macro invertebrates associated with the larval habitats of An. gambiae s.l. in different ecological environments for risk assessment purposes. It is in this context that this study proposes to inventory macro-invertebrates in mosquito breeding sites. From June 2021 to December 2022. The collection was carried out in two sites by monthly rentals at the level of the previously characterized cottages. Macro-invertebrates have been morphologically identified.Preliminary results show a total of 122 macro-invertebrates. The following species have been encountered: Lestidae, Bactidae, Corixidae, Dytiscidae, Gyrinidae, Hydrophilidae, Nepidae, Notonectidae, Physidae at both sites; Anisoptera, Belostomatidae, Haliplidae, Lestidae, Libellulidae, Lymnacidae, Naucoridae, Noteridae, Veliidae only in Ouassorola. The number varies according to the sites Ouassorola = 66%, Sogolombougou = 34% and over time. Notonectidae were most abundant in Sogolombougou (95%) and Noteridae was the most abundant species in Ouassorola, 97.94%. The rivers had the greatest diversity of macro-invertebrates compared to the other habitats studied (Brick Quarries, Footprints).

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## FEASIBILITY AND COMMUNITY ACCEPTANCE OF INSECTICIDE TREATED EAVE NETS AND INSECTICIDE TREATED WINDOW SCREENS IN TANZANIA

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Long-lasting insecticidal nets (LLINs) and targeted indoor residual spray (IRS) are used extensively in Tanzania and have significantly reduced malaria mortality and morbidity across the country. However, malaria remains a public health concern, calling for the development of supplementary intervention tools. This study aimed to understand community perceptions on the installation of insecticide treated eave nets (ITENs) and windows screens (ITEWs) for full house screening against mosquitoes. Fourteen In-Depth Interviews (IDIs) with local carpenters who installed the intervention in 440 households and six Focus Group Discussions (FGDs) with community members in both the treatment (with intervention) and control (without) arms were conducted to gain understanding of feasibility and community perceptions of the intervention against malaria in three villages at Chalinze district in Tanzania. Only two carpenters reported to get nasal congestion and a headache after the working with the intervention on day 1 and related it to the chemicals on the netting. However, none of the community members experienced any adverse effects after the intervention was installed in their houses. Community members reported the intervention reduced mosquito abundance in their houses and also protected them from insects, lizards and snakes. Due to an observed reduction in malaria incidences in their households, some residents reported to stop sleeping under LLINs. A willingness to buy the netting if sold at an affordable price range of TZS 1000-6000 (≤ USD 2.50) per square meter was also expressed among community members. Community feasibility studies provide insights to barriers and facilitators to the adoption of new interventions tools. Therefore, appropriate social behavioral change communication strategies can be developed prior to deployment newer vector control tools to ensure synergy with existing interventions.

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## COMBINING PYRETHROID-PIPERONYL BUTOXIDE (PBO) NETS WITH CLOTHIANIDIN-BASED INDOOR RESIDUAL SPRAYING IMPROVES CONTROL OF PYRETHROID-RESISTANT MALARIA VECTORS: AN EXPERIMENTAL HUT TRIAL IN SOUTHERN BENIN

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Coverage of pyrethroid-piperonyl butoxide (PBO) nets and indoor residual spraying (IRS) with the neonicotinoid insecticide clothianidin either as a solo-formulation or as a mixture with deltamethrin is being scaled up to improve control of malaria transmitted by pyrethroid-resistant mosquitoes. Most IRS campaigns are deployed against a background of moderate to high net coverage thus combined use of these tools is already an operational reality in many settings. However, no published trials have evaluated whether combining pyrethroid-PBO nets and clothianidin-based IRS would improve malaria control impact relative to use of either method alone. We performed experimental hut trials to evaluate the impact of combining different types of pyrethroid-PBO net with IRS formulations containing clothianidin both alone and as a mixture with deltamethrin against a pyrethroid-resistant vector population in southern Benin. We compared the impact of the combinations to each intervention alone and combinations with pyrethroid-only nets. The vector population at the hut site was susceptible to clothianidin but exhibited a high frequency and intensity of pyrethroid resistance that partially overcame by PBO pre-exposure. Mortality was significantly higher in huts combining pyrethroid-PBO nets with the clothianidin IRS solo-formulation (75%) and clothianidin-deltamethrin IRS mixture (77%) compared to those where pyrethroid-PBO nets (35–43%) and clothianidin-based IRS (42–48%) were applied alone. All combinations improved blood-feeding protection relative to the IRS alone but higher levels of blood-feeding inhibition were recorded with combinations containing pyrethroid-PBO nets compared to those containing pyrethroid-only nets (78–85% vs. 21–64%). Combining pyrethroid-PBO nets and clothianidin-based IRS shows potential to improve control of pyrethroid-resistant malaria vectors.

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## LONG-ACTING FORMULATION OF IVERMECTIN FOR EFFECTIVE MALARIA CONTROL: INSIGHTS FROM AN AGE-STRUCTURED MODELLING STUDY

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Controlling malaria consists of either killing the parasite within the human host or reducing the vector population. These two approaches have allowed to decrease the number of deaths by almost 40% during the 2 past decades. However, a stagnation since 2015 followed by an increase in the last years, highlight the limits of available tools. One of the known limits is the vector tolerance to insecticides. A complementary approach is to render the vector's blood meal toxic by treating hosts with a drug that impairs mosquito survival. Ivermectin (IVM), an endectocide, has been proven to possess such ability. Several studies have modelled the impact of mass drug administration (MDA) of oral ivermectin to human populations and have evidenced a promising effect in reducing malaria clinical cases. However, the mosquitocidal effect of a single oral IVM dose (150-200 µg/kg) is relatively short-lived, requiring multiple mass distribution to significantly decrease malaria prevalence. An original model was developed to investigate the effects of a Long-Acting Injectable Formulation of Ivermectin (LAIF-I) on malaria transmission. This population model considers multiple continuous structural variables: humans age, time since infection, and time post-IVM administration for both humans and vectors. Such an approach

allows targeting a specific human class, e.g. by excluding children under five years and women of childbearing age. Furthermore, the time post-IVM was required to properly capture the longitudinal dynamics of both (i) IVM systemic concentrations in the human bloodstream, and (ii) IVM effects on mosquitoes' life span after a blood meal on LAIF-I -treated human. The long-lasting insecticide treated net coverage associated to different malaria transmission profiles were used as baselines to evaluate the added value of using LAIF-I in realistic life conditions. The detailed effect of the LAIF-I on the reduction of malaria prevalence will be presented during the conference.

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## EXPLORATORY ANALYSIS OF THE EFFECTIVENESS OF INDOOR RESIDUAL SPRAYING WITH ACTELLIC 300CS AND FLUDORA FUSION TO REDUCE ENTOMOLOGICAL INDICATORS IN ALIBORI AND DONGA REGIONS, NORTHERN BENIN

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This study explores differences in the entomological indicators after Indoor Residual Spraying (IRS) with Actellic® 300CS (AC; pirimiphos-methyl) and Fludora® Fusion (FF; a deltamethrin-clothianidin mixture) in the Alibori and Donga Department of Benin. After the IRS campaigns with AC in 2019 and FF in 2020, respective monthly mosquito collections were performed over 10 months using both Human Landing Catches (HLCs) and Pyrethrum Spray Catches (PSCs) in Alibori and Donga, and adjacent untreated control areas (Bembèrèkè and Bassila). The Indoor Resting Density (IRD), the Human Biting Rate (HBR), Plasmodium falciparum Sporozoite Rate (SR), and the Entomological Inoculation Rate (EIR) of Anopheles gambiae s.l. were determined. The residual activity of AC and FF on the treated walls was also evaluated. A comparison of the indicators between IRS and control sites was done by calculating the percent reduction (Abbott's formula) and the P-value using poisson test function in R. Overall, the residual activity lasted 5 months for AC and 10 months for FF. The IRD was 0.30 An. gambiae s.l. per room (Ag/room) in AC-sprayed and 2.59 in control areas (Reduction [RD] = 88%, $p < 0.0001$), while the IRD was 0.92 Ag/room in FF-sprayed and 3.25 in control areas (RD = 72%, $p < 0.0001$); the HBR was 6.76 bites/person/night (b/p/n) in AC-sprayed and 19.93 b/p/n in control areas (RD = 66%, $p < 0.0001$), while the HBR was 18.33 b/p/n in FF-sprayed and 26.17 b/p/n in control areas (RD = 30%, $p < 0.0001$); the SR was 0.31% in AC-sprayed and 2.61% in control areas (RD=88%, p =0.0013), while the SR was 0.65% in FF-sprayed and 1.52% in control areas (RD = 61%, p = 0.0034); the EIR was 0.63 infectious bites person per month (ib/p/m) in AC-sprayed and 15.62 ib/p/m in control areas (RD = 96%, $p < 0.0001$), while the EIR was 3.52 ib/p/m in FF-sprayed and 12.00 ib/p/m in control areas (RD = 71%, p = 0.0019). When comparing sprayed and control areas, AC areas had a higher reduction in entomological indicators than FF. However, FF had longer residual activity than AC. More robust analyses and the use of epidemiological data are needed to compare these insecticides.

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## MOLECULAR TECHNIQUE FOR THE DETECTION OF WOLBACHIA (WANGA-MALI) WITHIN ANOPHELES GAMBIAE SENSU LATO IN MALI

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Malaria is a major public health problem for sub-Saharan countries including Mali. The progress made in the fight against malaria through the use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) is threatened by the emergence and progression of resistance of major vectors to commonly used insecticides, hence the need to develop new vector control methods such as the use of the endosymbiotic bacterium called Wolbachia.The aim of this work was to set up in Mali an efficient detection technique of Wolbachia within Anopheles gambiae s.l. as a prelude to the fight against malaria.This was an experimental study, carried out within ICER-Mali in the framework of a collaboration between the University of Science, Technology and Technology of Bamako (USTTB) and the National Institutes of Health of the United States of America (NIH). Mosquitoes were collected in Kenieroba by the "Spray Catch" insecticide spraying method in human dwellings. In order to obtain a good quality of mosquito DNA and the bacteria, two extraction methods were compared, namely the "old" Phenol-Chloroform extraction method and the "MasterPure" extraction kit. The molecular techniques used for the detection of Wolbachia was: quantitative PCR (qPCR) for the determination of the prevalence of Wolbachia in the An. gambiae s.l. population.The highest DNA concentrations and the lowest protein contamination were obtained with the MasterPure kit. This method was also the least toxic for the laboratory technician. The prevalence of Wolbachia infection was 45.16%. This prevalence was lower than previously reported. in the same locality in 2016 but who used a larger sample size. Our study showed that the Wolbachia wAnga-Mali strain of Wolbachia is still present in An. gambiae s.l. in the Kenieroba area, the prevalence of which may vary in time and space. The use of the Wolbachia bacterium remains a promising method in the eradication of malaria worldwide.

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## ENDECTOCIDES TO COMPLEMENT THE MALARIA VECTOR CONTROL TOOLKIT: EXPECTED AND UNEXPECTED SIDE-EFFECTS OF IVERMECTIN ON MALARIA VECTORS

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Mass treatment of livestock and/or humans with ivermectin has been proposed as a complementary vector control strategy to combat malaria. However, this strategy requires repeated mass treatments to cover the high transmission period during the rainy season. To overcome this, a Long-Acting Ivermectin Formulation (LAIF) is currently under development and its potential to release mosquitocidal concentrations of ivermectin for more than a month is under evaluation as part of the IMPACT project. Although broader use of ivermectin can pose some anticipated challenges (i.e. potential environmental contamination and effects on non-target organisms, withdrawal times in livestock and implications for milk or meat production, risk of inducing resistance in livestock or human parasites), other side-effects such as the selection and spread of ivermectin resistance in malaria vectors through physiological or behavioral modifications appear to be less obvious. The aim of this review was to decipher how resistance to ivermectin could be selected in malaria vectors and propose a research agenda to study and manage this phenomenon if it appears. We used relevant terms to search databases including PubMed and Web of Science and included studies that describe mechanisms of resistance in arthropods. Results were summarized in terms of resistance mechanism. We also proposed a research agenda that integrates 1) the characterization of resistance and/or pyrethroid-ivermectin cross-resistance mechanisms, 2) the development of molecular and phenotypic assays to monitor ivermectin resistance in the field, 3) the study of potential behavioral changes vis-a-vis to ivermectin-treated subjects, and 4) the study of potential strategies to mitigate ivermectin resistance in mosquitoes. Anticipating the research on physiological or behavioral resistance to ivermectin in mosquitoes will facilitate the development of effective resistance-management plans and

enable preparedness for monitoring and evaluating this strategy after its implementation. Selection of Anopheles coluzzii resistant to ivermectin is in progress in our research team.

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## ANOPHELES STEPHENSI IN TURKANA - PRELIMINARY FINDINGS ON THE LARVAL SURVEILLANCE IN TURKANA COUNTY, KENYA

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Anopheles stephensi has been identified by the WHO as a key threat to malaria control. Although endemic to Southeast Asia, it was detected in Djibouti in 2012 and has spread throughout the Horn of Africa. In 2020 it was detected in Nigeria. This invasive vector efficiently transmits both P. falciparum and P. vivax. Unlike other malaria vectors, An. stephensi thrives in urban settings where it can breed in manmade habitats such as tanks, tires and open sewers. In 2020, we established longitudinal larval surveillance in Lodwar, Turkana in the northwest corner of Kenya. We hypothesized that close proximity to Sudan and Ethiopia and the presence of a major transport route from the Horn into Kenya makes this region a likely environment for establishment of An. stephensi. We conducted bi-weekly surveillance in three types of sites - cisterns, river pans along the seasonal river and irrigation canals. Anopheline larval were collected, counted, and transferred to 95% ethanol for transport to the laboratory. Larvae were extracted in pools of 3 and tested by established molecular methods for An. gambiae, arabiensis, funestus and stephensi. In the first 5 months (March-July 2020), the selected sites were active in all collections. 557 larvae were tested by PCR and the major species was An. arabiensis (85%). The second surveillance period ran from November 2022 to March 2023. We collected a high number (2,387) of anopheline larvae across all sites in November-January. Out of 1483 larvae assayed, two pools from one site tested positive for An. stephensi. Larvae (5) from this site were sent for independent confirmation and sequencing at a partner laboratory. An. stephensi was a minority species in this river pan site which also hosted An. gambaie s.s. and arabiensis. Surveillance and testing are ongoing. The confirmation of An. stephensi in Kenya is critical information for malaria control efforts and underscores the need to expand vector surveillance even in parts of the country where malaria is epidemic/seasonal. The presence of An. stephensi could lead to increasing malaria transmission in northern Kenya, spreading further southwards to highly populated urban areas.

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## IMPACT OF INDOOR RESIDUAL SPRAYING ON ENTOMOLOGICAL INDICES IN SAKASSOU, CENTRAL CÔTE D'IVOIRE

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Indoor residual spraying (IRS) is a major vector control strategy for malaria prevention. Entomological parameters such as indoor resting density (IRD), human biting rate (HBR), and entomological inoculation rate (EIR) were monitored in Sakassou (sprayed with clothianidin-based insecticide) and Beoumi (unsprayed district) to monitor changes due to three consecutive years of IRS in Côte d'Ivoire. After one year of baseline data collection in 2019, IRS was implemented in August 2020, August 2021, and May 2022 using clothianidin-based insecticides. Adult mosquitoes were collected monthly using human landing catches (HLCs) and pyrethrum spray catches (PSC) from January 2019 through December 2022. Mosquitoes collected were identified morphologically while sibling species were further analyzed using PCR. A subsample of the mosquitoes collected were analyzed for sporozoite infection using ELISA for estimating the EIRs. At baseline in Sakassou, the mean IRD was 15.5 females/room/day (f/r/d), the mean HBR was 296.8 bites/person/night (b/p/n) and the mean EIR was 4.9 infective bites per person per night (ib/p/n). After three years of IRS the mean IRD, the mean HBR and the mean EIR decreased significantly to 5.8 f/r/d (p=0.0003), 142.6 b/p/n (p=0.0002) and 0.919 ib/p/n (p=0.0022), respectively. At baseline in Beoumi, the IRD was 3.8 f/r/d, the HBR was 35.5 b/p/n and the EIR was 0.61 ib/p/n at the baseline collection. After three years, there was a reduction of the IRD (2.2 f/r/d; p=0.0954), the HBR (24.9 b/p/n; p=0.5483) and the EIR (0.474; p=0.6776), but the differences were not statistically significant. Although Sakassou had higher vector density than Beoumi, the relative reduction of malaria entomological indicators post-IRS following three years of IRS was significant. These decreases in malaria transmission are consistent with preliminary findings on malaria case reductions after the 2020 and 2021 IRS campaigns, indicating that IRS was effective in Côte d'Ivoire.

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## IS THE UK PREPARED FOR A MOSQUITO-BORNE DISEASE EMERGENCE? A PROTOCOL FOR FIELD WORK

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In 2021, risks to health from vector-borne diseases were highlighted as an area requiring more action in the 3rd UK Climate Change Risk Assessment Technical Report. Currently, cases of mosquito-borne diseases (MBDs) are typically imported to the UK but there is a risk of arbovirus transmission from a number of vector-competent mosquitoes indigenous to the continent, as well as from an increasing number of invasive species establishing in Europe. A "mosquito watch" programme between the UK Health Security Agency (UKHSA) Medical Entomology team and the Chartered Institute of Environmental Health (CIEH) currently provides a portal for environmental health officers (EHOs) to submit samples to UKHSA and partners for identification of species. UKHSA also conduct a range of enhanced surveillance strategies for invasive mosquitoes in the UK at points of entry. The aims of this study are to i) identify current awareness, risk perception and engagement in local authorities, in England, ii) understand more about perceptions on the drivers of MBD and barriers to effective preparedness and iii) determine how they have changed over time. Current surveys distributed to EHOs have been carried out by the UKHSA every 10 years since the 1970s across 374 local authorities and include information on nuisance biting complaints, potential habitats for mosquitoes, local capabilities to control mosquitoes etc to determine two key aspects: experience with mosquitoes and mosquito control preparedness. For this study, additional scenario-specific questions related to potential Aedes albopictus incursions will be added to this survey, for the EHOs based in South-East England and the Greater London Regions, as previous research has forecasted those two regions as hotspots within England. This research will help determine the best survey tools that can be implemented more frequently, in more areas, to prepare for the risk of MBD emergence which is increasing with climate change on a global scale.

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## DETERMINING IMPACT OF DENGUE VIRUS INFECTION IN PREGNANCY ON MATERNAL AND CHILD OUTCOMES

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Dengue virus (DENV) is the most common arbovirus globally, with estimated 400 million infections yearly. Dengue in pregnancy has been associated with adverse maternal and neonatal health outcomes including preterm birth, low birth weight and miscarriage. Despite the abundance of literature for DENV in pregnancy, few studies examine this issue in Africa. In this study, we utilized IgG ELISA to identify DENV exposure (seronegative at antenatal visits and seropositive for anti-DENV IgG at delivery) and characterize the epidemiological burden of DENV among a Kenyan cohort of pregnant women. Seroconverted mothers were also tested by PCR to identify timing of DENV exposure and then assessed by logistic regression for any associations between DENV exposure and adverse maternal and/or infant health outcomes. At birth, neonatal development outcomes were assessed and children were followed every 6 months. The studied cohort comprised 454 pregnant women initially part of a 749-maternal child cohort followed for parasitic infections. Median maternal age was 25, primary delivery mode was vaginal (95%), and mean birth weight was 2,991 grams. The cohort experienced significant maternal and neonatal morbidity: one maternal death, 74% of women with anemia at delivery, 75% of women with parasitic infection during pregnancy, 20% preterm birth and 2% stillbirth. Using community-based estimates from our prior work, we expected approximately 18 women to have gestational DENV exposure; however 35 mothers (7.7%) seroconverted during their pregnancy, indicating nearly twice the expected burden of DENV for this vulnerable population. Using Fisher's Exact test, comparisons of DENV+ and DENV- mothers found no association between seroconversion and: Caesarian birth (5.7% vs 4.7%,p=0.68), adverse birth outcomes [Ex: stillbirth, maternal death, intrapartum fever] (0% vs 5.3%, p=0.62), or preterm birth (21% vs 20%, p=0.84). Although it was found that adverse fetal outcomes had no association with seroconversion, next steps are to complete assessments of fetal developmental outcomes, such as APGAR score, to better understand the burden of DENV in pregnancy.

5945

## YELLOW FEVER VACCINATION COVERAGE IN ARID AREAS OF KENYA AN ASSESSMENT FOLLOWING OUTBREAK, 2022

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Kenya reported a Yellow Fever (YF) outbreak in the arid pastoral counties in March 2022 following the confirmation of three cases. A reactive vaccination campaign conducted had a coverage of 52% in Garissa and 58% in Isiolo. However, the precise estimated coverage had not been determined. We sought to estimate vaccination coverage and evaluate strategies used in the campaign. We used the WHO-recommended strategies to conduct a cross-sectional population-based survey involving residents aged 9 months to 60 years. We used a multi-stage proportionate-to-size cluster sampling to determine the number of clusters and randomly selected 7 households per cluster. Data were collected using semi-structured questionnaires and analyzed using descriptive statistics. We visited 118 clusters and 818 households with 5187 eligible participants, of whom 76% (4020/5187) had been vaccinated during the campaign. Participants available for interview were 45% (2355/5187), among whom 81% (1927/2355) had been vaccinated, 60% (1419/2355) were female, and 28% (650/2355) were children aged 1–5years. Garissa county had a coverage of 77% (1093/1412), with 58% (818/1412) having the YF card. Isiolo county had a coverage of 89% (834/943), with 59% (555/943) having the YF card. Respondents who were vaccinated from mobile points were 88% (1696/1927). Community Health volunteers (CHVs) 55% (597/1090), Healthcare workers 56% (615/1090) and religious leaders 16% (170/1090) had the most influence on respondents taking the YF vaccine. Respondents who were aware of the YF campaign were 80% (1886/1927), with CHVs 48% (1135/2355), mobilizer/criers 23% (545/2355) and radios 13% (301/2355) providing the most information on the YF campaign. The respondents who experienced adverse events following immunization were 5% (102/1927). Garissa's overall coverage was below the desired threshold of 80%. CHVs and mobile vaccination points were integral in the vaccination campaign. The use of mobile vaccination points should be adopted in arid-pastoral areas.

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## ASSESSING ENTOMOLOGICAL IMPACT OF A LARVAL SOURCE MANAGEMENT PILOT USING AERIAL SPRAYING OF RICE FIELDS WITH DRONES IN TWO DISTRICTS OF MADAGASCAR

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As a complementary vector control intervention in Madagascar, the PMI VectorLink project and National Malaria Control Program conducted a larval source management pilot via drone in 17 fokontany of two mapped districts with high malaria incidence (144/1,000 in Morombe, 120/1,000 in Ankazobe in 2020) and abundant rice fields that are common larval habitats. The intervention occurred during the short rainy season, from February to July 2022, with Bacillus thuringiensis israelensis (Bti) larvicide twice per month (10 spray cycles).To assess the entomological impact of the intervention, larval collections, insecticide susceptibility bioassays, and adult mosquito collections were conducted before, during and after spraying in six sites per district. Bioassays using a range of Bti dilutions demonstrated larval susceptibility, with mortality above 80% after 24h at a dose of 0.07g (1/14 of larval habitat solution sample) and 0.05g (1/20). Larval density decreased by 96% and 97% in Morombe and Ankazobe, respectively, one day post spray. However, recolonization of larval habitats was observed one month after spraying ended with an increase from an average of 0.4 larvae/liter (l/L) one day post spray to 4.6 l/L in Morombe, and from 0.6 l/L to 2.6 l/L in Ankazobe. A reduction in mosquito biting rate was recorded in all sprayed sites compared to baseline. In Morombe, the Anopheles gambiae s.l. human biting rate decreased from 6.3 bites per person per night (b/p/n) at baseline to 1.3 b/p/n indoors, and from 8.4 b/p/n outdoors to 2.6 b/p/n after 10 spray cycles, but then increased one month after spraying stopped to 5.1 b/p/n and 6.4 b/p/n indoors and outdoors, respectively. In Ankazobe, the biting rate significantly decreased through the end of the spraying cycles from 2.1 b/p/n at baseline to 0.3 b/p/n after 10 spray cycles indoors and 2.4 b/p/n to 0.3 b/p/n outdoors, but then increased to 0.5 b/p/n and 0.9 b/p/n indoors and outdoors, respectively, one-month post-spray. This study suggests that biweekly spraying in rice fields reduced larval density and human biting rates and could be beneficial in areas with outdoor biting and seasonal malaria transmission.

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## DETECTION OF ANTIBODIES AGAINST SALIVARY PROTEINS OF AEDES ALBOPICTUS AND CULEX QUINQUEFASCIATUS IN NORTHERN CARDINALS IN LOUISIANA

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West Nile virus (WNV) is a mosquito-borne pathogen primarily transmitted by Culex mosquitoes. Since the emergence of WNV in the US in 1999, detecting infection in birds and mosquitoes have become the primary surveillance tools used as precursors for human infection and neuroinvasive disease incidence. WNV is normally transmitted between birds and mosquitoes in the environment; and humans are considered dead end hosts. WNV is transmitted to a vertebrate host through the bite of an infected mosquito during blood feeding. In this process, mosquitoes deposit salivary proteins that elicit antibody responses; previous studies have established that the concentration of such antibodies is directly related to the intensity of exposure to mosquito bites and a good proxy to determine risk of infection in the human population. Since WNV infection in birds has also been used to determine risk of transmission in some areas, we tested the levels of IgY antibodies against whole salivary glands of Aedes albopictus and Cx. quinquefaciatus in more than 700 Northern Cardinals captured via mist nets in Louisiana between 2018 and 2019. Our preliminary data (n=169) showed a significant negative correlation between the sample collection date and the IgY antibody levels against Ae. albopictus in younger hatch year Northern Cardinals (p=0.0375). This correlation was not shown in older after hatch year birds. We did not find significant difference in the antibody levels between males or females from any of the locations. In general, higher levels of antibodies against Ae. albopictus were observed in comparison to Cx. quinquefasciatus. We will discuss the proteins that were identified by immunoblot as the most immunogenic in these two different sets of samples. We are working towards designing new tools to track bird exposure to infective bites to effectively measure risk of WNV transmission.

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## ENTOMOLOGICAL SURVEILLANCE STRENGTHENING IN INDIA: MEETING THE CHALLENGES

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India has varying degree of challenges in entomological surveillance and vector control due to six major VBDs, multiple vector's influence and different eco-geographical zones. The core vector control measures include adult mosquito control through Indoor Residual Spraying, Long Lasting Insecticidal Nets and Larval Source Management for urban areas. The technical guidance to implement the VBD programme is based on knowledge on vectors and bionomics. National Programme established 72 entomological zones in 1977 to do entomological monitoring after massive resurgence of malaria in 1976. India has targeted for elimination of Malaria, Visceral leishmaniasis and Lymphatic Filariasis. During elimination phase, entomological monitoring becomes paramount to sustain the vector control impact and prevent re-establishment Shortage of entomologists are the major challenge in entomological monitoring which is highly skilled job. Though efforts have been made to bridge the gap by providing consultants and increasing number of Entomological Zones, high level advocacy is required to fill all sanctioned positions and support by development partners. Gates Foundation is supporting through integrated entomological surveillance in a few areas. Global funds also supporting by provisioning contractual entomologists. Insecticide resistance monitoring and operational research for programme are supported by two premier national institutions viz., NCDC, ICMR and WHO.

5949

## EVALUATION OF SPATIAL REPELLENT PRODUCTS AGAINST MALARIA VECTOR SPECIES IN PAPUA NEW GUINEA

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Malaria remains one of the most common and deadly diseases within tropical and sub-tropical countries with an estimated 229 million cases recorded by 2019 across 87 malaria-endemic countries. Papua New Guinea (PNG) accounts for about 80% of the malaria burden recorded within the Western Pacific region and controls vector populations through nationwide long-lasting insecticide treated bednets (LLINs). Early and outdoor biting behavior of vector species and the evidence of substandard LLIN products in PNG compromises the effectiveness of LLINs against the local vector populations. This study explores the potential of alternative vector control tools (VCTs) to complement LLINs and improve the effectiveness of malaria control within the country. A promising VCT are spatial emanators (SE), devices that passively emit insecticide volatiles to provide a protective space for users. This tool has the potential to be used both indoors to complement LLINs, and outdoors, particularly in the peridomestic space. This study aims to evaluate the efficacy of SE products against the Anopheles punctulatus group of mosquitoes in Papua New Guinea under laboratory and semi-field conditions. Secondly, it aims to understand the effects that sublethal exposure to insecticides may have on the local vector populations. Finally, this study aims to assess the feasibility, cost-effectiveness and impact on malaria transmission in a small-scale field study in two malaria-endemic villages in PNG. We will present data on the effectiveness of SEs against malaria vectors in PNG and explore the impacts of SE and spatial repellent insecticides against mosquito landing rates, feeding inhibition and the impacts of sublethal exposure to the local malaria vector species. Finally, we will discuss the effectiveness of SE in a proof of concept field trial that will guide the national malaria control program in consideration of SE products as malaria VCT for future control programmes in PNG.

5950

## NON-INFERIORITY EVALUATION OF PERMANET® DUAL TO INTERCEPTOR® G2 AND SUPERIORITY TO PERMANET® 3.0 AT THE 'DALA SUNA' EXPERIMENTAL HUTS IN SIAYA, KENYA

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The rise and spread of insecticide resistance threatens the gains already made in malaria control particularly with long-lasting insecticidal nets (LLINs). The development and evaluation of new generation LLINs is therefore critical in ensuring the constant supply of effective products. This study evaluated the non-inferiority of Permanet Dual against Interceptor G2 (IG2) and the superiority of Permanet Dual (PD) against PermaNet 3.0 (PN3.0) on mortality and blood feeding inhibition. The trial was conducted

at the 'Dala Suna' experimental huts located on the shores of Lake Kanyaboli, Siaya County, western Kenya. The study followed a 7 by 7 Latin square design with treatments employed as unwashed and 20 times washed. Each hut had sleepers daily. Mosquitoes were collected from the huts every morning and categorized based on collection site as roof, wall, net under bed, floor and window exit traps. The mosquito samples from experimental huts were monitored for immediate knockdown and delayed mortality after 24hours, 48hours and 72hours. This study was carried out in an area with high vectors' resistance to pyrethroids. Only An. funestus was used for the experimental hut evaluation due to high densities during the study period. A total of 15144 An. funestus mosquitoes were collected from the experimental huts over the 7 weeks' study period. Mortality at 72 hours was 37% for control net, and higher in the LLINs (washed and unwashed), at 56% for PermaNet 3.0, 66% & 64% for IG2 and 67% & 68% for PD. Blood feeding inhibition was highest with PN3.0 at 33% and 49% unwashed and 20 times washed respectively, and least with PD, at 3% and 12% unwashed and 20 times washed nets, respectively. PD and IG2 had differences in mortality with odds ratio of 1.096 at lower 95% CI: 1.001 – 1.199 and blood feeding odds ratio of 1.176 at upper 95% CI: 1.037 - 1.334. Additionally, PD was found to be superior to PN3.0 on mortality with an odds ratio of 1.805 at 95% CI: 1.654 - 1.969, and inferior in blood feeding, with odds ratio of 1.627 at 95% CI: 1.425 - 1.856. In conclusion, PD was found to be non-inferior to IG2 and inferior to PN3.0 LLINS following WHO efficacy criteria.

**5951**

## RESIDUAL BIO-EFFICACY OF ATTRACTIVE TARGETED SUGAR BAIT STATIONS TARGETING MALARIA VECTORS DURING SEASONAL DEPLOYMENT IN WESTERN PROVINCE, ZAMBIA

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The primary vector control interventions in Zambia are long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS). Challenges with these interventions includes insecticide resistance and the outdoor biting and resting behaviors of many Anopheles mosquitoes. Therefore, new vector control tools targeting additional mosquito behaviors are needed to interrupt transmission. Attractive Targeted Sugar Bait (ATSB) stations, which exploit the sugar feeding behaviors of mosquitoes may help in this role. This study was conducted to evaluate the residual laboratory bio-efficacy of Westham prototype ATSB stations deployed in communities throughout a 7-month deployment in Western Province, Zambia during the first year of a large cluster randomized phase-III trial (Clinical Trials.gov Identifier: NCT04800055). One undamaged bait stations that had been installed on outside walls of households were collected on a monthly (one per cluster per month) basis from each of twelve randomly selected intervention clusters among the 35 trial interventions clusters. Bioassays utilized mosquitoes from a laboratory reared colony of An. gambiaes.s.s, male and female mosquitoes, from December 2021 to June 2022 (rainy to dry season). In total, the study utilized 71 field deployed ATSB stations plus 12 new ATSB stations for comparability purposes. Field deployed ATSB stations had significant lower bio-efficacy than ATSB stations which had never been deployed in the field, but the field-deployed stations retained high levels of bio-efficacy mortality (over 80%) after more than six months in the field. Duration of deployment was also not associated with lower bio-efficacy. There was relatively little variation in mortality between month rounds for those ATSB stations which had been deployed to the field. Westham prototype ATSB stations can retain bio-efficacy even after deployment in the field for 7 months, provided they do not meet predetermined criteria for replacement.

**5952**

## IMPACT OF VOLATILE PYRETHROID SPATIAL REPELLANT ON THE ABUNDANCE OF OUTDOOR BITING ANOPHELINES IN A LOW MALARIA TRANSMISSION SETTING, SOUTHERN ZAMBIA

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Residual transmission in the catchment area of Macha Hospital, Southern Province, Zambia, is attributed to outdoor exposure to malaria vectors - spaces and times where indoor interventions are not as effective. This study aimed at evaluating the entomological impact of a volatile pyrethroid spatial repellant (VPSR) product for peri-domestic use (outdoor kitchens) in a semi-field-system (SFS) and in village structures. The transfluthrin-based VPSR tool enables spatial protection against host-seeking mosquitoes. The two phases of this study included 1) a SFS study utilizing replica outdoor kitchens. Here, two VPSR devices were hung from the eaves of each SFS- structure. Release-recapture (using human landing catches (HLCs) experiments enabled the evaluation of the impact of the VPSR device on laboratory reared Anopheles gambiae s.s. Additional secondary endpoints for mortality and reproduction were also collected. 2) a field study conducted in the Macha region during the transmission season. Forty households were selected from two villages for the study. HLCs in VPSR treated and untreated kitchens were conducted to assess the impact on human landing. SFS and filed results demonstrated a significant reduction (40-60%) of mosquito numbers entering VPSR-treated structures. In addition to a reduction in landing, there were also mortality and reduction of fitness impacts with mosquitoes exposed to the VPSR. In conclusion, this study demonstrates that VPSR can reduce the exposure of humans to vectors in both (SFS) and field (village) structures, with additional evidence for community impact through mortality and other impacts seen in transfluthrin-exposed mosquitoes. VPSRs are a potential gap filler as an alternative outdoor malaria intervention.

**5953**

## EXPERIMENTAL HUT AND FIELD EVALUATIONS OF THE THERMACELL® BASED METOFLUTHRIN SPATIAL REPELLENT AGAINST PYRETHROID RESISTANT ANOPHELES FUNESTUS IN SIAYA, WESTERN KENYA

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Spatial repellents (SR) are undergoing epidemiological evaluations with the aim of complementing current vector control tools This study conducted experimental hut and small-scale field trials to evaluate the protective efficacy of Thermacell® based metofluthrin SR against pyrethroid resistant Anopheles funestus in Siaya County, western, Kenya. Phase 1 of the study was conducted in the "Dala Suna" experimental huts located around Lake Kanyaboli, Siaya County, Kenya. The SR product emanator included a cartridge containing metofluthrin attached to liquefied petroleum gas (LPG) cylinder and included two experiments: one to evaluate whether fire from the LPG cylinder increased mosquito density indoors and the second to evaluate the effect of 2, 4 and 12-hour emanation periods had on indoor density and biting rates. Experiment 2 was further modified to include an hour's emanation between 0500-0600HRS the next morning. The second phase: was a field evaluation of an outdoor emanation of the SR product using human landing catches outdoors with volunteers sitting at 5ft,

10ft and 20ft from the emanator. Measured outcomes were deterrence, percentage feeding inhibition, mortality and mosquito landing rates. The SR had an 87.7% deterrence rate and knockdown of 95.5% of Anopheles funestus coming into the huts. Cooking with LPG cooker increased mosquito densities indoors by 52.2%. The 12-hour emanation period reduced Anopheles landing rate indoor by 99.3%. Using 5ft as reference, outdoor mean hourly biting rate were significantly lower than at 20ft (0.33 RR = 9.766(5.351-17.822) P<0.001) but were not significantly different from 10ft (0.025 RR=0.79 (0.349-1.79) P=0.573). SRs almost completely blocked biting indoors and led to 10X lower biting rates within 10ft of the emanator outdoors, the first product to demonstrate such potential. The use of LPG in house could increase exposure to Anopheles mosquito bites.

**5954**

## APPLICATION OF VECTOR CONTROL OPTIMIZATION MODEL ON EAVE RIBBONS FOR MALARIA VECTOR CONTROL IN KILOMBERO VALLEY, TANZANIA

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Despite significant progress against malaria, the use of two core interventions, namely insecticide-treated nets (ITNs) and indoor residual spraying (IRS), are becoming increasingly vulnerable due to insecticide resistance and outdoor biting by mosquitoes. One representative example is Kilombero Valley in Tanzania where transmission continues despite over 80% of the population using ITNs. Further progress in this and similar African settings will require identifying which supplementary tools combine most effectively with ITNs to reduce mosquito exposure. Using the Kilombero Valley as a case study, the Vector Control Optimization Model (VCOM) was adapted and extended to simulate the impact of adding eave ribbons treated with spatial repellent (ER) as supplementary intervention in an area of high ITN coverage. Simulation was conducted to assess the impact of varying coverage of this supplementary intervention on the entomological inoculation rates (EIR) generated by two common vectors in Kilombero, Anopheles arabiensis and Anopheles funestus. Finally, the impact of introducing this intervention on the combined EIR from An. arabiensis and An. funestus was assessed to identify scenarios in which values fell below 1; the likely threshold required for malaria interruption. ER was predicted to substantially reduce the EIR in Kilombero valley when combined with 80% ITN coverage. However, the nature of the impact varied notably between vector species. ER was predicted to have a much larger effect on transmission mediated by An. funestus than An. arabiensis. Additionally, in the situation where EIR from both An. arabiensis and An. funestus was combined, substantial coverage of this supplementary intervention was predicted to lower EIR to below one. Despite the significant impact ER in combination with ITNs on one of the two vectors (An. funestus or An. arabiensis), this intervention is insufficient when combined with ITNs to reduce the EIR to below one in settings like Kilombero Valley where both species contribute to malaria transmission.

**5955**

## COMMUNITY-BASED BIOLARVICIDING FOR MALARIA CONTROL IN TANGA REGION, TANZANIA

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Malaria remains a disease of public health importance globally. Prevalence of malaria in Tanzania among under-fives is 8.1% (MIS 2022) and the country accounted for approximately 3% of all malaria cases in 2021 (WHO – World Malaria Report). The country is deploying community-based biolarviciding in enhancing its efforts towards malaria control. Tanzania is conducting routine implementation of biolarviciding in three councils: Handeni DC, Tanga CC and Lushoto DC, representing 'high', 'moderate' and 'low' malaria risk strata respectively as well as representing both urban and rural settings. Implementation follows a community-based approach whereby trained community-owned resource persons (CORPs) identify the habitats and apply biolarvicide at the ground level. Officers at the village/street, ward, council and regional levels using existing local government structures supervise CORPs. The country is using two biolarvicide products produced in-country: Bacillus thuringiensis var. israelensis (Bti) and Bacillus sphaericus (Bs). Biolarvicide is applied to all identified breeding habitats for three rounds per year based on rainfall pattern. Each round comprises of eight weekly cycles of larvae monitoring and application of biolarvicide. The councils collected baseline data in February 2022 for four consecutive weeks. Handeni DC, Lushoto DC and Tanga CC had 12,203, 2,126 and 1,005 breeding habitats respectively. Handeni DC reported an average of 233,515 anopheles larvae per week, while Lushoto DC and Tanga CC reported 79,528 and 3,520 respectively. Two rounds of application have been completed: Jun-Jul 22 and Oct-Nov 22. Handeni DC showed anopheline larval reduction of 99.9% and 96.1% across the eight weeks of application between rounds 1 and 2 respectively. Lushoto DC reported a reduction of 97.2% and 98.5%, while Tanga CC reported a reduction of 84.4% and 89.6%. Programmatic monitoring highlights that biolarviciding reduces anopheline larval in all three councils. Next steps include conducting entomological and epidemiological evaluation to determine the impact of the intervention.

**5956**

## PILOT STUDY OF BACILLUS THURINGIENSIS ISRAELENSIS IN THE CONTROL OF PERSISTENT DRY SEASON BREEDING MALARIA VECTORS IN MALI

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One of the challenges to malaria elimination is residual malaria transmission. The concept of "residual malaria" expresses the contribution of other factors to the persistence of malaria transmission. Larval control has been poorly applied in African settings because of the huge number and type of larval habitats encountered. However, riverbeds constitute hot spots for maintaining malaria transmission throughout the long dry seasons in Sahelian West African countries with fewer numbers of larval habitats. In this study, we are pilot testing the biological larvicide Bacillus thuringiensis (Vectobac WG®) in malaria vector control along the River Niger during the dry season in Mali. An active search for major larval habitats was conducted during the dry season in localities along the Niger River. In total, 45 main larval habitats, including water pools, puddles, and brick pits were identified and treated with Bti. Just before treatment, the mean larval density was estimated through the standard WHO deepening technique. The mean number of larvae was 22.4 larvae per dip (SD = 32.75, 95% CI = 12.58—32.26). Using the same method 24 hours after treatment, we could not detect the presence of any larvae in all the identified larval habitats. This is except for a mixed Culex sp and Anopheline larval habitat, where the mortality rate was 97%. 48 hours after treatment, this larval habitat became negative. As a result of this trial, Bti has been shown to be effective in reducing larval density in residual riverbed transmission areas in sub-Saharan Africa.

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## CHARACTERIZATION OF THE PUTATIVE ANOPHELES FUNESTUS-CYP18A1 ORTHOLOG IN ANOPHELES GAMBIAE

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New targets for vector control are needed to reduce malaria transmission due to the spreading of insecticide resistance. In Anopheles gambiae, a likely candidate for genetic control is the 20-hydroxyecdysone (20E) hormone. Two genes CYP314A1 and CYP18A1 regulate respectively the activation and deactivation of the 20E. Although, CYP314A1 is conserved in all mosquitoes species, CYP18A1 is absent in An. gambiae genome. This work used in silico and an experimental approaches to characterize the putative 20E deactivating enzyme coding gene (Anopheles funestus-CYP18A1 ortholog) in Anopheles gambiae. Basic bioinformatics analyses were done to identify Af-CYP18A1 candidate ortholog in An. gambiae genome. To validate the ortholog, molecular docking was performed to compare the binding affinity of 20E to the candidate gene product in An. gambiae and to Af-cyp18A1 protein. The 20E deactivating ability of the candidate gene product in An. gambiae, was evaluated through loss of function experiment using RNA interference system. Two days old females of An. gambiae COGS colony were blood fed and injected with 138 nL of dsRNA+20E. 72 hours post blood meal, 20E titres was measured by High Liquid Chromatography (HPLC). Bioinformatics analyses revealed the CYP306A1 (AGAP004665) as the putative Af-CYP18A1 ortholog in An. gambiae with 54% and 32% protein sequence similarity and identity respectively. The 20E interaction (docking) showed an hydrogen bond between 20E carbon C3 and the Agap-cyp306A1 residue VAL321. While no 20E titre could be detected in control mosquitoes injected with the dsGFP at 72PBM, HPLC analyses showed that An. gambiae females were not able to successfully metabolize the injected 20E when the expression of the CYP306A1 was silenced. The findings of this study are preliminary results suggesting that CYP306A1 could be the Af-CYP18A1 ortholog in An. gambiae. Further investigations are needed to fully characterize the functional role of this candidate gene in 20E deactivation.

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## TRANSCRIPTOME-WIDE DISCOVERY AND QUANTIFICATION OF LNCRNA EXPRESSION IN VARIOUS CONTEXTS IN THE MALARIA MOSQUITO ANOPHELES GAMBIAE FROM RNA-SEQ DATASETS

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Long non-coding RNAs (lncRNAs) are a class of RNA transcripts longer than 200 nucleotides that cannot code for canonical proteins but play various roles in biological contexts. Despite being recognized in mosquito Anopheles gambiae, comprehensive profiling and characterization of lncRNAs remain limited. This study presents a transcript discovery pipeline to identify lncRNA transcripts from RNA-seq datasets in public domains. First, RNAseq reads are mapped against the An. gambiae PEST genome reference using parameters allowing(intron spanning alignment. Then, transcripts from the resulting read mappings are inferred. To identify lncRNAs, non-mRNA transcripts are filtered by the following parameters: a minimum of 10 reads, relative confidence of >10%, a minimum length of 200 nucleotides, and no protein-coding potential. Transcripts that meet these criteria are considered putative lncRNAs. Notably, up to 30% of RNAseq reads are non-mRNA reads. The pipeline predicted thousands of putative lncRNAs. We validated 5 predicted lncRNAs using RT-PCR. The predicted lncRNAs are integrated into the existing transcript annotation to enable simultaneous quantification of mRNA and lncRNA abundance. The lncRNAs identified from different transcriptomes can be merged to update lncRNA annotations. We applied the pipeline to RNAseq datasets in different conditions, such as immune responses to bacterial and Plasmodium challenges and hemocytes, midgut, salivary gland, and whole body. In the datasets of different hemocytes upon bacterial challenges, principal coordinates analysis (PCA) revealed mRNA and lncRNA co-expression patterns. In the dataset that profiled polysome-associated transcripts in the midgut infected with P. falciparum, some lncRNAs demonstrated a differential association with polysomes between the infection and control samples, suggesting that some lncRNAs may encode micropeptides, and/or play roles in regulating protein translation. Our pipeline facilitates lncRNA discovery and quantification to enhance our understanding of transcriptomic response in different contexts.

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## ENDOGENOUS NON-RETROVIRAL RNA VIRUS ELEMENTS IN ANOPHELES DARLINGI

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The rise of metavirome research has positioned insects as important virus reservoirs, mainly dominated by insect-specific RNA viruses (ISV). The discovery of the ISVs and the sequencing of a variety of genomes have allowed the identification of RNA virus sequences integrated into genomes denominated non-retroviral endogenous viral elements (nrEVEs). This work aimed to identify nrEVEs in the Anopheles darlingi reference genome (idAnoDarlMG_H_01) and RNA-seq data obtained from An. darlingi natural populations of three regions of Colombia. A database was built with all virus sequences identified in arthropods available in the NCBI-virus repository and a tBLASTn was performed against the An. darlingi reference genome. The detection of nrEVEs in the An. darlingi transcriptome was performed by mapping the RNA-seq reads on the nrEVEs identified in the reference genome; additionally, contigs assembled from RNA-seq data were aligned against nrEVEs. As a result, 44 nrEVEs of negative-strand RNA viruses (ssRNA-) of the families Rhabdoviridae (n= 22), Chuviridae (n= 11) and Phasmaviridae (n= 3) and double-stranded RNA virus (dsRNA) of the families Partitiviridae (n= 6) and Reoviridae (n= 1) were identified in the An. darlingi genome. Thirty-four nrEVEs were detected on chromosome 3, eight on chromosome 2 and two on chromosome X. A differential nrEVE read count was observed in the Colombian An. darlingi metatranscriptomic data. Finally, the multiple alignment showed an identity greater than 98% in three of the sequences assembled from the metatranscriptome against the nrEVEs identified in the reference genome. Knowledge of the presence and expression of nrEVEs in An. darligi contributes to elucidating the dynamics of the Anopheles virome and its endogenization in the genome. Future studies may explore possible antiviral mechanisms associated with the nrEVEs identified.

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## ANALYSIS OF THE GENETIC VARIATION OF THE FRUITLESS GENE WITHIN THE ANOPHELES GAMBIAE (DIPTERA: CULICIDAE) COMPLEX POPULATIONS IN AFRICA

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One promising strategy for genetic control is to reduce the reproductive potential of disease vectors or pests by targeting genes involved in sexual determinism. However, targeting these genes requires a better understanding of their polymorphism in natural populations to ensure good stability and persistence of the transgene in nature. The genomic data of Anopheles gambiae s.l. used in our study were sequenced by the An. gambiae 1000 Genomes (Ag1000G) project in which the Institut de Recherche en Sciences de la Santé is partner. We used Jupyter notebooks to analyze the genetic variation and conservation score of the fruitless gene

in 18 populations across Africa. A total of 34339 SNPs were identified including 3.11% [1071 SNPs] of non-synonymous polymorphic sites. The overall nucleotide diversity of the gene was low (0.0036) and the Tajima neutrality test (-2.52) was negative indicating an excess of low frequency SNPs. Allelic frequencies of non-synonymous mutations were low except for SNPs at position X: 1309218 (C>G) and X: 1300290 (C>G) that were identified at high frequencies (0.8 - 1) in all populations. The conservation score was variable throughout the fruitless gene with maximum values in the exonic regions compared to the intronic regions. These results would be a good indicator for the spread and persistence of a transgene targeting the fruitless gene in wild populations of An. gambiae s.l.

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## UNRAVELLING THE GENOMIC AND PHENOTYPIC DIVERGENCE WITHIN SUB-POPULATIONS OF TWO MAJOR MALARIA VECTORS: ANOPHELES GAMBIAE AND AN. COLUZZII

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Although a significant reduction in malaria incidence over the decades, this mosquito-borne disease remains a major public health concern. The use of insecticides is a cornerstone in malaria vector control, but widespread insecticide resistance coupled to increasing drug resistance and high costs of implementation, is jeopardizing the efficacy of this strategy making malaria resurgence a grim reality. New strategies with novel tools that complement LLINs and IRS to target the major vectors could prevent the resurgence of disease and hasten malaria elimination. Despite the central role of oviposition preference in selecting suitable environments in blood-feeding insects, its contribution to ecological specialization and local adaptation remains elusive. Population studies at early stages or ecological/genetic divergence provide an excellent opportunity to assess the role of oviposition preference in local adaptation of mosquitoes. Combining laboratory dual choice experiments and whole genome sequencing, we conducted the first assessment of phenotypic variation among some of the subpopulations of two major malaria vectors that are emerging along gradients of anthropogenic disturbance in sub-Saharan Africa. When offered choice, Anopheles gambiae gravid females released individually in cages under standard conditions, lay eggs almost exclusively in water collected from their locality of origin while An. coluzzii mosquitoes did not show any preference. This extreme source-specialization prevails in populations belonging to the same ecological biome and displaying very low levels of genome-wide divergence. Interestingly, An. gambiae females maintained in laboratory conditions for several generations using regular water retained water discrimination and were able to choose between source water and exogenous water. We conclude that favourable aquatic oviposition sites though highly heterogeneous in form, space and time are strong enough to drive ecological specialization in the presence of extensive gene flow in mosquitoes and act as signature cues at early stages of divergence in gravid Anopheles mosquitoes seeking to lay.

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## IDENTIFICATION OF SEX-SPECIFIC PATTERN OF THE DOUBLESEX GENE IN THE MOSQUITO CULEX PIPIENS

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Gaining insight into the molecular mechanisms behind sex differentiation in the mosquito species Culex pipiens might lead to the identification of genes that could be used to achieve selective male sterility in transgenic lines or to regulate the expression of deadly genes. In many insects, sexual dimorphism is controlled by doublesex (dsx), a double-switch gene located at the end of the somatic sex-determination cascade. Here, we report the isolation and characterization of sex-specific transcripts and isoforms for the Cx. pipiens dsx homologue. The CpdsxF and CpdxM transcripts in females and males. The exon/intron structure of Cpdsx shows that it employs a mechanism for sex-specific splicing distinct from that of Drosophila melanogaster dsx. Insect transgenic technology may be used to alter Cx. pipiens sex ratios, and these results will be useful for sterile insect-based vector control initiatives.

**5963**

## CHIP-SEQ STUDY IDENTIFIES TARGETS OF THE CLOCK GENE, PAR DOMAIN PROTEIN 1 (PDP1), THAT REGULATE DIAPAUSE IN CULEX PIPIENS

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Multivoltine insects use diapause to endure adverse seasons. Insects employ environmental cues like short day length in late summer and early fall to enter diapause, but how they measure day length is unknown. The circadian clock is necessary for photoperiodism in many insects, including the northern house mosquito, Culex pipiens, which enters diapause, stops egg follicle development, and gains fat. We employed ChIP sequencing to identify PDP1's downstream targets that contribute to diapause's features and how circadian clock genes regulate diapause. We identified the nearest genes in a 10-kb region of the anticipated binding sites, listed prospective targets, and searched for PDP1-specific binding sites. We then examined the genes for functional significance to diapause-specific behaviors and alterations such as metabolic pathways, lifespan extension, cell cycle regulation, and stress tolerance. We validated PDP1 targets from those candidate genes using ChIP-qPCR. In addition, qRT-PCR demonstrated increased expression in diapausing females compared to non-diapausing counterparts. Our investigation uncovers PDP1-controlled diapause-specific genes.

**5964**

## ASSESSING THE FEASIBILITY OF TWO 'MULTIPLEXED' STRATEGIES IN ANOPHELES STEPHENSI MOSQUITOES

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Anopheles stephensi is a major malaria vector mainly present in southern Asia and the Arabian Peninsula. Since 2012 it has invaded several countries of eastern Africa, requiring urgent efforts to develop more efficient strategies for vector control such as CRISPR/Cas9-based homing gene drives. One of their challenges is to ensure efficient recognition of the target site by the gRNA. Unfortunately, when a double-stranded break is repaired by NHEJ, it can lead to the creation of mutations. These can destroy the target site, making it difficult for the gRNA to recognize the homologous chromosome. Recent studies have explored the use of multiple gRNAs to solve the issue of target site resistance. In theory, 'multiplexing' would require the individual to become simultaneously resistant to all gRNA target sites for it to be completely resistant to the drive. A potential downside to this strategy is that homing efficiency may depend on perfect homology near the cut site. Thus, the optimal distance between gRNA target sites must be considered. We performed different set of crosses to assess the homing and cutting efficiency of two different 'multiplexing' strategies targeting the cardinal locus: the classic multiplexing and the additive strategies. To assess the feasibility of the first strategy, we generated a gRNA-expressing line with 4 gRNAs separated by a maximum distance of 142bp, which was crossed to a Cas9-expressing line. No significant difference was observed in homing in comparison to a single gRNA-expressing line targeting the same locus. Furthermore, a mutation altering one of the gRNA target sites was introduced to observe changes in the drive's performance. For the additive strategy, we tested the homing efficiency of two independent

gRNA-expressing drives by crossing them to a Cas9-expressing line. Then, we repeated these crosses while adding a mutation in the other gRNA-expressing drive target site found in a 481bp distance to see if homing was affected. We don't expect to see any significant reduction in homing efficiency for any of the performed crosses, showing that these are feasible strategies to tackle target site resistance in An. stephensi.

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## GEOGRAPHICAL DISTRIBUTION AND GENETIC POPULATION STRUCTURE OF AEDES ALBOPICTUS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Aedes albopictus, a major vector of dengue, zika, and chikungunya viruses, has recently been introduced to central Africa, including the Democratic Republic of the Congo (DRC). Although it is well-known that the establishment of this mosquito poses a serious public health threat in the invaded area, ecological and genetic data for the invading populations in DRC are still scarce. Therefore, our study aimed to reveal the nation-wide geographical distribution of Ae. albopictus and differences in genetic structure among populations. We conducted two entomological surveys at 308 sites within 104 cities from May 2017 to September 2019 and from March to August 2022. We sequenced and analyzed the mitochondrial cytochrome c oxidase subunit 1 gene (COI) (1,434bp) of 498 samples from 16 populations. Neutrality tests and genetic distance analyses were performed on the populations which were classified into three distinct geographical groups. Aedes albopictus was found at 193 (62.6%) sites within 82 (78.8%) cities, mainly in the western and northern DRC, as well as in the western part of the central DRC. This species has become the preponderant Aedes species in these areas, but was not found in the southeastern DRC. Nine COI haplotypes were detected, with one being widely distributed and the most frequent (49%). Aedes albopictus exhibited moderate haplotype diversity (Hd = 0.69 ± 0.02) but low nucleotide diversity (Pi=0.00174 ± 0.00004). Neutrality test revealed Tajima's D (2.14; $p<0.05$) and Fs values (Fs = 3.078; p=0.044) indicating an excess of intermediate-frequency alleles, which can be due to either positive selection or a recent population expansion. Low to moderate genetic differentiation (Fst = 0.016, 0.067 and 0.1328) and high level of gene flow (Gamma St, Nm = 3.75, 7.1 and 24.15) were observed among the groups. Phylogenetic analyses showed that all the nine haplotypes clustered with the populations from Vietnam. The results suggest that Ae. albopictus has established in the western and the northern DRC and was recently introduced to DRC from Southeast Asia.

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## GENETIC VARIATION AND TRANSCRIPTIONAL ENHANCER ACTIVITY IN THE MALARIA VECTOR, ANOPHELES COLUZZII

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Enhancers are an important class of non-coding regulatory elements, and genetic variation in enhancers can underlie phenotypic variation. A genome-wide map of transcriptional enhancers for the malaria vector Anopheles coluzzii was filtered to highlight 16 candidates with potential influence over differential malaria susceptibility. The effect of candidate enhancer alleles from malaria susceptible and resistant mosquitoes was assessed by site-directed mutagenesis and Dual-Glo luciferase assays, with 7 enhancers displaying significant differences between resistant and susceptible mosquitoes. Computational prediction of transcription factor binding sites (TFBS) in the enhancers identified 82 enriched motifs that represent i) TFBS conserved with Drosophila melanogaster and ii) predicted mosquito-specific TFBS. DNA-protein pulldowns were implemented to confirm TFBS identification and distinguish enhancer allele-specific differences in TFBS binding that could be associated with differential transcription regulation.

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## IDENTIFICATION OF H3K27ME2 SITES THAT CAUSE VARIOUS DIAPAUSE PHENOTYPES IN THE MOSQUITO CULEX PIPIENS

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To survive winter, the mosquito Culex pipiens enters diapause, a state that enables adult females to accumulate fat, cope with stress, and survive longer. Epigenetic controls, specifically histone changes, have been linked to several diapause phenotypes. In our recent Western blot investigation, we observed significantly fewer H3K27me2 marks in the fat bodies of diapausing females, suggesting that H3K27me2 may regulate diapause, although the regulatory mechanisms remain unclear. Using ChIP-sequencing, we identified H3K27me2's direct targets in the mosquitoes' fat bodies and proposed that epigenetic modification of these histone methylation marks may activate diapause-programmed genes. We selected and evaluated diapause-related genes and prioritized the top 300 candidate genes with the highest fold enrichment for functional annotation based on their proximity to the peaks within 1 kb of the promoter's transcription start site. We then verified a collection of candidate genes of H3K27me2 ChIP-seq using ChIP-qPCR and qRT-PCR.

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## GENETIC AND NEURAL BASIS OF ATTRACTION OF GRAVID AEDES AEGYPTI TO AFRICAN BERMUDA HAY INFUSIONS

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The yellow fever mosquito Aedes aegypti is a prolific disease vector with explosive population dynamics. Its ability to thrive in urban environments lies in the ability of female Ae. aegypti to seek out standing pools of water to lay their desiccation-resistant eggs. Of note, females are highly attracted to the scent of decomposing botanical matter in water. Currently, hay infusion preparations made from African Bermuda Grass are widely used to bait the CDC autocidal gravid ovitrap for mosquito surveillance and control in the field. However, the molecular and cellular basis of how mosquito attraction to this potent oviposition attractant is unknown. To gain insight into olfactory preferences of female Ae. aegypti for oviposition stimuli, we established a two-choice behavioral assay that quantifies attraction to odors emitted by fermenting African Bermuda hay. Mated and blood fed females of different Ae. aegypti strains were more attracted to hay infusion odor than water alone. In addition, the preference for hay infusion is state-specific as it was not observed in unmated blood-fed, nor mated unblood-fed females. To understand the molecular and neural basis of this attraction, we first tested mutants for olfactory co-receptors to understand which are necessary for female olfactory choice. Next, we generated a mosquito line with an inward rectifying potassium channel (Kir2.1) downstream of the QUAS binary expression sequence. In concert with olfactory co-receptor QF2 driver lines, we are testing the role of different subsets of olfactory co-receptor-expressing cells in hay infusion attraction. This research will fundamentally improve our understanding of biological basis of oviposition site search behavior and catalyze development of novel approaches that target this important aspect of female mosquito reproductive biology. Furthermore,

the generation of a line for neural silencing in Ae. aegypti can be used more generally to investigate the neural basis of mosquito behaviors that underly its population dynamics and capacity to transmit arboviral diseases.

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## LEVERAGING FIELD DNA SEQUENCING TO MEASURE SPATIOTEMPORAL VARIATION IN MOSQUITO COMMUNITY COMPOSITION AND FEEDING BEHAVIOR IN RURAL MADAGASCAR

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Understanding how climatic variables impact mosquito distribution in tropical highlands is particularly paramount, as human populations in regions with historically low mosquito-borne disease burdens are potentially immunologically naive relative to nearby lowland groups and may lack genetic or behavioral adaptations to pathogens such as Plasmodium. Despite this pressing public health need, baseline data from mosquitoes in tropical highlands are scarce due to the historically low vector-borne disease burden of these regions. Critically, this gap in information hampers fine-scale modeling of disease likelihood in areas where the predicted risk of climate-linked disease increase is highest. Here, we report longitudinal sampling of mosquitoes along altitudinal gradients in the montane tropical rainforest of southeastern Madagascar to understand the ecological drivers of malaria disease dynamics. We measured mosquito community composition and distribution in six villages surrounding Ranomafana National Park in southeastern Madagascar that vary in altitude and intensity of land use change. We additionally applied new portable sequencing technology to sequence individual mosquitoes in-country for assessment of population connectivity and vertebrate host species selection. Such data across seasons and altitudes will allow us to understand the human health impacts of the predicted shift for higher elevation areas to the vector dynamics of the lowlands.

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## CHARACTERIZATION OF THE VIROME IN AEDES AEGYPTI VECTOR OF CONDORCANQUI PROVINCE, AMAZONAS REGION, THROUGH SHOTGUN METAGENOMIC SEQUENCING

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The total virome in mosquitoes has yet to be explored in the population of Aedes vector in Condorcanqui province, Amazonas region, Peru. Since the last few years, dengue cases have been considerably increasing in this tropical area, mainly through A. aegypti bites. There is an urgency in scrutinizing A. aeypti viral ecology and interrelations due to its public health relevance. Here, we characterized for the first time, through untargeted shotgun metagenomic sequencing, the virome of Aedes mosquitoes collected in the Rio Santiago district of Condorcanqui. A total of 137 individuals morphologically identified as A. aegypti were collected using mouth aspirators. These were grouped into twelve pools of ten to fifteen individuals. A protocol based on RNA extraction, reverse transcription, metagenomic library preparation, and sequencing, using the Illumina NextSeq 500 system, was performed. Bioinformatic analysis for screening shotgun metagenomic data was used. This included filtering out low-quality reads, assembling reads into contigs and viral classification using the ViralRefSeq Protein NCBI database. The results indicated a complex diversity of viral family members in A. aegypti, mainly Phenuidiridae, Flaviviridae and Totiviridae; also, Rhabdoviridae, Chuviridae, Baculoviridae, Mimiviridae and other viral families were found. Phenuidiridae, Flaviviridae and Totiviridae viruses were consistently found in the pools, suggesting these might be members of the core virome of A. aegypti in Amazonas. Some viruses detected have been identified as pathogenic for plants, ruminants and insects; a few might be used as biopesticides for biological control of insects due to their high pathogenicity and host specificity. Anopheline-specific viruses were also found, suggesting that there might be an exchange of viruses during their blood feeding. Additionally, targeted methods are needed for a more sensitive and precise search of human pathogenic viruses in vectors of these endemic areas.

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## EXAMINING WEST NILE VIRUS INFECTION OF CULEX TARSALIS MIDGUTS AT SINGLE-CELL RESOLUTION

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In mosquito vectors, the midgut functions as a critical interface between pathogen and vector. Several studies have demonstrated that the mosquito midgut plays an important role in systemic infection, host immune response, intrahost virus population dynamics, and vector competence. Recent work examining mosquito hemocytes and midgut epithelium at the single-cell level has uncovered distinct functions within immune cell populations and identified key markers in the midguts of Aedes aegypti that overlap with major cell types found in Drosophila midguts. However, studies characterizing cell populations and their functions in the mosquito midgut are scarce and, in Culex tarsalis mosquitoes, nonexistent. Cx. tarsalis is a main enzootic vector of West Nile virus (WNV) and one of the most important arthropod-borne virus (arbovirus) vectors in North America. The Cx. tarsalis midgut is a stringent escape barrier that impacts the diversity of WNV populations that escape the midgut, infect the salivary glands, and escape into the saliva where transmission occurs. To gain a better understanding of how Cx. tarsalis midgut tissue functions as the interface between the vector and WNV, we performed single-cell RNA sequencing (scRNA-seq) on dissociated midgut cells from both WNV infected and mock-infected Cx. tarsalis mosquitoes. Examining WNV infection of the midgut at single-cell resolution will allow us to identify distinct cell populations and differentially expressed genes associated with WNV infection. Further, by using an approach to scRNA-seq that is flavivirus inclusive, we hope to identify cell types that serve as either sites of virus replication or cellular bottlenecks. Identification of midgut cell populations and genes impacted by WNV infection, will provide key insight into the physiology of WNV infection within a critical vector, and further characterize mechanisms that influence intrahost evolution of WNV.

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## EVOLUTIONARY HISTORY OF SYLVATIC POPULATIONS OF ANOPHELES GAMBIAE AND IMPLICATIONS FOR MALARIA TRANSMISSION

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Apart from the increasingly reported adaptation to urban environments, the adaptation of malaria vectors to sylvatic, non-anthropogenic environments remains poorly studied. Recent observations on the existence of stable populations of Anopheles coluzzii in the Lopé National Park, Gabon, led us to investigate the evolutionary history of this population and their potential involvement in pathogens transmission. We analyzed the genome of 96 individuals of An. coluzzii from 3 different biotopes: urban, rural and sylvatic. We revealed the existence of structure between the populations of Libreville and those found in La Lopé (rural and sylvatic). However, despite the existence of important gene flow between the sylvatic and rural populations, we highlighted the existence of selection signals that suggest local adaptations of these different populations of An. coluzzii. In addition, the carrying of human and non-human Plasmodium by mosquitoes found in the

park, revealed by an extensive field collection, could be a threat to malaria control. The park could then be a reservoir of vectors and vector-borne pathogens. Further analyses are underway to better characterize these different populations from a genetic and phenotypic perspective.

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## TEMPORAL AND COEVOLUTIONARY ANALYSES REVEAL THE EVENTS DRIVING THE EMERGENCE AND CIRCULATION OF HUMAN MAMASTROVIRUSES

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Characterized by high genetic diversity, broad host range, and resistance to adverse conditions, coupled with recent reports of neurotropic astroviruses circulating in humans, mamastroviruses pose a threat to public health. The current astrovirus classification system based on host source prevents determining whether strains with distinct tropism or virulence are emerging. By using integrated phylogeny, we propose a standardized demarcation of species and genotypes, with reproducible cut-off values that reconcile the pairwise sequence distribution, genetic distances between lineages, and the topological reconstruction of the Mamastrovirus genus. We further define the various links established by co-speciation and resolve the dynamics of transmission chains to identify the sources from which different mamastrovirus species circulating in humans have emerged. The well-known 'human' astrovirus, defined here as mamastrovirus species 7, has co-speciated with humans, while there have been two additional host-jumps into humans from distinct hosts. Newly defined species 6 genotype 2, linked to severe gastroenteritis in children, resulted from a marmot to human jump taking place ~200 years ago while species 6 genotype 7 (MastV-Sp6Gt7), linked to neurological disease in immunocompromised patients, jumped from a bovine source only ~50 years ago. Through demographic reconstruction, we determined that the latter reached coalescent viral population growth only 20 years ago and is evolving at a much higher evolutionary rate than other genotypes infecting humans. This study constitutes mounting evidence of MastV-Sp6Gt7 active circulation and highlights the need for diagnostics capable of detecting it.

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## A COMPARATIVE ANALYSIS OF COMMERCIAL ANTI-DENGUE VIRUS IGG TESTS TO AID DENGUE IMMUNIZATION PROGRAMS

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The Advisory Committee on Immunization Practices (ACIP) recommends screening with anti-dengue virus (DENV) IgG tests to confirm prior DENV infection in children aged 9-16 years living in dengue-endemic areas of the United States before vaccination with Dengvaxia®. The minimum recommended test performance is ≥75% sensitivity and ≥98% specificity. To ensure safe implementation of Dengvaxia®, it is necessary to evaluate commercial anti-DENV IgG tests to assess which meet these criteria. We evaluated the performance of seven tests using serum panels from early convalescent specimens paired with acute specimens characterized by DENV and Zika virus (ZIKV) RT-PCR. The five best performing tests along with two additional tests specifically designed for pre-vaccination screening were evaluated with a panel of 44 specimens collected from healthy 9-16-year-old children in Puerto Rico. Four of these tests had promising discriminatory capacity for past DENV infections and were further evaluated using 400 specimens from the same population. Specimens from this population were classified as DENV exposed, ZIKV exposed, DENV and ZIKV exposed or unexposed using DENV and ZIKV virus Focus Reduction Neutralization Tests in combination with an in-house DENV IgG ELISA. No single test met the recommended performance to identify children eligible for Dengvaxia®, but the Euroimmun anti-DENV NS1 Type 1-4 ELISA combined with the CTK OnSite Dengue IgG rapid test R0065C with a visual test read yielded 80.3% sensitivity and 100% specificity. Manufacturers of these two tests modified their tests to meet the recommended performance standards with a single test. Using the automated ALTA rapid test reader, the modified CTK OnSite Dengue IgG rapid test R0065C yielded 76.2% sensitivity and 98.1% specificity. The preliminary performance for the modified Euroimmun anti-DENV NS1 Type 1-4 ELISA was 76.6% sensitivity and 99.1% specificity. These modified tests allow the benefit of vaccination while reducing the risk of vaccinating individuals without prior DENV infection and provide additional options for dengue pre-vaccination screening.

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## CHALLENGES TOWARDS CLINICAL ALPHAVIRUS ENCEPHALITIS DIAGNOSTICS IN A DENGUE ENDEMIC COUNTRY

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Febrile undifferentiated diseases are often treated empirically in arboviral endemic regions due to the lack of available commercial diagnostics for highly diverse and sometimes unknown pathogens. Therapeutic decisions rely on clinical symptoms and sympatric circulation of multiple etiologic agents underscore the need to generate strategies for accurate diagnostics. Madariaga (MADV) and Venezuelan equine encephalitis virus (VEEV) have been associated with severe and sometimes fatal disease. We explored potential symptoms as predictors of alphavirus encephalitis infection. A total of 78 MADV and VEEV cases that occurred between 2010-2019 were compared to 1152 Dengue (DENV) controls. Case-patients and controls were compared based on reported symptoms registered using epidemiological forms. A total of 40 symptoms were collected from alphavirus encephalitis patients. The most common symptoms were fever, myalgias and petechiae. We found no major differences in symptoms between cases and controls. Vomiting (OR 2.9; p=≤0.001, 95% CI 1.7-4.8) and diarrhea (OR 11.9; p=≤0.001, 95%: CI 3.3-40.2) were the only non-specific early symptoms that increase in cases compared with controls. Symptoms associated with advanced clinical progression such as seizures (OR 70.3; p= ≤0.001, 95%: CI 25.8-218.8) and altered mental status (OR 5.2; p=≤0.001, 95% CI 1.43-15.5) were associated with alphavirus encephalitis, while respiratory symptoms (OR 7.8; p=≤0.001, 95%: CI 2.0-25.8), an uncommon symptom were found increased with alphavirus infection. Taken together, our results suggest that alphaviral encephalitis infections remain a diagnostic clinical challenge in dengue-prone regions, because of similarities in acute symptoms and the difficulty of predicting severe outcomes such as encephalitis, which can have significant morbidity and mortality. This underscores the necessity for building diagnostic capacity and lab surveillance in such areas.

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## POST-DISCHARGE DEATHS AMONG SEVERE ACUTE RESPIRATORY INFECTION PATIENTS WITH SARS-COV-2 IN BANGLADESH DURING 2020-2022

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To understand the real burden of SARS-CoV-2-associated deaths among patients with severe acute respiratory infection (SARI), it is crucial to consider post-discharge deaths along with in-hospital deaths. We aimed to describe the characteristics of SARS-CoV-2-infected SARI patients who died at post-discharge in Bangladesh excluding accidental deaths. From March 2020-December 2022, we conducted COVID-19 surveillance among WHO-defined SARI patients at nine tertiary-care hospitals. We tested nasopharyngeal and oropharyngeal swabs for SARS-CoV-2 by rRT-PCR. We collected information on demographics, clinical characteristics and outcome at discharge and 30 days post-discharge. We used Chi-square test to compare post-discharge decedents and survivors. We identified 10,886 SARI patients with a median age: 16 years (IQR:1-50), 63% were male and 12% (1,319) were laboratory-confirmed SARS-CoV-2. Among patients with SARS-CoV-2, 10% (132) died during their hospital stay and 9.5% (112) died within 30 days of post-discharge. Of 112 post-discharge deaths, 4% (5) were discharged as fully recovered, 72% (80) as partially recovered, and 24% (27) were referred to specialized hospitals for further treatment. Adults aged ≥60 years were more likely to die at post-discharge compared to those aged <60 years [22% (74/330) vs. 4% (38/853); p<0.001]. Males were more likely to die at post-discharge compared to females [11% (79/698) vs. 7% (33/485); p=0.009]. Compared to the SARS-CoV-2 infected survivors, a higher proportion of SARS-CoV-2 infected post-discharge death cases had difficulty breathing on admission (94% vs. 77%, p<0.001), received in-hospital oxygen (92% vs. 64% p<0.001) and had at least one co-morbid condition (50% vs. 38%, p=0.017). One in five SARS-CoV-2-associated SARI patients died; of these, half occurred within 30-days post-discharge commonly among males, elderly, and patients with comorbid condition. Thus, post-discharge deaths might be unrecognized contributor to the true SARS-CoV-2 mortality in Bangladesh. Limiting premature discharge among high-risk groups should be considered to reduce these post-discharge deaths.

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## RATS' FEEDING BEHAVIOR AT FRUIT TREES IN BANGLADESH AND IMPLICATIONS FOR PATHOGEN SPILLOVER

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Rats are the reservoir of important zoonotic pathogens like hantavirus, lymphocytic choriomeningitis virus, Salmonella, and agents of rat-bite fever. Food contaminated with rat saliva, feces or urine is a transmission route for these pathogens. Raw date palm sap and fruits dropped on the ground are consumed by humans and animals in Bangladesh, and sap consumption is a known route of Nipah transmission from bats to humans. Here, we describe rats' feeding behavior in date palms and fruit trees (jujube, banana, star fruit, sapodilla, olive, tamarind, fig, guava) in home gardens in Bangladesh captured using motion sensor-activated infrared cameras in two different studies. One study was conducted during the winter of 2011 and 2012, in 137 villages across Bangladesh, and the second in a village in the northern part of Bangladesh, from March 2013 to February 2016. We investigated how rats may contaminate date palm sap and fruits that may be consumed by humans. We recorded a total of 1002 rat visits with 150 nights at date palm trees and 118 nights at fruit trees during the winter season. We used descriptive statistics and a generalized linear model to identify conditions associated with rat visits. Among the 1002 rat visits observed, 831 (84%) were detected at date palm trees, 67 (7%) at jujube trees, 48 (5%) at banana plants, and 35 (4%) at start fruit tree. Camera images showed that rats were licking date palm sap either alone or in pairs or in some cases alongside bats. Rats ate larger fruits in the tree, like star fruit, and took away smaller fruits, like jujube. The longest duration of contact/night was recorded at date palm trees (78 sec) followed by olive trees (37 sec), star fruit trees (23 sec), and jujube trees (17 sec). Our study has some limitations as the cameras recorded the events only in the focused area and we do not have any evidence of rats urinating or defecating either in the sap or on the fruits or dropping fruits on the ground. However, the opportunities for pathogen transmission through shared food deserve additional follow-up to identify if rat-borne pathogens are infecting humans through shared food sources.

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## PREVALENCE AND PREDICTORS OF PERSISTENT SYMPTOMS POST-ACUTE COVID-19 INFECTION AMONG A COHORT OF FRONTLINE HEALTHCARE WORKERS IN BANGLADESH

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Despite evidence of a wide range of persistent symptoms among COVID-19 survivors, commonly known as long COVID, their frequency, clinical spectrum and risk factors are not well characterized. We assessed the prevalence and predictors of long COVID among healthcare workers (HCWs) in Bangladesh. Between July 2021-March 2023, we enrolled a cohort of HCWs from purposively selected hospitals of four divisions across Bangladesh to prospectively record COVID-19 illness. At enrolment, we captured data on HCWs' demographics, co-morbid conditions and COVID-19 illness. The study physician followed the participants biweekly to record any new and persistent symptoms following acute illness. We used the WHO case definition for long COVID (symptoms occurring 3 months from the acute COVID-19 infection and persisting for at least 2 months). We performed a multivariable logistic regression to identify the predictors of long COVID. The analysis included 875 HCWs with lab-confirmed SARS-CoV-2 infection: 30% (261) doctors, 53% (468) nurses, and 17% (146) support staff. The median age of the HCWs was 35 (IQR, 29-44), and 69% (601) were female. Of the 875 HCWs, 462 (53%) reported persistent symptoms, with fatigue being the most common (83%), followed by brain fog (14%), cough (5%), breathing difficulties (4%), and joint pain (4%). HCWs with co-morbidities (aOR 3.39, 95% CI 2.32-4.95; p=0.0001), breathing difficulty during the acute phase (aOR 2.84, 95% CI 1.77-4.55; p=0.0001), and those who required hospitalization during acute infection (aOR 2.25, 95% CI 1.53-3.04; p=0.0001) were more likely to develop persistent symptoms than HCWs without a history of co-morbidities, respiratory symptoms, or hospitalization. Nurses (aOR 1.36, 95% CI 1.01-1.85; p=0.04) were more likely to develop persistent symptoms than doctors. More than half of the HCWs in our cohort experienced long-term symptoms of COVID-19, with a greater risk observed among nurses and those with the co-morbid condition. These findings underscore the pressing need for long-term care and rehabilitation strategies with a standardized guideline to enhance the post-acute recovery of COVID-19 patients.

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## PSYCHIATRIC SEQUELAE AND PSYCHOSOCIAL IMPACT OF LASSA FEVER IN SURVIVORS IN EDO STATE, NIGERIA

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Lassa fever (LF) is a viral haemorrhagic fever with high mortality rates endemic to West Africa. Psychiatric sequelae in survivors of other high consequence infectious diseases have been reported before; in particular, high rates of post-traumatic stress disorder (PTSD) were found in survivors of Ebola virus disease. Yet, there is a paucity of data on the psychological impact of LF. This comparative cross-sectional study aimed to determine the occurrence of psychiatric diagnosis and psychosocial aspects of LF in survivors in Edo State, Nigeria. From May 2022 to date, 123 cases and 51 age- and sex- matched controls were enrolled. The mean age was 33.2 ± 11.4 years, 46% were female. Validated and established psychiatric screening tools were administered to screen for PTSD, depression and anxiety. In case participants scored above cut-offs, further psychiatric evaluation using the Mini Neuropsychiatric Interview (MINI) was conducted to confirm diagnosis. To investigate the psychosocial impact of LF, survivors completed a 15-item Likert scale questionnaire on perceptions related to the infection. There was no statistically significant difference in the frequency of anxiety and depression between survivors and controls. However, a higher rate of PTSD (7%) was found among survivors. All confirmed PTSD cases received counselling, yet most required pharmacotherapy. In the Likert scale, a majority of survivors stated that they were scared of the severity of LF and feared for their life when on the ward. Most respondents reported that they were anxious to infect others and that admission was stressful for both themselves and their families. In addition, 76% of survivors found being admitted with LF to be a financial burden. Our findings suggest that LF causes considerable psychosocial stress, in some cases even leading to clinical diagnosis of PTSD. This is the first study to show the perceptions of LF survivors regarding the impact of the disease on their life. These results indicate the importance of integrating mental health services into the care of LF patients. Moreover, knowledge of the perception of LF will greatly aid in public health interventions.

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## SECONDARY ATTACK RATES AND DETERMINANTS OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 (SARS-COV-2) HOUSEHOLD TRANSMISSION IN PAKISTAN: A CASE-ASCERTAINED PROSPECTIVE, LONGITUDINAL STUDY

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Households are considered ideal settings for studying the transmission dynamics of an infectious disease. A prospective study was conducted, based on the World Health Organization FFX protocol from October 2020 to January 2021. Household contacts of laboratory-confirmed index cases of SARS CoV-2 were followed up for their symptomatic history, nasal swabs for RT-PCR and blood samples for anti-SARS CoV-2 antibodies were collected at enrollment and days 7, 14, and 28. We estimated the secondary attack rate (SAR), effective reproduction number (Re), and determinants of secondary infection among susceptible household contacts using multivariable logistic regression. We enrolled 77 index cases and their 543 contacts. Out of these, 252 contacts were susceptible at the time of enrollment. There were 77 household clusters, out of which, transmission took place in 20 (25.9%) giving rise to 34 cases. The acquired secondary attack rate (SAR) was rate 14.0% (95% CI 9.0-18.0). The average effective reproduction number (Re) was 0.44 (95% CI 0.33-0.60). Reported symptoms of nausea and vomiting (OR, 7.9; 95% CI, 1.4-45.5) and fatigue (OR, 9.3; 95% CI, 3.8-22.7) were associated with SARS-CoV-2 transmission. We observed low SARS-CoV-2 transmission in the backdrop of high seroprevalence among households in Karachi, Pakistan. Symptomatic history influences infection transmission.

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## COMPARISON OF THE PERFORMANCE OF RNA EXTRACTION KITS USED IN THE DIAGNOSIS OF COVID-19 AGAINST THE INHOUSE TRIZOL RNA EXTRACTION METHOD

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The Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiologic agent of the COVID-19 pandemic which has since spread across the globe, placing a burden on society. Measures taken to reduce its spread depend on timely and accurate diagnosis done using various RNA extraction techniques and RT-PCR. Many commercial RNA extraction kits have been manufactured and released globally on the market however, due to growing demand, a shortage in kit supplies could be experienced in several labs. For these reasons, the use of different commercial or in-house protocols for RNA extraction may provide a basis for various labs to choose reliable extraction kits from the available pool and pave the way for seeking alternative procedures to replace commercial kits using common reagents found in a basic molecular biology laboratory. This study aimed to compare the analytical performance of different SARS-CoV-2 RNA extraction kits used in the diagnosis of SARS-CoV-2 in Uganda against the conventional TRIzol extraction method to address the existing knowledge gap. This work retrieved SARS-CoV-2 positive nasopharyngeal swabs from -80°C storage at biobank and Makerere University. SARS-CoV-2 RNA was then extracted from the samples using four commercial kits and the TRIzol method. The study demonstrated variations in the performance of the different kits/methodologies; The TRIzol extraction method generated the highest concentration of RNA; however, its purity was significantly lower than that of the commercial kits. When comparing the CT values, there was no statistically significant difference between TRIzol and the commercial kits except one. This research concludes therefore that although the TRIzol method recovered RNA with relatively lower purity, its performance in diagnosing SARS-CoV-2 using RT-PCR did not differ significantly from the tested commercial kits.

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## CHANGING PATTERN OF DENGUE SEROTYPE IN THE SINDH REGION OF PAKISTAN, 2006-2022

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Dengue virus infection is a major threat to public health in Pakistan (PK). over the past few decades, the risk of infections with severe disease outcomes is increasing with the changing environmental and climatic conditions making PK a hotspot for mosquito breeding the disease has become hyper-endemic, with different serotypes causing epidemic outbreaks with severe clinical presentation. we analyzed the DENV serotypes circulating in the Sindh region of PK between 2006 -2022. Archived serum samples of two Filed surveillance studies conducted in 2006-2011 and 2014-2017 for patients with acute febrile illness along with prospective random selected clinical laboratory samples collection (2020-

2022) of DENV positive samples were used to assess the changing trends. All samples were tested by rapid NS1- antigen and PCR using serotype-specific primers, a significant changing trend of the predominant DENV serotypes in the three cohorts studied. From 2006-11 samples (n=200), DENV-3 was the predominant serotype tested in 60% (n= 57) of the total 94 DENV PCR positive samples followed by DENV-2, 38.3% (n=36) and DENV-4 1.1% (n= 1) we did not find DENV-1. The 2015-17 samples (n=168),34.52% wereDENV-2 followed by DENV-1 5.9%, DENV-3 8.92%, and DENV-4 3.57%. The results of 2020-22 showed changing trend with a progressive predominance of DENV-1 over other serotypes, accounting for 74% of all DENV positive (n=50) samples. Our study confirms that PK is hyperendemic for DENV as all four serotypes are found to be circulating and the temporal trend suggests the fluctuation in the predominant type circulating during the epidemic season. These findings are significant as changing pattern makes the population vulnerable to secondary infections with severe clinical outcomes warrant large-scale genomic surveillance of the DENV to better understand the role of factors responsible for varying trends such as human migration, climatic changes, and virus evolution. such surveillance is also required to better predict and prepare for the outbreaks outcomes as an entry of new serotype in the community after a gap of few years creates a risk for severe secondary infections.

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## SEVERE MORBIDITY AND MORTALITY FROM RIFT VALLEY FEVER DISEASE BETWEEN NOVEMBER 2017 AND MARCH 2020 AMONG HUMANS IN UGANDA

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Rift Valley fever (RVF) is a zoonotic viral disease affecting animals and humans. Over the last two decades the disease has occurred with increased intensity in humans. In Uganda, cases reported prior to 2016 were either mild or under-reported. Here, we report on the morbidity and mortality of human cases identified in Uganda between November 2017 and March 2020. Human cases reported to the Uganda Virus Research Institute (UVRI) were identified and confirmed by polymerase chain reaction (PCR). Ethical and regulatory approvals were obtained to enrol survivors into a one-year follow-up study. Data were collected on patient socio-demographics, medical history, laboratory tests, and potential risk factors, and analysed using Stata software. Forty (40) cases were confirmed in four outbreak clusters that occurred over 29 months in 20 districts. Nearly all confirmed cases were male (39/40; 97.5%), median age 32 (range 11-63). Over three-quarters (31/40; 77.5%) of patients presented to the health care system with fever and bleeding. Twenty-eight (70%) cases were hospitalised, and more than half (21/40; 52.5%) of all confirmed cases died. Mortality was highest among admissions in regional referral (11/16; 68.7%) and district (4/5; 80%) hospitals, patients with bleeding at case detection (17/31; 54.8%), and older than 44 years (9/9; 100%). Survivors presented with mild disease manifesting commonly as gastro-intestinal syndrome with nausea (83.3%), anorexia (75%), vomiting (75%), abdominal pain (50%), and diarrhoea (41.7%). Symptom duration varied between two to 120 days. In conclusion, RVF causes high mortality and prolonged morbidity among humans that present to the health care system and are confirmed positive for RVF by PCR. Interventions should be developed to prevent infections, promptly detect disease outbreaks, and improve patient outcomes. 1

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## KNOWLEDGE, AND PERCEPTIONS OF COVID-19 INFECTION AMONG PEOPLE REPORTING FOR COVID-19 VACCINATION IN HEALTH FACILITIES IN MALAWI

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Knowledge and perceptions of COVID-19 play a significant role in the uptake of preventive measures within communities. Understanding the knowledge and perception of COVID-19 infection among subjects willing to be vaccinated allows planning additional health education campaigns to be included in the routine COVID-19 vaccination. These subjects may act as role models, motivating other community members to take up vaccine and other prevention measures. An ongoing cross-sectional study interviewed 138 adults at the time they received first dose of the COVID-19 vaccine in a peri-urban and urban health facility in Blantyre, Malawi. A score summarized information on four domains of knowledge on COVID-19: symptoms, prevention, transmission and people at risk. This score was dichotomized into low vs. moderate/advanced knowledge. Through logistic regressions, determinants of low vs. moderate/ advanced knowledge were investigated including age, gender, educational level, and employment. We also investigated the source of the subject's information about COVID-19. Only 71 (52%) and 52(37%) had moderate/advanced knowledge on symptoms and prevention of COVID-19, respectively, whilst 125 (91%) had moderate/advanced knowledge on transmission. A total of 52 (37%) understood the highest risk groups. Females were more likely to have moderate/advanced knowledge on prevention than males (odds ratio ([OR] 2.1; P=0.04); subjects > 40 years had higher moderate/advanced knowledge at identifying highest risk groups (OR 3.8; P=0.01). Being a farmer was associated with low knowledge on at risk groups compared to office work/manual unskilled workers (OR 0.4; p value = 0.05). However, poor knowledge and understanding of symptoms and transmission of COVID-19 were uniformly spread in the population. Radio stations were the major sources of COVID-19 information (80% subjects). Our results suggest that after three years of the start of pandemic, information about COVID-19 has not been widely disseminated in Malawi. This may be negatively impacting uptake of preventive measures. There is a need to integrate COVID-19 health education in routine vaccination.

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## A SIMULATION-BASED METHOD TO INFORM SEROSURVEY DESIGN TO ESTIMATE DENGUE FORCE OF INFECTION USING EXISTING BLOOD SAMPLES

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Limited knowledge exists on the extent of dengue virus circulation in Africa. Cross-sectional serological surveys provide the ideal data to investigate historical dengue transmission, to reconstruct the age-dependent immunity profile of a population, and to estimate transmission intensity, as measured by the force of infection (FOI), the per-capita risk of infection for

susceptible subjects. However, owing to limitations in capacity for arboviral disease surveillance, according to the published literature, only 17 dengue serosurveys have been conducted in the African region so far. A convenient strategy to overcome this limitation is to retarget existing blood biobanks, e.g., from previous serosurveys, for dengue surveillance by secondary testing for anti-dengue antibodies. Here we present a new simulation-based method developed to identify both the optimal number and age-distribution of samples required to obtain informative FOI estimates through secondary testing of existing blood samples. We discuss its application to the sample sizes previously collected during a SARS-CoV-2 serological survey conducted in Ghana, and show that the method is effective in reducing sample sizes required for testing without affecting the accuracy of the FOI estimates.This study highlights how existing blood samples from cross-sectional serosurveys can be leveraged for dengue surveillance and provides a framework for generating new immunological data to understand dengue historical circulation while optimising resources. The methods developed in this study can be adopted to investigate multiple viruses and diseases in different transmission settings around the world.

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## LASSA FEVER OUTBREAK IN GHANAIAN COMMUNITIES, FEBRUARY 2023

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Lassa fever (LF) is an acute viral haemorrhagic illness, endemic in rodent populations in parts of West Africa such as Benin, Ghana, Guinea, Liberia, Mali, Sierra Leone, Togo, and Nigeria with case fatality of 15% among patients hospitalized with severe clinical manifestations. Infection can occur by exposure to food or household objects contaminated with the urine or faeces of infected Mastomys rats; person-to-person infections and laboratory transmission can also occur. On February 24th, 2023, the Noguchi Memorial Institute for Medical Research (NMIMR) confirmed two cases of LF and alerted the Ghana Health Service. As of February 28th, 2023, there were 14 confirmed cases, with one fatality. The other cases were contacts of the index cases, and Ghana had previously reported an epidemic in 2011. Korle-Bu Teaching Hospital submitted two probable viral haemorrhagic fever (VHF) patients, 33- and 40-year-old women with symptoms indicative of LF, including fever, vomiting, diarrhoea, abdominal pains, difficulty in breathing and bleeding from the eyes and nose.
The samples were submitted to viral RNA extraction, purification, and amplification using molecular testing methods for recognized endemic VHFs such as LF, Ebola, Yellow Fever, Dengue, and Marburg virus. The molecular testing methods employed yielded presumptive positive findings for LF. The initial close relationships in 12 other cases were confirmed as well as 156 contacts were observed for the stipulated period of 21 days. The number of confirmed patients for this epidemic is 26 with one fatality as of March 20th, 2023, with 512 samples received. All 25 patients are alive and well and were treated and subsequently discharged in authorized health institutions. In conclusion, there is a need to continuously strengthen public health systems to ensure effective and timely responses to outbreak situations or future emerging pathogens. The VHF testing capacity established by NMIMR as well as the GHS public health disease control unit has proven to be active and sensitive to detecting emerging pathogens for public health response.

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## CLINICAL EVIDENCE ON DISEASE BURDEN OF THE MOSQUITO-BORNE CHIKUNGUNYA VIRUS (CHIKV) : A SYSTEMATIC LITERATURE REVIEW

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Chikungunya virus (CHIKV) is a re-emerging arbovirus that causes an infection characterized by an acute phase frequently including severe polyarthralgia, myalgia, and fever, which can progress to chronic sequelae and consequently result in productivity losses and a significant decline in health-related quality-of-life (HRQoL). To investigate the available clinical evidence on the disease burden associated with CHIKV, we conducted a systematic literature review (SLR). The SLR of clinical evidence was performed in Medline and Embase databases (no date restriction) and congress abstract repositories (2019-2021). The search adhered to Preferred Reporting Item for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Acute and chronic polyarthralgia and myalgia, two of the most prevalent CHIKV infection symptoms, have been identified as severely impairing the physical capacity of the patients and further affecting their daily functioning and HRQoL. Since the patients experience severe pain symptoms, their mental health is also significantly impacted, which often results in several psychological disorders. A chronic CHIKV infection may lead to long-term disability due to multiple long-term sequelae associated with a reduction in HRQoL. The low number of identified interventional studies (i.e., 17) displays the shortage of available interventions for the treatment of CHIKV infection. CHIKV is a serious threat to global public health and causes considerable disease burden worldwide. The rapid geographic expansion of CHIKV outbreaks and distribution is a result of a combination of travel, CHIKV mutations, global warming, and the spread of the mosquito vector. Currently, no antiviral treatment exists for chikungunya, and vector control is suboptimal and challenging. Due to the increase in the global spread of CHIKV and the paucity of treatment options for chikungunya, an effective preventive measure such as a vaccine is urgently needed.

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## MONKEYPOX VIRUS OUTBREAK IN GHANA

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Monkeypox viral infection is a zoonotic disease caused by the Monkeypox virus from the family Poxviridae. It is usually a self-limiting disease with its clinical presentation like that of the smallpox virus. Reservoirs for the Monkeypox virus is unknown however, some animals that are susceptible to this virus include tree squirrels, and non-human primates. Since 1970 when the first human Monkeypox virus was detected, 11 African countries have reported cases of the virus with Nigeria recording the largest outbreak of 200 confirmed cases in 2017.The 2022-2023 Mpox outbreak in Ghana is a part of the larger outbreak of human Mpox caused by the West African clade of the MPXV. Ghana, as compared to its neighbors including Nigeria, had hitherto no endemic presence or active search and diagnosis for Mpox, until the 2022 outbreak. The first 5 cases of Mpox in Ghana were detected on 8th June 2022 and as of 20th March 2023, 569 suspected cases have been investigated. Here we sought to establish the molecular evidence and elucidate on the characterized circulating strains of MPXV of the cases investigated. Viral DNA was processed from lesion exudate or crust on a swab and/or serum specimens of patients identified by clinicians to have met the case definition of an initial febrile prodrome accompanied by headache, and fatigue prior to rash development. Polymerase chain reaction was then performed at the NMIMR to amplify the viral DNA. As of 2March 2023 a total of 569 suspected MPXV cases have been received from health facilities across the country and tested. Of the total, 65 (11.4%) have been confirmed to be MPXV cases whilst 87 (15.3%) were other orthopoxviruses. Nearly 60% of all confirmed cases were children under 15 years old and 3 fatalities have been recorded. Sequenced and characterized MPXV positives were found to belong to the West African clade. This work documents molecular evidence of monkeypox virus circulation in Ghana. It is worth to consider vaccinating affected areas with vaccinia (smallpox vaccine), and to intensify education on good personal hygiene as well as heightened surveillance for monkeypox and other orthopoxviruses in the country.

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## NEUTRALIZING ANTIBODY TITER AFTER COMPLETE SARS-COV-2 VACCINATION

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Vaccination against SARS-CoV-2 is a priority and fundamental strategy to prevent the spread of this disease and avoid the collapse of the health system. The recommended vaccination schedule for this vaccine is 2 doses with an interval of at least 21 days between them. Neutralizing antibodies are considered a good marker for measuring humoral responses. To evaluate the humoral response following COVID-19 vaccination by measuring the antibody titer against the receptor binding domain (RBD) of the spike (S) protein. We conducted an observational study in volunteer health care personnel who had received two doses of vaccines. We detected binding antibodies against SARS-CoV-2 by chemiluminescence immunoassay test (CLIA) for quantitative determination of specific IgG antibodies against the trimeric spike protein. We log-transformed the amount of SARS-CoV-2 IgG. We performed ANOVA tests to evaluate by months of follow-up (every 30 days) and linear regression between elapsed days with antibody titers. We considered a p-value less than 0.05 as significant. RESULTS: A total of 224 persons who completed the vaccination schedule (2 doses) were evaluated. Every 10 days that elapsed since the first vaccine dose, the coefficient of difference of the Log difference of the IgG titer of binding antibody decreased on average 0.25 log(IgG) (p-value<0.001). Patients started with an average SARS-CoV-2 binding antibody titer at 9.33(±1.5) log(IgG SARS-CoV-2) during the first 30 days, and at the end of follow-up (days 150-180) on average had 5.12(±1.1) log(IgG SARS-CoV-2). A history of COVID-19 resulted in increased antibody titers during the 61-180 day post-vaccination period relative to those without a history of COVID-19 (p<0.001). There was no difference in mean log(IgG SARS-CoV-2) according to sex (p=0.0.274). In conclusion, antibody production against SARS-CoV-2 virus has shown to have good humoral response the first 30 days, but has been decreasing up to 54% in the sixth month post-vaccination. The COVID-19 background sustained SARS-CoV-2 IgG antibody levels above those who had not been infected.

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## LOW SEROPREVALENCE OF EBOLA VIRUS IN HEALTH CARE PROVIDERS IN AN ENDEMIC REGION (TSHUAPA PROVINCE) OF THE DEMOCRATIC REPUBLIC OF THE CONGO

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A serosurvey among health care providers (HCPs) and frontliners of an area previously affected by Ebola virus disease (EVD) in Boende, Democratic Republic of the Congo (DRC) was conducted to assess the seroreactivity to Ebola virus antigens. Serum samples were collected in a cohort of HCPs and frontliners (n=698) participating in the EBL2007 Ebola vaccine trial (December 2019 to October 2022). Specimens seroreactive for EBOV were confirmed using either the Filovirus Animal Nonclinical Group (FANG) ELISA or a Luminex multiplex assay. The seroreactivity to at least two EBOV-Mayinga (m) antigens was found in 10 (1.4%: 95% CI, 0.7-2.6) samples for GP-EBOV-m + VP40-EBOV-m, and 2 (0.3%: 95% CI, 0.0 - 1.0) samples for VP40-EBOV-m + NP-EBOV-m using the Luminex assay. Seroreactivity to GP-EBOV-Kikwit (k) was observed in 59 (8.5%: 95%CI, 6.5-10.9) samples using FANG ELISA. In contrast to previous serosurveys, a low seroprevalence was found in these HCPs and frontline population. The use of various tests, of different seroprevalence cut-offs, and of distinct geographical populations with different risk of exposure to EBOV, would contribute to this difference. This underscores the high need for standardized antibody assays and cutoffs in EBOV serosurveys to circumvent the wide variability in reported EBOV seroprevalence rates in EBOV endemic areas. Furthermore, it is unclear whether based on lessons learned from previous EVD outbreaks, the majority of HCPs and frontliners participants in the EBL2007 Ebola vaccine trial would have adequately adopted the EVD prevention key messages or were practicing good EVD prevention. This would have kept them safe, free of EBOV exposure and then explain the low seroprevalence. Similarly, other filovirus infections that can generate cross-reacting antibodies not investigated in this serosurvey may account for this low seroprevalence.

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## MOLECULAR CHARACTERIZATION OF SARS-COV-2 IN HEALTHCARE PERSONNEL WITH THIRD GENERATION SEQUENCING IN LIMA, PERU, 2021-2022

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Genomic surveillance of SARS-CoV-2 variants has enabled the study of the emergence of new viral mutations during this pandemic. It is thought that mutations are emerging from infected persons with more than 14 days of viral shedding. We did a molecular characterization of SARS-CoV-2 virus detected in workers of a health facility that had a nasopharyngeal or nasal sample taken weekly until it became RT-PCR negative. RT-PCR positive samples obtained from 2021 and 2022, stored at -80°C, were sequenced in the MinION Mk1C device from Oxford Nanopore Technologies. Extracted viral RNA was processed following the PCR tiling of SARS-CoV-2 virus with rapid barcoding and Midnight RT-PCR Expansion (SQK-RBK110.96 and EXP-MRT001) protocol. 78 samples from 50 workers were processed for sequencing. Samples from 44 workers (88%) were able to be sequenced. All samples from 2021 were Lambda 21G (C.37) SARS-CoV-2 variant. In 2022, all SARS-CoV-2 belonged to the Omicron variant from different sub-lineages, mainly BA.2 (20%), BA.1.1 (17%), BA.1 (15%), BA.5 (13%) and BA.5.1 (10%). Samples from 10 workers with SARS-CoV-2 positivity over 1 week were processed; however, we only were able to sequence the first samples during the first week of episode from 8 workers and samples from both the first and second week of only 2 workers. All others with samples up to 7 weeks positive for SARS-CoV-2, were unable to be sequenced. Bioinformatic analysis of the 2 workers with sequenced samples for week 1 and 2 showed identical variants. These results suggest that prolonged RT-PCR positivity for SARS-CoV-2 may not represent viable viruses. We concluded that the identified SARS-CoV-2 variants detected in 2021 and 2022 were similar to those detected elsewhere, and that in this small series of cases in healthy individuals, prolonged positivity by RT-PCR may not represent viable viruses with potential to generate new variants. These results require confirmation in larger studies.

5992

## ENTERIC VIRAL PATHOGENS AND CHILD GROWTH: INSIGHTS FROM SOUTH ASIA AND SUB-SAHARAN AFRICA

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Enteric viral pathogens such as rotavirus, norovirus, adenovirus, astrovirus, and sapovirus are associated with a significant burden of childhood morbidity and mortality. We investigated the relationship between viral

pathogens and child anthropometric outcomes among under-5 children in South Asia and sub-Saharan Africa. We analyzed data from 5,572 children enrolled in the Global Enteric Multicenter Study (GEMS) across seven study sites between December 2007 and March 2011. Viral pathogens: rotavirus and adenovirus were detected using stool immunoassays (ELISA) and norovirus, astrovirus, and sapovirus by RT-PCR. Multiple linear regression was used to examine the association between the viral pathogens and length/height-for-age (HAZ), weight-for-age (WAZ), and weight-for-length/height (WHZ) z-scores, stratified by diarrheal symptoms and adjusted for potential covariates. Rotavirus (18.51%) and norovirus (7.33%) were the most prevalent pathogens among symptomatic and asymptomatic under 5 children, respectively. Among asymptomatic children, viral pathogens were associated with lower WAZ: rotavirus (β=-0.09; 95% CI: -0.17, -0.01), norovirus (β=-0.12; 95% CI: -0.18, -0.06), adenovirus (β=-0.28; 95% CI: -0.47, -0.10), and sapovirus (β=-0.14; 95% CI: -0.23, -0.06). Among the symptomatic children rotavirus (HAZ:β=0.12; 95%CI:0.07,0.17 and WAZ:β=0.05; 95% CI:0,0.11), norovirus (HAZ:β=0.08; 95% CI: 0.01,0.15 and WAZ:β=0.14; 95% CI: 0.06,0.21), and sapovirus (WAZ:β=0.13; 95% CI:0.02,0.24) were associated with higher HAZ and WAZ, but astrovirus was associated with lower HAZ (β=-0.16; 95% CI: -0.28, -0.04). While previous studies hypothesized that several viral pathogens had a controversial role in child growth, our findings indicate that enteric viral pathogens are associated with growth shortfalls among asymptomatic children. This highlights the need for preventive strategies targeting enteric viral pathogens among asymptomatic young children, which could potentially reduce the burden of childhood growth faltering.

**5993**

## PILOT SURVEILLANCE EVALUATION USING LEFTOVER MEASLES/RUBELLA NEGATIVE SURVEILLANCE SPECIMENS TO DETECT ARBOVIRUS INFECTIONS

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Dengue, chikungunya, and Zika virus infections remain a threat in areas where the Aedes (Stegomyia) mosquito vectors are established. Limited arbovirus surveillance results in underestimates of disease burden and delayed recognition of transmission activity. WHO recommends an integrated public health approach to Aedes-borne arboviruses. A proposed strategy for case detection in some regions is arbovirus testing of suspect measles and rubella cases, as patients present with clinically similar febrile rash illness and blood specimens are routinely collected. We describe the evaluation of a pilot sentinel surveillance strategy using this approach for infection detection and feasibility of implementation. Between 2019 and 2022, the national public health laboratories of Burkina Faso, Indonesia, Myanmar, and the Philippines tested stored specimens for measles and rubella testing from the preceding 1-12 years for evidence of acute dengue, chikungunya, and Zika infections using a validated RT-PCR molecular assay and IgM-capture enzyme-linked immunosorbent assays. Where feasible, IgM-positive specimens were transferred to the Uganda Virus Research Institute and the National Environment Agency Singapore, respectively, for further testing by repeat IgM and plaque reduction neutralization tests. In total, 8,648 leftover measles/rubella surveillance specimens were tested. Molecular testing detected either chikungunya or dengue RNA in 330 (4%) of all specimens and in three of four countries. All countries detected IgM antibodies to all three viruses, with overall seropositivity of 13% to at least one virus. Confirmatory testing demonstrated the continued challenges of flavivirus serologic cross-reactivity in endemic countries. Arbovirus testing of measles-rubella surveillance specimens leverages a widespread, robust collection of samples from patients with clinically compatible illness. However, the resources required to yield accurate results suggest that this approach should be targeted to specific objectives and sentinel locations for optimal utility.

**5994**

## ESTIMATING THE INCIDENCE OF DENGUE IN INTERNATIONAL TRAVELERS FROM NON-ENDEMIC COUNTRIES

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We estimated the risk of dengue infection among travelers from non-endemic countries to inform public health preparedness, especially in non-endemic countries with suitable vectors where infected travelers can spark outbreaks. We used data from 2010-19 on ~30,000 traveler cases that were reported to national authorities in 21 non-endemic countries, hazards of infection in 102 endemic countries based on local seroprevalence studies, and the numbers of flights and trip durations between countries. From these, we estimated the total numbers of infections in travelers (i.e. regardless of symptoms) and the fractions of infections reported in travelers' home countries. We tested two simplifying assumptions: assuming time-constant country-specific reporting rates, and assuming the reporting rate was determined by endemic and non-endemic terms only (i.e. no interaction). We analyzed data using Bayesian Markov Chain Monte Carlo and used the leave-one-out information criteria to compare models. From 2010-19, nearly 30,000 traveler cases with known country of infection were reported in the 21 non-endemic countries. Australia, USA, and Germany reported the highest case numbers. Indonesia, Thailand, and the Philippines were the destinations giving rise to the most cases. The model assuming time-varying reporting and no interaction performed best. Estimated reporting rates varied across country pairs (median 1.7%, range 0.07% to 38.2%). We estimated that an average of 170,800 annual dengue infections occurred among travelers from these 21 countries, with most occurring among travelers from the USA (85,100), Japan (20,400), and South Korea (16,300). Thailand (18,700), Brazil (15,300), and India (14,300) were the destinations with the highest numbers of traveler infections. In conclusion, this study shows that reported dengue cases among travelers represent a small fraction of infections in this group and that this fraction varies greatly across countries. These estimates can guide public health preparedness, including quantifying the benefit of dengue vaccination in travelers.

**5995**

## ARBOVIRUS DISEASE SURVEILLANCE AMONG FEBRILE PATIENTS IN KILIMANJARO, TANZANIA, 2016-2019

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Arthropod-borne flaviviruses and alphaviruses are emerging infectious diseases and causes of febrile illness in East Africa. A prospective cohort study enrolled febrile patients admitted to 2 referral hospitals in Moshi, Tanzania, September 2016 through May 2019. Acute serum was obtained at enrollment, and convalescent serum 28-42 days later.

Convalescent serum underwent antibody screening by ELISA for the following arboviruses: dengue (DENV), Zika (ZIKV), West Nile (WNV), yellow fever (YFV), Sindbis, o'nyong-nyong (ONNV), and chikungunya (CHIKV). Those with positive, uninterpretable, or equivocal screens underwent plaque reduction neutralization testing (PRNT) on acute and convalescent sera. PRNT results were expressed as the reciprocal of the serum dilution yielding >80% reduction in the number of plaques (PRNT80). Acute arboviral disease (ABD) was defined as having ≥4-fold increase from acute to convalescent PRNT80 titer with an end-titer PRNT80 >40 and ≥4-fold greater than other viruses in the same genus. Prior infection or vaccination (against arboviruses with available vaccines) was defined as PRNT80 titer >10 without a ≥4-fold rise in convalescent titer. Both for acute and prior infections, if there was not an end titer 4-fold higher for one species compared to others within the same genus, this was designated at the genus level (e.g., flavivirus disease). Of 1,132 patients enrolled, 430 (38.0%) were screened for ABD by convalescent ELISA and 110 reflexed to PRNT80. Eleven (2.6%) participants had ABD: 1 CHIKV, 4 DENV, 2 ZIKV, and 4 flavivirus. Twenty-five (5.8%) participants had evidence of prior infection or vaccination: 1 alphavirus, 7 flavivirus, 3 DENV, 2 ONNV, 7 WNV, 1 ZIKV, and 4 YFV. DENV was the most frequent acute ABD and WNV the most frequent prior infection. YFV seropositivity likely reflected prior vaccine exposure. While we found acute ABD to be a relatively uncommon cause of febrile illness, these serologic case detections suggest circulation or arrival of flaviviruses and alphaviruses of public health significance in northern Tanzania.

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## ACCEPTANCE AND HESITANCY TOWARDS COVID-19 VACCINE AMONG HEALTHCARE WORKERS IN BUKAVU, EASTERN DEMOCRATIC REPUBLIC OF CONGO

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The Democratic Republic of Congo (DRC) is falling behind the global COVID-19 vaccination effort in achieving herd immunity against SARS-CoV-2 infection due to vaccine hesitancy, which poses a threat to global health and hinders the implementation of a successful vaccination program. Healthcare workers in the DRC were highly hesitant towards the COVID-19 vaccine even before its deployment, further complicating efforts to prevent and contain the pandemic. This study aimed to identify the factors associated with COVID-19 vaccine hesitancy among DRC's healthcare workers one year after the availability of the vaccine. From March 1st to March 31st, 2022, we conducted face-to-face interviews and web-based surveys using the WHO's modified Behavioral and Social Drivers of Vaccination instrument among healthcare workers in Bukavu, eastern DRC. Logistic regression models were built to identify predictors of vaccine hesitancy, defined as uncertainty or intention to refuse an available COVID-19 vaccine. Of the 380 participants, with a mean age of 35.0 (28.0-48.0) years and 198 (52.1%) males, 266 (70%) were hesitant to accept the COVID-19 vaccine, while only 29 (7.6%) were willing to accept it. A majority of participants (63.9%) believed that COVID-19 vaccines were not safe, and 77.9% and 81.6% distrusted central and local government authorities, respectively, in their ability to deliver a safer vaccine. While 42.4% trusted in the science behind the vaccine development, distrust in the central government (aOR=3.543, IC1.351-9.289), distrust in the science that proposed the COVID-19 vaccine (aOR=4.360, IC 1.351-9.289), perceiving that COVID-19 vaccines available are not safe (aOR=4.330 IC 1.854-10.111), and lack of knowledge of COVID-19 vaccination locations (aOR= 10,87, IC 3.785-31.246) were the main factors associated with vaccine hesitancy in an adjusted model. The study found a high and persistent prevalence of COVID-19 vaccine hesitancy among Congolese healthcare workers. Strategies to increase vaccine uptake need to include interventions that address trust in vaccine safety and public authorities.

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## SOURCES OF INCONSISTENCIES BETWEEN DENGUE INFECTION INTENSITIES ESTIMATED FROM SEROLOGICAL AND PASSIVE CASE SURVEILLANCE STUDIES

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Force of Infection (FOI) is an important metric when designing and implementing interventions. Ideally, FOI would be calculated using serologic data from highly powered prospective cohort studies, directly measuring rates at which naive individuals seroconvert to the pathogen of interest. However, because this approach is resource intensive, FOI is typically inferred from mathematical models applied to cross-sectional seroprevalence or passive surveillance case datasets. Consistency of FOI estimates from these different approaches remains poorly understood. Here, we use data from a series of cohort studies in Kamphaeng Phet, Thailand (1998-2016: total 10159 individuals, 59801 bleeds with hemagglutination inhibition antibody measurements to all four dengue serotypes) and case data from the provincial hospital (1994-2019, n=12222). Considering the cohorts as both longitudinal measures (multiple samples per individual) and cross-sectional data (single sample per individual), we estimated the annual FOI between 1998 and 2018 using standard models for each data type. We found strong incongruence between the estimates with seroincidence-derived FOI being systematically higher than estimates from case data, and seroprevalence-derived FOI being systematically lower. To uncover sources of these inconsistencies, we performed simulations to study effects of commonly violated model assumptions on the inferred FOI. We found that noise in the assay alone leads to extreme inflation of FOI inferred from seroincidence data and dampened FOI inferred from seroprevalence data. While waning of monotypic antibody titers alone does not affect seroincidence-derived FOI, it can further exacerbate the inflation of FOI in noisy assays. Non-independence between observations and non-uniform infection risk in age affects the FOI estimates from each data type differently in non-trivial ways. Finally, we developed methods to correct for these biases and describe the extent at which these corrections can be done in empirical settings to guide future inferences and interpretations of empirical data.

5998

## CIRCULATING NON-DENGUE FLAVIVIRUSES IMPACT DENGUE VIRUS DIAGNOSTIC TESTING AND DISEASE RISK IN CAMBODIA

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Mosquito-borne viruses are a growing global public health threat. Some areas have co-circulating flaviviruses that induce cross-reactive antibodies, which can increase risk of disease caused by dengue virus (DENV) but could also provide partial cross-protection. Cambodia is highly endemic for dengue virus (DENV), and there is serological evidence of West Nile virus (WNV) and Japanese encephalitis virus (JEV) in birds. Pre-existing immunity to JEV, WNV, and Zika virus (ZIKV) may impact DENV testing results and disease risk. Healthy children (n=771) aged 2-9 years living in Chbar Mon, Kampong Speu, Cambodia were enrolled in a prospective cohort study between July and August 2018 and followed for three years. DENV antibodies were measured bi-annually and with each febrile illness by an indirect dengue IgG ELISA (PanBio; Brisbane, QLD, Australia) and focus reduction neutralization test (FRNTs) to detect neutralizing antibodies (nAbs). The ELISA had a high false positive rate (13%) and specificity of 82%, which was lower than the 90-100% reported by others, including the manufacturer. Of 100 children ("discordants") with high DENV ELISA titer (>1.1) and low DENV nAbs (FRNT≤40), half had DENV nAbs levels below 10. After adjusting for age and sex, discordants had a higher odds of going on to experience a DENV infection compared to naïve (ELISA≤1.1 and FRNT≤40) children (OR 1.96, 95% CI 1.24-3.11). Interestingly, their infections were more likely to be inapparent (OR 3.95 [1.39-11.32]). To evaluate the high DENV ELISA false positive rate, we tested for nAbs against other flaviviruses in DENV naïves and discordants. ZIKV nAbs were present in 10% discordants vs 4% naïves (p = ns). JEV nAbs were also identified in discordants and these, along with WNV nAbs, will be compared with the naïve group. We found that immunity to other circulating flaviviruses altered the specificity of dengue testing and disease risk in Cambodian children. Investigators and clinicians in dengue endemic areas should consider co-circulating flaviviruses when testing children.

**5999**

## FORECASTING DENGUE INCIDENCE: REVIEW OF METHODOLOGY AND COVARIATES

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Globally, there is an increase in dengue morbidity and mortality, resulting in a significant burden on health systems. Having accurate predictions for future dengue incidence is important to inform public health response and guide interventions. This study summarizes methodologies and covariates used to forecast dengue cases over time. Embase, Global Health, Medline, and Web of Science databases were reviewed using keywords related to dengue and forecasting. Only peer-reviewed articles in English, which predicted any aspect of human dengue transmission including case counts and incidence, used any methodology and conducted out-of-sample validation such that time-series nature of data was preserved were included. No geographical or time limitations were imposed. Following de-deduplication, 5,412 records were identified. Two reviewers reviewed each title and abstract and screened full text of articles, identifying 206 studies for inclusion. A further 17 studies were identified through search of references of previous reviews related to this topic. Data was extracted on study setting, data source, spatial and temporal scale of prediction, forecasting models, variables tested and variables included in final models, and evaluation methodology. Models identified were machine learning and statistical models, followed by mathematical models. Most common covariates identified included temperature and humidity. Review of this literature will inform current and future forecasting efforts and ensures public health responses incorporate latest methodological advances appropriate for context.

**6000**

## COMPARISON OF REPORTED PRIOR DENGUE INFECTION WITH LABORATORY-CONFIRMATION OF SEROSTATUS AMONG 9 TO 14-YEAR-OLD CHILDREN IN CEBU, PHILIPPINES

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Dengue is an acute febrile illness that is an important public health problem in tropical Asia and Latin America. There are four dengue virus serotypes (DENV-1 to 4); infection produces durable homotypic immunity against the same serotype but waning cross-protection against the other serotypes resulting in repeat dengue infections. We aimed to assess the association between laboratory-confirmed presence of dengue antibodies with the reported history of prior dengue infection. In 2017, we enrolled 2,996 children who were 9- to 14-years of age and eligible to participate in a government dengue vaccination program in Cebu, Philippines. We collected a baseline blood sample from each participant, which was assessed for dengue antibodies by indirect ELISA and focus reduction neutralization test (FRNT). 1790/2996 children received a dose of CYD-TDV during the mass vaccination in July 2017. After an additional safety concern of CYD-TDV was announced, the dengue vaccination program in the Philippines was discontinued. We actively monitored the children in the cohort for febrile illness from November 2017 to October 2022. Data on past history of dengue illness was collected using a brief questionnaire when a child in the cohort had a febrile illness. 674/2996 (22.5%) children had at least one febrile episode detected during the five-year period. Of the 674 children, 458 (68%) received a single dose of CYD-TDV. The majority (593/674 or 88.7%) were dengue seropositive at baseline: 512/593 or 76% with a multitypic profile and 81/593 or 12% with a monotypic response. Of the 593 children who were seropositive at baseline, 520 (87.7%) did not recall previous dengue illness. In 2018, the World Health Organization has recommended a pre-vaccination screening strategy for the dengue vaccine, CYD-TDV, whereby the vaccine should only be given to those who are dengue seropositive. We found that recall of previous dengue illness is unreliable in establishing dengue serostatus in our setting.

**6001**

## DETECTION OF OTHER HUMAN CORONAVIRUSES (HCOVS) AND CROSS- REACTIVITY AGAINST SARS-COV-2 IN CLINICAL SAMPLES

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The emergence of SARS-CoV-2 ignited a pandemic with reported 633 million cases and over 6.6 million deaths. There is evidence of disparity between reported SARS-CoV-2 infections and the actual infections in African populations. These have been attributed to many factors such as lack of accurate data on the rates of infections and death. As compared to other populations however, lower morbidity and mortality rates have been reported in Africa. Some studies have attributed it to possible immune protection conferred by viral and parasitic infections in the Ghanaian population. This study set out to determine cross-reactivity of SARS-CoV-2 in clinical samples collected before and during the COVID-19 pandemic and investigate the presence of other human coronaviruses (HCoVs) circulating within the Ghanaian population. Naso-pharyngeal swabs were collected from 999 consenting individuals aged ≥5 years for the detection of SARS-

CoV-2 and the other human coronaviruses (HKU1, OC43, NL63, 229E and SARS) infections by RT-qPCR. Preliminary results show that overall positivity for SARS-CoV-2 was 20% (200/999) and other HCoVs was 23.8% (238/999). SARS accounted for 60% (143/238) of the HCoV positives while, OC43 made up 9% (23/238) of positives. One sample (0.42%;1/238) was positive for four HCoVs and another (0.42%;1/238) was positive for all 5 HCoVs. Males were observed to be 1.47 times more likely to be positive for SARS-CoV-2 [95% CI: 0.98 – 2.20, p-value=0.06] as compared to females. Living in an urban area gives a higher risk of being positive for all HCoVs and SARS-CoV-2 [aOR: 1.67, 95% CI: 0.97 – 2.66, p-value=0.07] [aOR: 2.49, 95% CI: 1.49 – 4.14, p-value<0.001] respectively. Positive HCoV participants were approximately 4 times more positive for SARS-CoV-2 [95% CI: 2.51 - 5.68, p-value<0.001]. The co-presence of HCoV and SARS-CoV-2 may be a possible indicator of cross immune protection. This may play a role in the high numbers of asymptomatic SARS-CoV-2 infections during the peak of the pandemic in Ghana.

**6002**

## PREVENTION AND CONTROL OF VIRAL HEMORRHAGIC FEVER IN LEARNING INSTITUTIONS IN UGANDA

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Uganda is located in East Africa. On the 22nd September, 2022 an outbreak of Ebola Virus Disease was confirmed in Mubende District located in the Central region of the country West of the capital city Kampala. Uganda has approximately 15 million learners concentrated in close to 50,688 education institutions with an average of about 1,000 learners or more in a learning institution. Many schools are high volume congregate settings and are mixed Day and Boarding posing an increased risk of infection transmission. To control spread interventions in learning institutions were jointly carried with the key line ministries and partners. The multi sectoral committee supported the different pillars with the National School Health Task force as a sub -pillar for the coordination of the activities including development of guiding documents. Ebola Virus Disease surveillance was built on the existing COVID-19 school-based surveillance system. Risk Communication team played a key role in the development of Information Education and Communication materials for the general public and school settings. Confirmed cases were evacuated to the Ebola Treatment Unit. Arrangements were made for continuity of learning by the school. Contact listing of the close playmates and classmates were isolated from home or a designated place with daily monitoring of symptoms for a period of 21 days. Infection prevention measures at the school together with daily fever screening. By 14 October 2022, 58 cases had been confirmed among which was a Primary school pupil from Mubende Municipal Council. By 23 October2022, a total of 28 cumulative cases of EVD were registered amongst persons aged 0-19 years, of these 19 were attending school in the three districts of Kampala, Mubende and Kassanda that had been greatly affected. Of these seven died resulting in a case fatality rate of 37%. A total of about 10 learning institutions were affected during the outbreak. The prevention and control of EVD was made possible through strict compliance to the standard operating procedure, political commitment, continuous monitoring and support supervision by the different administrative levels.

**6003**

## A COHORT STUDY IN GHANA REVEALS HIGH SEROPREVALENCE OF MONKEYPOX IN GHANA

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Monkeypox virus (MPXV), an orthopox virus endemic to West and western Central Africa, is the etiological agent of Monkeypox (Mpox) disease. While the recent global outbreak has been fuelled primarily through human-to-human contact, primary transmission in endemic areas is most likely through contact with reservoir host species with secondary transmission to caregivers and close relatives. Prior to the year 2022, Ghana had not reported any human cases of Mpox, however, the 2003 Mpox outbreak in the US was initiated by rodents that were exported from Ghana. This preliminary study was done to determine the seroprevalence of Mpox after about 40 years of global eradication and discontinuation of smallpox vaccination in Ghana. Direct ELISA was conducted on a total of 1507 archived sera collected prior to 2022 from the 16 regions of Ghana and 281 samples collected from clinical patients and their contacts to detect the presence of anti-monkeypox IgG antibodies. The overall seroprevalence of the mpox virus estimated by the study was 29% (526/1788). People living in rural communities were also found to be 0.62 times [95% CI:0.47 – 0.81, p=0.001] more likely to be exposed to the virus which explains the seroprevalence of participants from the Upper East region having a 71% (12/17). Participants younger than 35 years accounted for 66% (1185/1788) of the samples tested and were considered to be unvaccinated with the smallpox vaccine. The likely odds of monkeypox exposure in participants aged >19years, between 20-29 years and 30-39 years were higher compared to other age groups (aOR 1.89: 95% CI 1.23 – 2.91, p=0.004), (aOR 2.04: 95% CI 1.30 – 3.21, p=0.002) and (aOR 1.89: 95% CI 1.19 – 3.01, p=0.007) respectively. The study results indicate a fairly high seroprevalence suggesting existing MPXV circulation, especially among unvaccinated people living in the rural part of Ghana.

**6004**

## SHIFTS IN THE SEASONALITY OF DENGUE ASSOCIATED WITH THE TRANSITION TO ENDEMICITY

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The incidence of many pathogens in humans is affected by seasonality due to climate factors. However, the timing of peak incidence of emerging pathogens often differs from post-emergence seasonal patterns. This study examines the seasonality of dengue in Brazil over a 16-year period to determine if the timing of the dengue season has changed as immunity to dengue in the population accumulates. The hypothesis is that there is a shift in the seasonal timing of dengue due to the accumulation of immunity to dengue over time and a shift towards endemic transmission after the re-emergence of dengue in Brazil in 1986. Alternatively, the seasonality of dengue may be changing due to climatic change. The study analyzed monthly time series from 1999 to 2014 of reported cases of dengue from Brazil surveillance data and associated changes in the timing of the dengue season with climate factors such as temperature, precipitation, and humidity. The age-specific rates of immunity over time were reconstructed using infectious disease transmission models and the potential impact of accumulation of immunity on the timing of seasonal peaks was simulated. The study found that 25 of 27 provinces across Brazil experienced a delay in the timing of seasonal peaks in dengue incidence with an average

delay across all provinces of 2.5 days per year. Temperatures increased throughout Brazil, but only subtle changes in the timing of season have occurred. Mechanistic models incorporating changes in immunity show shifts in the timing of the dengue season consistent with observed data whereas increases in temperature shift the season earlier. The study concludes that the shift in dengue seasonality towards later times of year is consistent with the slow accumulation of immunity in the Brazilian population over the last thirty years. The findings may help to understand changes in seasonality for other emerging pathogens such as Zika, influenza, and SARS-CoV-2.

6005

## FACTORS ASSOCIATED WITH CHIKUNGUNYA INFECTION AMONG PREGNANT WOMEN IN GRENADA, WEST INDIES

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Neonates are vulnerable to vector-borne diseases given the potential for mother-to-child congenital transmission of arboviruses and associated complications of neonatal infection. To determine factors associated with chikungunya virus (CHIKV) infection among pregnant women in Grenada, West Indies, a retrospective cohort study enrolled women who were pregnant during the 2014 CHIKV epidemic. 520/688 women (75.5%) were positive for CHIKV IgG. Low incomes, use of pit latrines, lack of home window screens, and subjective reporting of frequent mosquito bites were associated with an increased risk of CHIKV infection in bivariate analyses. In the multivariate modified Poisson regression model, low income (aRR 1.05 [95%CI 1.01-1.10]) and frequent mosquito bites (aRR 1.05 [95%CI 1.01-1.10]) were linked to increased risk of infection. In Grenada, markers of low socio-economic status are associated with CHIKV infection among pregnant women. Given that Grenada will continue to face vector-borne outbreaks in the future, interventions dedicated to improving the housing and living conditions of the most disadvantaged will help to reduce the incidence of a range of arboviral infections and positively impact the health of the population.

6006

## FILOVIRUS VIRUS GLYCOPROTEIN - EPITOPE MAPPING, PSEUDOTYPING, AND INFECTIVITY TARGETING

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Recent disease outbreaks highlight the need to characterize the immune response to filoviruses to develop vaccines and therapies. We have used extensive GP mutation libraries to map the epitopes for >200 monoclonal antibodies (MAbs) targeting the EBOV surface glycoprotein, GP. We have extended these studies to Marburg virus (MARV) by generating an Ala-scan library of MARV Δmucin GP (Uganda strain). Initial maps of anti-MARV MAbs include those of two non-neutralizing MAbs MR228 and MR235, targeting the wing region of MARV GP, that showed therapeutic protection in animal models (MR228) or that increased binding (MR235) by neutralizing MAbs. The variety of EBOV MAbs mapped, many from survivors of ebolavirus infection, include cross-neutralizing MAbs targeting the GP membrane-proximal external region (MPER); a broadly cross-reactive MAb that blocks GP interaction with its endosomal receptor Niemann-Pick C1; and MAbs who synergistically transform a non-neutralizing MAb into a potent neutralizer. The epitope maps have expanded our understanding of how the immune system recognizes EBOV GP, and allow correlation of epitopes with MAb neutralizing capabilities, to develop anti-EBOV therapeutics and vaccines. Mapping also identified mutations that increase the exposure of neutralizing epitopes, impacting future anti-ebolavirus vaccine strategies. To provide critical reagents for analyses of antibody or serum immune responses to ebolaviruses, we have developed a pseudotyped lentiviral reporter virus (RVP) system for EBOV and MARV, expressing the appropriate viral GP. These replication-incompetent RVPs perform one round of infectivity and enable safe (BSL-2) and reproducible virus neutralization assays with luminescent or fluorescent readout. Using our knowledge of EBOV GP we have also retargeted pseudotyped lentivirus to multiple cell surface receptors using antibodies inserted into GP1. In mixed primary cell populations, pseudotyped engineered GPs containing B- or T-cell-specific antibodies showed specificity for the targeted cell lineages. Such targeted EBOV GPs enable transduction of specific PBMC cell types.

6007

## TRANSMISSION DYNAMICS OF DENGUE VIRUS IN LARGE AND SMALL POPULATION CENTERS IN NORTHERN ECUADOR USING A PHYLOGENETIC ANALYSIS

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Although dengue is considered an "urban" disease, rural communities are also at high risk. To further our understanding of dengue virus (DENV) transmission in settings with characteristics generally considered rural (e.g., lower population density and remote), we conducted a phylogenetic analysis in 6 communities in the northwestern province of Ecuador that have distinct landscape, ecological and social variables that we identify as contributors to transmission risk. During household-based active fever surveillance, we collected 488 serum samples with suspected dengue from participants between 2019 and 2021. One hundred and twenty one had detectable DENV RNA by PCR. Twenty seven samples with CT under 30 were selected for whole genome sequencing (MinION nanopore technology) and phylogenetic analysis that included available DENV sequences from Ecuador and South America. Our data confirmed that DENV-1 circulated from May 2019 to March 2020 and DENV-2 from December 2020 to July 2021. Combining locality and isolation dates, we found strong evidence that DENV entered Ecuador into the northern province of Esmeraldas from neighboring country Colombia, and that viral isolates were related to Colombian and Venezuelan DENV. Phylogenetic patterns suggest that within this province communities with larger populations and commercial centers were more often the source of DENV but that smaller remote communities also play an important role in the regional transmission dynamics acting as sources or sinks of DENV.

6008

## DETECTING AND MONITORING THE RE-EMERGENCE OF DENGUE VIRUSES IN PUERTO RICO WITH GENOMIC SURVEILLANCE

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The Americas experienced the highest numbers of dengue cases in 2019 and 2022. The four dengue virus serotypes have recently been reported throughout the Americas, and the emergence of the DENV-2 Cosmopolitan genotype and recent emergence of DENV-3 underscore the importance

of maintaining genomic surveillance. After a period of absence, DENV-1 re-emerged in Puerto Rico in 2019, followed by DENV-2 and DENV-3 in 2022 and we have continued to detect transmission in the island. To characterize the genomic diversity circulating in Puerto Rico, we conducted next-generation sequencing (NGS) of representative samples (N>350) collected through entomological and human surveillance between 2019 and 2023 across Puerto Rico. Complete genomes were obtained directly from clinical samples and mosquito collections using a PCR-targeted NGS method developed by the CDC Dengue Branch. Phylogenetic diversity and the evolutionary dynamics of the circulating DENV-1, -2, and -3 were inferred using maximum likelihood and Bayesian methods. Phylogenetic trees were reconstructed with the addition of genomes from viruses of contemporary circulation in the region for context. Our analyses determined that the emergent DENV-1 variant is phylogenetically related to viruses circulating in the Caribbean but distinct from the lineages previously on the island. Wide-spread transmission of this new variant was confirmed in humans and mosquitoes. Similarly, the emergent DENV-2 is closely related to variants recently detected in Brazil and the emergent DENV-3 is closely related to a variant of Asian origin that caused a recent epidemic in Cuba. We also infer that the three serotypes circulated in the island before detection by laboratory surveillance, suggesting cryptic transmission. Despite detection of new variant introductions, new genotypes have not yet been detected in the island. Our genomic surveillance findings revealed that the re-emergence of dengue viruses in Puerto Rico was caused by the introduction of variants new to the island. We continue to monitor the evolution and spread of these variants to understand the impact to dengue epidemiology in the island.

**6009**

## LOCATION AND TIME ARE DRIVERS OF VIRAL DIVERGENCE DURING ACUTE PHASE ZIKA VIRUS INFECTION

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The high error rate of the RNA-dependent RNA polymerase drives variation in the genome of RNA viruses as they replicate, generating a swarm of viral variants closely related to the original inoculum. A viral swarm is shaped by host and environmental factors which can modify the diversity of the population, and ultimately shape evolution. In the current study we evaluate the impact of T cells, as well as physical barriers, sites of replication and time on the population genetics of Zika virus during acute infection using a mouse model. We ultimately found that time and location of viral replication within the mammalian host are critical factors driving viral swarm divergence, with minimal detected effects of CD4+ and CD8+ T cells over eight days. Using different inoculation routes, we demonstrate that entry of the virus within the CNS acts as a bottleneck, restricting viral variants upon entry. However, once entry into the CNS, novel single nucleotide variant replication was driven by viral replication within the CNS (as opposed to peripheral tissues). Ultimately, this study highlights time and location of replication as important factors in driving divergence of viral populations from the original parent sequence.

**6010**

## NEAR-COMPLETE GENOME SEQUENCES OF DENGUE VIRUS 3 ISOLATES ASSOCIATED WITH OUTBREAKS FROM DIFFERENT REGIONS OF KENYA IN 2011 AND 2019

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Dengue viruses (DENVs) are mosquito-borne viruses which can cause disease ranging from mild fever to severe dengue infection. These viruses are endemic in several tropical and subtropical regions. It is the most prevalent arbovirus in terms of human public health importance globally. Since 2011, Kenya has experienced increased number of outbreaks in mainly the north eastern and coastal regions. Despite the increased number of outbreaks, there is limited information on the genomic epidemiology of DENV in Kenya. In this study, we performed whole genome sequencing (WGS) on Dengue serotype 3 that was isolated from patients in Mandera and Mombasa counties of Kenya, to understand the genetic diversity of Dengue serotype 3 across time in the country and compare with other contemporaneous sequences across the globe. Twenty nine outbreak samples were received at the Arbovirus/VHF laboratory in KEMRI between 2011 and 2019 Ribonucleic acid (RNA) was extracted using QIAmp RNA kit (Qiagen, AG, Hombrech-tikon, Switzerland). Eighteen samples that tested positive and had cycle-threshold (Ct) values below 30 were selected for WGS. The RNA was reverse transcribed using Lunascript were then sequenced by next-generation sequencing using GridION Genome Sequencer (ONT, UK). We obtained 13 near complete genomes (78-97%). Genotyping showed that the Dengue 3 isolates sequenced fall within genotype III. The phylogenetic analysis indicates a variation in the clustering pattern of 2011 and 2019 outbreak isolates. This suggests the outbreak experienced in 2019 was as a result of a new introduction. The 2019 sequences clustered with an isolate from China, while the 2011 sequences clustered in a unique clade, which also shares a common ancestor with other African strains. The findings in the study reveals the existence of two different strains of Dengue 3 genotype III in Kenya. One of this was associated with the outbreak in 2011 and the other caused dengue outbreak in 2019. There is need to sequence more samples to help understand dengue virus trends in the country and in the region at large.

**6011**

## DEVELOPMENT AND CHARACTERIZATION OF BARCODED POWASSAN VIRUS TO ANALYZE BOTTLENECK EVENTS DURING TICK TRANSMISSION

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Powassan virus (POWV) is an emerging tick-borne virus that can cause severe neurologic disease including encephalitis and meningitis. POWV is classified into genetically defined lineages: Powassan virus (POWV, lineage I) and deer tick virus (DTV, lineage II). DTV poses a significant threat to human health because it is transmitted by black-legged ticks (Ixodes scapularis), which display human-biting behavior in highly trafficked wooded areas of the northeastern and north central US. The evolutionary forces exerted on POWV during transmission and pathogenesis are poorly understood. To assess virus population structure during tick transmission, we developed a barcoded virus to quantitatively measure population bottlenecks in cell culture, arthropods, and vertebrates. Barcoded viruses are engineered to contain synonymous nucleotide substitutions that facilitate efficient and cost-effective measurements of the stochastic reductions in virus populations that occur during virus transmission by arthropods. Barcoded POWV (bcPOWV) was created by changing the third nucleotide of 11 consecutive codons in the NS2a coding sequence, resulting in 411 (~4.2

million) possible unique barcodes. DNA containing the synthesized barcode region was inserted into a DTV infectious clone and virus was successfully rescued and propagated in baby hamster kidney (BHK-21) cells to high titers (105 PFU/mL). To confirm the barcode sequence in bcPOWV, we sequenced the barcoded region and observed 11 degenerate nucleotides in sequence chromatograms as expected. In addition, we are performing deep sequencing to quantify barcode diversity. Using bcPOWV, we will assess POWV population dynamics during transstadial and vertical tick transmission, where we will perform deep sequencing to quantify the extent of bottlenecks during the tick life cycle. We expect to see very few changes in genetic diversity from larval to nymphal life stages and major reductions in diversity from adult females to their eggs. Overall, barcoded viruses are a powerful genetic tool, which we will use to assess POWV population dynamics during various transmission modalities.

**6012**

## GENOMIC SURVEILLANCE OF SARS-COV-2 VARIANTS DURING DIFFERENT WAVES OF COVID-19 IN MALI

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Genomic epidemiology of SARS-CoV-2 has been important in the control of COVID-19 pandemic. SARS-CoV-2 genome sequencing has made it possible to detect new variants and inform the COVID-19 control strategies. However, in Mali there are very few genomic data available on SARS-CoV-2. Therefore, we sought to generate more genomic data to contribute the monitoring of SARS-CoV-2 variants circulating in Mali during different waves of the pandemic. A retro-prospective study was conducted on samples collected between March 2020 and September 2022 in Mali representing samples from the first four waves. RNA was extracted using the Qiagen kit. Libraries were prepared using either an Illumina or Nanopore kit and subsequently sequenced on an Illumina MiSeq and MinION respectively at the MRTC. Sequence data was analyzed on a local server. We successfully sequenced 89 viral genomes. In addition, we downloaded 21 Malian sequences from the GISAID repository. We detected ten (10) variants: A, A.1, A.21, A.27, B, B.1, B.1.525 (Eta), B.39, Delta, Omicron (BA.2, BA.5). The most recent increase of cases corresponded to the occurrence of BA.5 Omicron sublineage. Except the A.21 variant, which might have emerged locally, the other variants detected were all cases introduced into Mali. Our results highlight the importance of sequencing SARS-CoV-2 locally and provide information on variants circulating during the first waves of the COVID-19 pandemic. We are monitoring the evolution of variants and updated data will be presented.

**6013**

## SPATIOTEMPORAL DYNAMICS OF CIRCULATING DENV-1 IN LA VIRGINIA, RISARALDA BETWEEN 2019 AND 2021

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Dengue virus (DENV) in Colombia has an endemo-epidemic transmission pattern characterized by circulation of multiple serotypes and lineages with a heterogeneous spatiotemporal distribution. It is not clear how inter-epidemic transmission is sustained, and the triggers for epidemic seasons in the country. This work aims to understand how a recent dengue serotype 1 outbreak is related to transmission dynamics between near and distant endemic regions of Colombia. We performed sequencing and assembly of complete genomes of dengue virus circulating in La Virginia, Risaralda (2019-2021) and Santiago de Cali, Valle del Cauca (2021). Genomes were aligned with sequences from other cities that were available in GenBank. We performed a Maximum-likelihood and Bayesian phylogenetic analyses and identified the best-fit molecular clock and population growth model. Subsequently, we reconstructed the spatial dispersal of the virus using a discrete diffusion phylogeographic model with Markov chains.The lineage of DENV1 that circulated in La Virginia between 2019-2021 is related to the lineages that circulated in the department of Antioquia and share ancestry with viruses introduced in the 1990's from Venezuela. By contrast, the lineage detected in Cali in 2021 is more closely related to a lineage that circulated in the department of Santander in 2008. The reconstructed dispersal routes show that distinct DENV1 lineages from two nearby locations experiencing recent DENV1 transmission (La Virginia and Cali) likely derive from different sources. We hypothesize that introduction and re-introduction of new lineages can be related with sustained transmission and epidemics in our setting. Our results provide insights about the heterogenous dispersal of DENV1 in Colombia, and its potential impact on transmission dynamics.

**6014**

## INTRODUCTION OF A NEW CLADE OF ECSA GENOTYPE DURING THE LARGEST OUTBREAK OF CHIKUNGUNYA VIRUS IN PARAGUAY

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Chikungunya virus (CHIKV) is an arthropod-borne virus (arbovirus) of epidemic concern. CHIKV was introduced to Paraguay in 2015 and caused minor outbreaks in some regions of the country. However, in late 2022 a major outbreak started. Between October 2022 and mid-March 2023, more than 27.700 cases were reported, also cases of meningoencephalitis and deaths caused by CHIKV infection were registered. In this study, we sequenced 30 serum samples collected in Asunción and Central Department in January, 2023. Nanopore technology was used to generate CHIKV near-complete genomes (average coverage nt: 94,06% - CDS: 98,87%). Consensus sequences were assigned as East/Central/South African (ECSA) lineage by Genome Detective tool. Maximum Likelihood phylogeny showed that the current Paraguayan strains grouped separately from the Paraguayan ECSA strains detected in 2018, but within the same clade of Rio de Janeiro ECSA strains from 2019, indicating the introduction of a new clade to Paraguay. Interestingly, CHIKV detected in Paraguay in 2023 formed a monophyletic cluster with a larger branch within the clade. All Paraguayan strains presented new amino acid substitutions: Q368L and S405P at the N-terminal domain of nsp2; A366V at the hyper variable domain of nsp3; L129 and Q175K at RdRp domain of nsp4. Even though E2:V264A was detected at low frequency in sequences from Brazil in 2019, this substitution was present in all the sequences analyzed in this study. Altogether, this data suggest that this ECSA clade of CHIKV this clade may have circulated undetected since 2019 (or the data is not publicly available at the moment). Further studies are needed to address whether the mutations observed in non-structural proteins may have functional consequences for CHIKV replication and/or infectivity, evasion from the immune system or pathogenesis. This study reinforces that continued genomic surveillance strategies are needed to support the monitoring of CHIKV epidemics in order to better understand changes in the incidence, severity of the disease and to shed light onto the highest outbreak caused by CHIKV in Paraguay.

6015

## PURIFYING SELECTION DECREASES THE POTENTIAL FOR BANGUI ORTHOBUNYAVIRUS OUTBREAKS IN HUMANS

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Pathogens carried by insects, such as Bunyaviruses, are frequently transmitted into human populations and cause disease. Knowing which spillover events represent a public health threat remains a challenge. Metagenomic next-generation sequencing (mNGS) can support infectious disease diagnostics by enabling detection of any pathogen from clinical specimens. mNGS was performed on blood samples to identify potential viral co-infections in HIV+ individuals from Kinshasa, Democratic Republic of Congo (DRC) participating in an HIV diversity cohort study. Time-resolved phylogenetics and molecular assay development assisted in viral characterization. The nearly complete genome of a novel orthobunyavirus related to Nyangole virus, a virus previously identified in neighboring Uganda, was assembled from an HBV+ patient. A quantitative PCR assay was designed and used to screen over 2,500 plasma samples from Cameroon, DRC, and Uganda, failing to identify any additional cases. Recent sequencing of a US CDC Arbovirus Reference collection revealed that this same virus, now named Bangui virus, was first isolated in 1970 from an individual in the Central African Republic. Time-scaled phylogenetic analyses of Bangui with the related Anopheles and Tanga serogroup complexes indicate that this virus emerged nearly 10,000 years ago. Pervasive and episodic models further suggest this virus is under purifying selection and that only distant common ancestors were subject to positive selection events. This study represents only the second identification of a Bangui virus infection in over 50 years. The presumed rarity of Bangui virus infections in humans can be explained by its constraint to an avian host and insect vector, precluding efficient transmission into the human population. Our results demonstrate that molecular phylogenetic analyses can provide insights into the threat posed by novel or re-emergent viruses identified by mNGS.

6016

## PROFILING OF DENGUE SEROTYPE -2 SPECIFIC MICRORNA EXPRESSION IN THE SERUM SAMPLES OF DENGUE PATIENTS IN SABAH, MALAYSIA

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Dengue is an Aedes mosquito-transmitted human arboviral disease which remains endemic in Asia Pacific countries including Malaysia The Dengue virus is a single-stranded, positive-sense RNA virus with four antigenically distinct serotypes namely DENV-1, DENV-2, DENV-3 and DENV-4. MicroRNAs (miRNAs) are a class of small non-coding RNA molecules. There has been substantial prior report which showed the association of MicroRNA (miRNAs) in many viral infections. However, data on circulating microRNA expression patterns in dengue patients are scanty. This study aims to identify the circulating DENV serotype-specific microRNAs in patient's serum. Here we investigate the microRNA expression profiles in the serum samples of DENV-2 serotype patients from Sabah, Malaysia. We have subjected to high-throughput small RNA (sRNA) sequencing. Total RNA was isolated and small RNA sequencing was performed using Illumina MiSeq high-throughput next generation sequencing collected clinical samples from a total of 30 patients with DENV-2 serotype infection and 30 apparently healthy individuals as controls. The serum RNAs was isolated from these subjects and platform to identify deferentially expressed miRNAs. After quality control of the sequence reads, we identified 19 miRNAs that were expressed in DENV-2 serotypes. Of these, 13 were upregulated (hsa-miR-122-5p, hsa-miR-92a-3p, hsa-miR-451a, hsa-let7b-5p, hsa-miR-619-5p, hsa-miR-652-3p, hsa-miR-16-5p, hsa-miR-191-5p, hsa-miR-22-3p, hsa-miR-26a-5p, hsa-miR-320a-3p, hsa-miR-423-3p and has-miR-486-5p) and 6 were down regulated (hsa-miR-197-3p, hsa-miR-27a-3p, hsa-miR-449a, hsa-miR-342-3p, hsa-miR-574-3p and hsa-miR-204-5p). Our preliminary findings seem to suggested significant alterations in the miRNA levels in patients with dengue infection. In conclusion, the differential expression of DENV-2 serotype specific miRNA may provide a better understanding the disease and can be used as a potential biomarker candidate for monitoring dengue viral infection.

6017

## METAGENOMICS ANALYSES REVEALS PRESENCE OF THE MERIDA-LIKE VIRUS IN GEORGIA (COUNTRY)

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Emerging arbovirus infections can rapidly expand and adapt in new geographic and environmental conditions. For this reason, mosquito surveillance serves as a tool to detect, monitor, and prevent pathogens which present a potential threat of human infection. In this study, we evaluated the presence and abundance of viral pathogens in mosquitoes of the Culex pipiens specie. Supporting this effort, a total of 467 mosquitoes, were collected from the country of Georgia between 2018 and 2019. We used an unbiased total RNA-seq approach and conducted a Heat Map analysis to explore the mosquito virome. The viral reads from this analysis were mostly aligned to insect-specific viruses from two main families, the Iflaviridae; a positive-stranded RNA virus and the Rhabdoviridae; a single-stranded, negative RNA virus. It was intriguing to find in our heat map analysis viral reads aligning to the Merida-like virus Turkey (MERDLVT) strain. The Merida like-virus is a single-stranded RNA that's known to have high levels of amino acid identity. A Blast sequence analysis of our positive samples aligned to the MERDLVT strain showed an 96-100% sequence identity and an 99.7-100% of sequence coverage. We proceeded to use a phylogenetic tree to further evaluate the evolutionary relationship among these positive pooled specimens with the (MERDLVT) strain and other Merida-like virus strains. As expected, the Merida like- virus found in Georgia mosquitoes clustered with two strains from Turkey; the Merida-like virus KE-2017a isolate 139-1-21 and the Merida-like virus Turkey isolate P431. Collectively, these results imply presence of the MERDLVT strain in Georgia.

6018

## CO-INFECTION OF DENGUE AND CHIKUNGUNYA IN BENGALURU CITY, SOUTHERN INDIA - A MOLECULAR SURVEILLANCE APPROACH

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Dengue virus (DENV) and Chikungunya virus (CHIKV) are RNA viruses belonging to families Flaviviridae and Togaviridae transmitted by Aedes sp. Mosquitoes. Globally, the monoinfections and coinfections result in fatalities in the tropical and subtropical regions. Detection of DENV and CHIKV primarily relies on Enzyme Linked immunosorbent assay (ELISA) and rapid antigen tests, which are nonconfirmatory detection methods. Our molecular surveillance study across Bangalore, with local municipal

corporation, aims to understand the prevalence of infections from DENV, CHIKV and coinfections with both arboviruses in ELISA tested patient samples. We screened 892 serum samples from July 2022 to November 2022 collected across the Urban Primary Health Centres, Referral hospitals and Maternity homes. Samples were initially screened for DENV and CHIKV using ELISA. Subsequently, RNA extractions were conducted followed by TaqMan Probe based RTPCRs for serotype-specific detection of DENV and CHIKV. We found 304/892 (34.08%) samples tested positive for DENV and 167/ 892 (18.72%) for CHIKV whereas 77/892 (8.63%) showed presence of coinfection with DENV and CHIKV. Among DENV infections, DENV-serotype-2 showed 56% of infections followed by DENV-serotype 1 showed 46% and DENV-serotype -3 showed 23% of infections. We found 3% of samples infected with multiple serotypes. Total prevalence from DENV and CHIKV exhibited a significant decreasing trend from July to November (Wald's $\chi 2$= 239.66, df =4; P &lt 0.001). This trend was also reflected in infection from DENV (Wald's $\chi 2$= 171.2, df =2; P &lt 0.001) and CHIKV (Wald's $\chi 2$=175.33, df=4 P &lt 0.001) across months. There was no significant variation in viral prevalence by gender. Comparatively, the total prevalence of DENV and CHIKV using ELISA and RTPCRs based detections was found to be 28.14% and 64.70% respectively. Our study represents real-time disease detection using two methods which helps in strengthening the existing the public health surveillance system and contributes towards developing cost-effective protocols in low-resource settings.

**6019**

## SEROLOGICAL EVIDENCE OF PRIOR EXPOSURE TO EMERGING PATHOGENS IN RURAL LIBERIA, WEST AFRICA

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West Africa is a source of emerging pathogens, largely zoonotic, that can cause intra- and extra-regional outbreaks. The extent to which emerging pathogens are transmitted to and by humans undetected is unclear. We assessed serological status to several emerging viruses using serum collected at baseline from a purposefully sampled (age and gender distribution) cohort of individuals >2 years of age living in rural Liberia participating in the observational Coalition for Epidemic Preparedness Innovations (CEPI) ENABLE Study. Samples were analyzed in duplicate using the AFRICOM bead panel for detection of IgG on a Luminex MAGPIX. Viruses included were Ebola Virus (EBOV), Crimean-Congo Hemorrhagic Fever Virus (CCHFV), Lassa Virus (LASV), Marburg Virus (MARV), Rift Valley Fever Virus (RVFV), and Panflavivirus (Panflavi) and Panalphavirus (Panalpha) panels. Median ratios of signal to noise (S:N) and the proportion above selected thresholds were calculated for each virus. Two S:N thresholds were considered including an empirically selected S:N of 10 and a threshold of 40, which in a separate West African cohort was associated with viral neutralization. Serum from 461 participants (age: median = 18; range 2-97. 54% female) were analyzed. The median S:N for individual viruses ranged from 3.6 for MARV to 55.9 for LASV. The proportion of participants exhibiting S:N >10 ranged from 16.7% for MARV to 72.2% for LASV; using a threshold of S:N ratio >40 the proportion ranged from 3.9% for MARV to 55.5% for LASV. Approximately a third of participants exhibited seropositivity for Panflavi or Panalpha antibodies at the >40 S:N threshold. Correlation of seropositivity across the pathogens was observed but while significant, was moderate. Overall, serological evidence of prior infection with emerging pathogens was not uncommon among residents of rural Liberia and, although Lassa fever is endemic, the rate of LASV seropositivity was greater than expected. The detection of seropositivity to the other emerging pathogens tested suggest unrecognized spillover events and the need for enhanced diagnostic capacity to identify these infections in real time.

**6020**

## MAYARO VIRUS EXPOSURE IN FREE-RANGING BATS OF ANIMAL-HUMAN INTERFACE AREAS, MIDWEST BRAZIL

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Arboviruses as Dengue (DENV), Zika (ZIKV) and Chikungunya (CHIKV) are maintained in epidemic cycles of transmission among humans and Aedes aegypti mosquitoes in Brazil, and are of great importance to public health. Other arboviruses such as Yellow Fever (YFV), Mayaro (MAYV) and Oropouche (OROV), that are maintained in enzootic cycles of transmission involving wild vertebrate and diptera species, have also great medical importance in the country. The circulation of these arboviruses in wild vertebrate species at the human-animal interface in Brazil remains poorly investigated. Bats are among the synanthropic vertebrates found in large populations in these areas and their exposure to these arboviruses is still obscure. Here, we aimed to search for the circulation of the main epidemic and enzootic arboviruses in Brazil in specimens of bats collected in forested areas of the metropolitan regions of the Midwest region of Brazil between 2017 and 2018. RT-qPCR assays were used for the investigation of active DENV, ZIKV, CHIKV, YFV, MAYV and OROV infections in liver, kidney and brain tissues of euthanized bats (n=168). Carollia perspicillata, Artibeus lituratus and Artibeus planirostris were the most common species collected. Previous exposure to MAYV and OROV was also evaluated by the investigation of specific neutralizing antibodies by plaque reduction neutralization test (PRNT) in 79 bats from several species. No active infections were identified and none of the bats showed neutralizing antibodies for OROV. On the other hand, 2.6% (2/76) of the bats were seropositive for MAYV, one Noctilio albiventris (PRNT50 titer 40) and one Molossus rufus (PRNT50 titer 80). The role of bats in arbovirus cycles of transmission remains unclear worldwide, and the exposure of bats to MAYV in Midwest Brazil merits further investigation.

**6021**

## EVIDENCE OF CORONAVIRUS TRANSMISSION AMONG PTEROPUS MEDIUS IN BANGLADESH, 2019-2021

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Pteropus bat species are the known reservoirs for henipaviruses; humans are infected with the Nipah virus by P. medius in Bangladesh through the consumption of contaminated date palm sap. The ecological drivers and transmission dynamics of these viruses within the reservoir hosts remain poorly understood. The objective of this study was to investigate coronavirus transmission among P. medius in Bangladesh, which could pose a risk for spillover. Serum samples from 3,023 P. medius in eight colonies across seven districts in Bangladesh were collected between 2019 and 2021 and screened by multiplex immunoassay (MIA) for antibodies reactive to the viral spike glycoprotein (S) from the Severe acute respiratory syndrome coronavirus (SARS-1), Severe acute respiratory syndrome coronavirus-2 (SARS-2), Middle East respiratory syndrome coronavirus (MERS), Bat SARS-like coronavirus isolate Rs4874 (Rs4874), Bat coronavirus isolate PREDICT/PDF-2180 (PDF2180), and Rousettus bat coronavirus HKU9 (HKU9). In addition, pooled oral and fecal swabs from 307 of these bats were screened for coronaviruses using conventional PCR. Two percent (62/3023) of bats tested had coronavirus IgG antibodies from four of the seven districts. Adult seroprevalence (3%, 51/1943) exceeded juvenile (1%, 10/1021) and pup (2%, 1/60). Among the 62 IgG

positive samples, 60 samples (97%) indicated reactivity to Bat-CoV Rs4874 suggesting prior exposure to Bat-CoV Rs4874 or an antigenically similar virus. Bat seroprevalence was highest (4%, 39/876) from Cox,s Bazar, in southeastern Bangladesh. The PCR results revealed that 1% (3/307) of the samples were positive for betacoronavirus, (nobecovirus), which were closely related to bat coronavirus previously identified in the South East Asian region and Bangladesh. These three bats were negative in the serology test for all coronaviruses. The seroreactivity and PCR results (active infection) indicate continuous exposure of the bats to different coronaviruses. The finding of this study added information to support the role of bats as natural reservoirs of coronaviruses.

**6022**

## DETECTED ARBOVIRUSES IN EASTERN MEDITERRANEAN REGION AND SOUTH EAST ASIAN REGION MOSQUITO POPULATIONS: A SYSTEMATIC REVIEW

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The circulation of arboviruses in mosquitoes implicates many arboviruses as causes of undifferentiated fever that remain undiagnosed due to lack of available testing methods and short viremia in humans. We aim to determine what arboviruses have been discovered circulating in mosquito species in Eastearn Mediterranean Region and South East Asian Region. We used the PubMed database to identify 3100 related articles on surveillance of mosquitoes for arboviruses. Following data, among other datapoints, from included studies was extracted: country of surveillance, duration of collection, number of mosquitoes collected, number of different mosquito species identified, detection technique used, viruses screened for and viruses detected. 34 included studies were done in EMR and SEAR, including 3 from Pakistan. Culex are the most prevalent mosquito species. Dengue, West Nile, Japanese Encephalitis and Rift Valley Fever viruses have been detected; dengue and West Nile in Pakistan. The most common method of detection is pooled PCR of mosquitoes using virus-specific primers. Several viruses not currently implicated in human infection such as Tembusu virus, Bagaza virus, Barkedji virus, cell-fusing agent virus, Phlebotomus-associated flavivirus and Culex-specific flaviviruses have also been detected.

**6023**

## FACTORS RESPONSIBLE FOR POST-DISCHARGE DEATH IN COVID PATIENTS

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The post-discharge all-cause mortality of COVID-19 disease is known, but predictors for the same are not much studied. The current research was a single-center unmatched case-control study conducted at a tertiary care center in northern India, between April and September 2022. The data were extracted retrospectively from the hospital's electronic medical records of patients with the assistance of trained physicians using a standardized data extraction sheet. A total of 184 patients were enrolled and were segregated into two groups cases and control with 92 in each. The mean age of patients was 49.3 ±17.53 years. The mortality group had a higher mean age (53.24 ± 18.53 yrs) as compared to the control group (45.37 ± 15.58 yrs, p - 0.002). Bivariate analysis revealed a significant difference in the two groups with respect to O2 saturation at the time of admission (Case - 91.12 ± 12.49 %, control - 95.46 ± 5.01 %, p - 0.003); Maximum O2 flow rate [L/min] (Case - 11.01 ± 22.2, Control - 6.41 ± 13.31, P - 0.04); ICU need (p - 0.005), Cancer (p - 0.001), O2 requirement at discharge (p - 0.001) and AKI (p - 0.007). On multiple regression analysis, Cancer (aOR- 2.469; 95% CI,1.183- 5.150, p-0.016), ICU admission (aOR- 2.446; 95% CI, 1.212-4.938, p- 0.013), Oxygen at discharge (aOR- 2.340; 95% CI, 0.971-5.640, p-0.0586) and Acute kidney injury (aOR- 5.6; 95% CI, 2.351-13.370, p-0.00) only found to be significant. Among the patients discharged from the hospital post-COVID-19 treatment, the following aspects oxygen requirement (2.3 times), Malignancy (2.4 times), ICU admission (2.4 times), and Acute Kidney Injury (5.6 times) are risk factors of mortality. The presence of these variables would warrant a close follow-up for these patients in order to decrease post-COVID mortality.

**6024**

## ROLE OF TELEHEALTH AND COMMUNITY MOBILIZATION IN MANAGING COVID -19 WITHIN THE CONTEXT OF A DISTRICT HEALTH SYSTEM IN MALAWI

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The COVID-19 pandemic has impacted health systems globally. High demand for health services and staff absenteeism due to illness are some of the barriers that negatively affected healthcare delivery. To reduce pressure on healthcare services, academics from Blantyre implemented the "Strengthening COVID-19 Response in Blantyre District" (SCORE) project, which established a remote case management system (phone clinic) and community mobilization workforce in fighting COVID-19. A cross-sectional study was done to evaluate the pilot implementation of the project. Qualitative and quantitative methods were used to collect data from community leaders (n=27), volunteers (n=49), and members (n=201) from communities served by the project: Chileka, Limbe, Mpemba and Ndirande. Data was also collected from District Health Management Team (DHMT) (n=5) and Health Surveillance Assistants (HSAs) (n=6). Focus Group Discussions, in-depth interviews and structured questionnaires were used to collect data. Quantitative data was analyzed descriptively using SPSS 26. Qualitative data was transcribed and analysis was done using Nvivo 12. Phone calls data showed 58% of inquiries were for COVID-19 followed by general healthcare (25%). Of COVID-19 enquiries, 41% were for general information, 29% for preventive measures, and 14% for signs and symptoms. 99% of participants said the SCORE project helped spread COVID-19 preventive measures, 92% raised awareness of risk factors, and 90% encouraged vaccination. Qualitative data showed volunteers had a good working relationship with community leaders, HSAs, and members. Both interventions were recommended by communities and District Health Office staff for scale up and extension to other health conditions like HIV and Cholera. The project showed its potential to enhance remote case management, information sharing and community mobilization efforts related to COVID-19. In conclusion, the project has shown that effective remote case management and community mobilization can reduce pressure on healthcare systems and improve health outcomes, providing a model for future health crisis response efforts.

**6025**

## DISTRIBUTION AND OUTCOMES OF ANIMAL BITES IN THE MBALE REGION OF EASTERN UGANDA

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Animal bites can lead to rabies, tetanus, and skin/soft tissue infection, and are a significant cause of global morbidity and mortality. Although an average of 16,414 animal bites are reported each year in Uganda, there is a paucity of data on animal bites in the country. In this cross-sectional study, we aimed to determine the distribution and outcomes of animal bites in the Mbale region of eastern Uganda. We collected data on demographic characteristics of those with animal bites in the Uganda District Health

Information Software 2 database maintained by the Uganda Ministry of Health. We included data that were reported from January-December 2022 from outpatient departments in the 16 districts of the Mbale region. We determined frequencies and proportions of each variable and used the QGIS to present the analyzed data. Animal bites were documented in 984 of 4,656,700 patient visits to health centers in the Mbale region. During the same period, a total of 15,261 rabies exposures were reported in the country with 833 (5%) reported from the Mbale region. Of those bitten by animals in the Mbale region, 540 (55%) were male and the median (interquartile range) age was 17.5 (7.5-25.5) years. The frequency of animal bites ranged from 10 to 20 per week. Bukwo, Tororo, and Busia districts had the highest prevalence rates of animal bites in the Mbale region with 1.74, 1.68, and 0.81 bites per 1000 population, respectively. Of those bitten, there were 7 deaths (case fatality ratio 0.71%), all of which were attributed to rabies. Animal bites and rabies exposure are common in the Mbale region. Our findings emphasize the need for the availability and administration of routine and post-exposure vaccination to prevent rabies and tetanus following animal bites in the high prevalence at-risk Mbale region of eastern Uganda.

6026

## MOSQUITO IDENTIFICATION: NANOPORE SEQUENCING OUT OF A SUITCASE LAB AS AN EARLY WARNING SYSTEM FOR EMERGING INFECTIOUS DISEASES

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Mosquito-borne diseases are responsible for spillover to around 700 million people each year, including many neglected tropical diseases such as Dengue fever and Chikungunya virus disease. Case reports are rising over the last decades, as mosquito species flare-up as a result of climate change and globalization. Surveillance tools and early warning systems are essential to prevent disease spread to humans and animals. Next generation sequencing technologies offer great advantages for disease outbreak investigation. The aim of this study was to develop a rapid and field deployable sequencing platform to identify potential mosquito-borne pathogens, mosquito species and host in blood meals. First a rapid extraction reverse purification method was developed. Nucleic from mosquito specimens, including Culiseta longiareolata, Culex pipiens, Aedes albopictus, Ae. cretinus and Ae. aegypti were isolated using a rapid "all-in-one" extraction protocol based on lysis buffer, glass beads, magnetic beads, heating and vortexing. Nucleic acid from 30 mosquito samples were extracted using commercial purification kits as a control. Rapid barcoding 96 sequencing (Oxford Nanopore Technologies) was performed using a MinION Mk1c device. For RNA targets, a reverse transcription step was performed using random hexamers. All handling steps were carried out in the fully equipped suitcase lab. A specific offline BLAST database was created to semiautomatically identify mosquito species, host in blood meal and pathogens. In all samples, the species was correctly identified. Both animal and human DNA could be detected. Interestingly, only mosquito origin viruses could be detected in the pool. The nucleic acid extraction and detection protocol performed in the suitcase lab allows fast mosquito "footprint" analysis directly in the field. This could pave the way as an early warning tool for mosquito-borne diseases and on-site outbreak investigation. Ultimately, the point-of-need generated data can lead to more accurate and fast preventive measure implementation. We are testing the whole system in a highly endemic region in Bangladesh.

6027

## SIMIAN ARTERIVIRUSES: A ZOONOTIC THREAT?

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Simian arteriviruses are endemic in African nonhuman primates yet remain largely unstudied and uncultured in vitro. These viruses can cause Ebola-like diseases that are fatal to Asian macaques but are not yet known to infect humans. To evaluate the risk for a spillover event, we tested the ability of a simian arterivirus, simian hemorrhagic fever virus (SHFV), to enter human cells and replicate within them. First, we substantiated CD163 as the cellular receptor for SHFV entry into host cells. Next, we showed that CD163 acts as an intracellular receptor, a rare mode of virion entry that is shared with other high-consequence viral pathogens (e.g., Ebola virus and Lassa virus). Alarmingly, we found that the human version of CD163 is a fully functional receptor for SHFV entry into cells. Further, SHFV can replicate robustly in human cells, showing full functionality of the orthologs of all host proteins required for the SHFV lifecycle. Our study raises concern about future possible spillovers of simian arteriviruses to humans and the potential public-health risk that such spillover events would pose. Indeed, it is possible that people in Africa are already being infected with simian arteriviruses but that these cases remain undetected in the way HIV-1 infections did for decades. For elucidation, development of serology tests for human surveillance should be a priority.

6028

## CASE AND WASTEWATER SURVEILLANCE TO MONITOR COVID-19 AND OTHER INFECTIOUS DISEASES IN ATLANTA K-12 SCHOOLS

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For schools, timely responses to outbreaks of infectious diseases rely on information from disease surveillance systems. Epidemiological case surveillance information for schools can be extracted from public health databases at county/city level or collected from schools that use a self-reporting system. Wastewater surveillance has been recognized as a valuable tool to complement case surveillance. Wastewater samples are typically collected at wastewater treatment plants, but can also be collected at manholes adjacent to schools. This study compares information from wastewater surveillance at the city and school level to reported case data at the county and school level for 14 K-12 schools in Atlanta, GA. COVID-19 epidemic curves for Fulton County, the City of Atlanta, and specific school zones were generated using geocoded case information reported to the Georgia Department of Public Health. Self-reported case information was obtained for K-12 schools within Atlanta Public School district. Wastewater surveillance data from wastewater treatment facilities in Atlanta was also obtained. From 09-07-2021 to 03-14-2023, we collected and analyzed 489 Moore swab samples from manholes that only received wastewater from the study schools. All samples were tested for SARS-CoV-2 RNA, and after 11-29-2022, 88 samples were also tested for Influenza A and RSV, and 74 were tested for Influenza B by qPCR. 41% (201/489) of samples tested positive for SARS-CoV-2. Temporal and school variation were observed with high schools having the greatest proportion of wastewater samples

positive for SARS-CoV-2 and the lowest reported COVID-19 incidence. Very few samples were positive for Influenza A (5.7%), RSV (2.3%), and Influenza B (0%). We found increasing discordance between results from different case and wastewater surveillance systems which may reflect greater underreporting of cases, changing SARS-CoV-2 fecal shedding patterns, and decreasing sensitivity of assays for emerging variants. The results from this study provide insights for how K-12 schools can use information from different surveillance systems to guide public health measures.

6029

## DETECTION OF HUMAN CORONAVIRUSES AMONG PATIENTS WITH RESPIRATORY TRACT INFECTIONS IN GHANA

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Acute Respiratory infections are caused by various microorganisms particularly viruses including influenza, human metapneumovirus, adenoviruses and coronaviruses; human coronaviruses (HCoVs). HCoVs account for up to 18.4% of ARIs burden globally and between 3.5-12.4% in Ghana. During the COVID-19 pandemic, individuals presented with febrile-like-illnesses exhibiting respiratory symptoms yet, tested negative for SARS-CoV-2 and influenza viruses. HCoVs and influenza viruses are known to exhibit symptoms similar to SARS-CoV-2 hence there is the plausibility that during the pandemic, the symptomatic but negative SARS-CoV-2 and influenza samples could have tested positive for these other HCoVs. To investigate the presence of other human coronaviruses from archived negative SARS-CoV-2 and Influenza samples. The study will adopt a retrospective cross-sectional study which will involve archived samples collected from influenza sentinel surveillance sites in Ghana. RNA will be extracted from samples and and amplified by RT-qPCR to detect the other human coronaviruses (OC43, NL63, HKU1 and 229E) and sequenced using MinIon sequencing to detect variations in the strains. Conclusions, this investigation will contribute to an understanding of the causes of ARI in Ghana and aid health authorities enact policies to improve quality health care delivery and patient management.

6030

## USE OF LIGHTWEIGHT GPS DATA LOGGERS TO TRACK HORSESHOE BAT MOVEMENT PATTERNS IN EASTERN UGANDA

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Bats are reservoirs for pathogens with zoonotic potential; therefore, it is crucial to investigate aspects of bat ecology that may facilitate spillover events. Bats in the genus Rhinolophus, commonly called horseshoe bats, are known to harbor SARS-like coronaviruses. Geospatial investigations of horseshoe bat ecology are often hindered by the small size of these insectivorous bats. To evaluate seasonal movement and foraging patterns of horseshoe bats in Eastern Uganda, we fitted 36 bats with ultra-lightweight (<1.1g) GPS data loggers in January and May of 2022 as part of a larger viral surveillance program targeting bat caves in the region. We demonstrate the successful deployment and recapture (9/36) of data loggers collecting high-resolution short-term foraging data from cave roosting Rhinolophus spp. in Eastern Uganda, and share recommendations on their future use in other bat-related applications.

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## IGG HYPORESPONSIVENESS AFTER DENGUE VIRUS INFECTION IN KENYAN CHILDREN

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An important serologic hallmark for dengue virus (DENV) infection is the development of DENV specific IgG. Previously, we observed IgG hyporesponsiveness after DENV infection in a cohort of febrile Kenyan children who had DENV viremia, documented by RT-PCR, who failed to seroconvert from negative to positive serum anti-DENV IgG after one month (median interval 31 days between febrile and follow up visits, interquartile range 28-36 days). We hypothesized that the serum IgG response to DENV infection may be transitory or delayed. To investigate our hypothesis, we designed a study to measure anti-DENV IgG in cases of DENV infected children at 6 different timepoints over the course of a year: at the febrile visit, 14 days, 30 days, 3 months, 6 months, and 12 months. We enrolled 1046 subjects and identified 12 cases of acute DENV infection (by RT-PCR), along with temporally and geographically matched controls without DENV infection. One case developed anti-DENV by 3 months post-infection. Another case did not develop anti-DENV until 3 to 6 months later. The remaining DENV cases and non-infected controls did not develop anti-DENV IgG at any time point throughout the 1 year follow up period. Ongoing characterization of the peripheral memory B cell population by mass cytometry may yield insights into the mechanisms that govern DENV IgG hyporesponsiveness. These examples of delayed development of anti-DENV IgG or complete anergy after infection imply that previous cross-sectional serologic surveys based on anti-DENV IgG prevalence may have grossly underestimated DENV exposure in our population. IgG hyporesponsiveness to DENV infection also may underlie the low incidence of severe dengue disease previously observed in our population. Finally, IgG hyporesponsiveness to DENV must factor into the design of future vaccine studies and/or immunization campaigns.

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## CHIKUNGUNYA VIRUS SPECIFIC T CELLS PREDOMINANTLY RECOGNIZE VIRAL STRUCTURAL PROTEINS

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Chikungunya disease (CHIK) is a mosquito-borne re-emerging viral disease, that is defined by its capability to induce incapacitating chronic arthralgia and inflammation in patients' months to years following infection. Despite CHIKV outbreaks occurring worldwide and several vaccines currently in development, CHIKV immune response remains largely understudied. Chikungunya virus (CHIKV) the causative agent for CHIK, is an alphavirus, coded in five structural proteins (CP, E3, E2, 6K, E1) and four non-structural proteins (nsP1, nsP2, nsP3, nsP4).We have tested pools of overlapping peptides spanning each of these viral protein in peripheral blood mononuclear cells (PBMCs) collected from patients diagnosed with CHIKV during the 2014-2015 outbreak in Colombia. The majority of these patients still experienced diseases symptoms 7 years after infection, representing a chronic infection with CHIKV. To characterize the CHIKV specific T cell response we performed high resolution flow cytometry analysis utilizing the Activation Induced Marker (AIM) and Intracellular Cytokine Staining (ICS) assays, to evaluate CHIKV-specific T cell responses directly ex vivo,

without the need for intensive manipulation.Interestingly, we detected CHIKV specific CD4+ T cell responses in the majority of patients 7 years after CHIKV infection. Memory CD8+ T cell responses were also observed but at much lower frequencies. Overall, two thirds of antigen-specific CD4+ T cell responses were directed against structural protein while one third was directed against non-structural proteins. In conclusion, this study comprehensively characterizes the T cell response against CHIKV during the chronic phase and provides insights in the possible role of T cells in CHIK disease.

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## INVESTIGATING THE IMMUNE PROFILES ELICITED BY CLINICALLY APPARENT AND CLINICALLY INAPPARENT DENGUE VIRUS INFECTIONS

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Dengue virus (DENV) is a flavivirus transmitted by Aedes mosquitoes that can cause symptoms ranging from febrile illness to hemorrhagic fever. There are an estimated 400 million cases of dengue every year, with the majority being clinically inapparent. While much is known about the immunological differences between mild and severe dengue infection, the immunological correlates of clinically inapparent and clinically apparent infections are currently unclear. To fill this knowledge gap, our team initiated a prospective, hybrid cohort-cluster study designed to characterize DENV transmission in multigenerational households in Kamphaeng Phet, Thailand. Enrollment requirements for this study include a household containing a pregnant female, an adult 50 years or older, and a child, with the newborn being enrolled in the study at birth. Annual blood draws from all study participants are obtained starting upon enrollment, supplemented with blood draws taken from each of the family member upon confirmation of a dengue illness in the household through active surveillance. Currently in its eighth year, this study design allows for the investigation of pre- and post-infection DENV-specific immunity, and the detection of inapparent DENV infections. Using virologic and serologic assays we have identified 148 acute infections through active surveillance. Of these, 43 out of 65 primary infections and 16 out of 83 secondary infections were inapparent. Preliminary data has shown a lower titer of IgG and IgM in inapparent samples from both primary and secondary infections when compared to total antibody titers of the same samples, suggesting that there may be more IgA present in the serum from clinically inapparent cases. Using a multiplex serology assay we are assessing the contribution of IgA to the antibody profile following apparent and inapparent DENV infections, as we propose that IgA may be a biomarker associated with less severe DENV infections. These findings would have implications for the identification of correlates of protection from dengue illness, urgently needed for the design and evaluation of DENV vaccines and potential therapeutics.

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## MALARIA-EXPOSED UGANDANS EXHIBIT A DIFFERENTIAL SARS-COV-2-SPECIFIC T CELL RESPONSE

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While SARS-CoV-2 and its interaction with the immune response has been well-studied in resource-rich areas, there are still many unanswered questions about how it affects malaria-endemic areas in sub-Saharan Africa. Compared to other global regions, during the pandemic, hospitalization and death rates have been reported to be much lower in these areas. One possible explanation could be differential immune responses due to different infectious exposures such as malaria. Using samples collected from SARS-CoV-2 exposed Ugandan adults, and similarly aged adults from California, we investigated the SARS-CoV-2-specific T cell response by intracellular cytokine staining (ICS) and an activation-induced marker (AIM) assay. Overall, we found that COVID-19 seropositive Ugandans have a diminished SARS-CoV-2-specific T cell response compared to convalescent Californians. IFNg- and TNFα-producing T cells were predominant in the Californian cohort early on and months after initial infection. However, very low or no IFNg and TNFα production was found in the Ugandan cohort. Furthermore, Ugandans have a heightened IL-10-producing CD4+ regulatory response after polyclonal stimulation. Ongoing research will investigate whether and how other pathogenic exposures, specifically malaria, may influence the T cell response. We hypothesize that previous exposure to malaria mitigates infection with SARS-CoV-2 due to a more 'tolerized' immune response. Potential explanations for our findings include epitope cross-reactivity or down-modulation of an inflammatory response that is implicated in severe COVID-19. Identifying differences in immune responses across populations will be important for future therapeutic innovations and vaccine development.

6035

## 17DD-BASED YELLOW FEVER INACTIVATED VACCINE IN ASSOCIATION WITH THE NS3 HELICASE DOMAIN INDUCES T LYMPHOCYTE RESPONSES AND SEROCONVERSION TO YELLOW FEVER VIRUS IN A MURINE MODEL

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Yellow fever is a disease caused by the Yellow Fever virus (YFV), an arbovirus that belongs to family Flaviviridae, genus Flavivirus. Like other flaviviruses, YFV RNA genome encodes a polyprotein that is cleaved into 7 non-structural and 3 structural proteins that harbor the majority of epitopes involved with the cellular and humoral immune responses. Yellow Fever clinical manifestations range from a mild disease to a severe disease characterized several organ impairment. However, different from other arbovirus diseases, a live-attenuated virus vaccine (17D/17DD) is available to prevent against YFV infections but, in rare cases, the immunization with this vaccine can cause serious adverse effects, such as viscerotropic or neurological diseases. Furthermore, it is not recommended for immunosuppressed individuals. An inactivated YFV vaccine was manufactured using YFV-17DD virus, either in its pure preparation [inactivated (i17DD)] or in combination with a recombinant NS3 helicase peptide (i17DD+NS3Hel). The safety and immunological responses to these inactivated vaccines were evaluated in C57BL/6 mice. Our preliminary results showed that the combination of i17DD+NS3Hel promoted TCD4+ and TCD8+ lymphocyte expansion undistinguishable from the groups that received either inactivated or attenuated YFV-17DD virus. When investigating the response profile of TCD4+ cells, it was observed that this population is more associated with a Th1 profile, which grants cellular immunity to viruses, since no IL-4 expression was detected and IL-2 and IFN-gamma levels were similar to those observed with both YFV-17DD viruses. Regarding the antibody response, 100% of the mice that received the formulation i17DD+NS3Hel seroconverted to YFV. Neutralization and survival testing are ongoing to finalize the vaccine validation. Up to this moment, it was observed that, in mice, the i17DD+NS3Hel vaccine formulation elicits an immune response compared to that observed with

both YFV-17DD vaccines and might be an alternative to vaccination against Yellow fever in individuals in whom the attenuated vaccine is not recommended.

6036

## ANTIBODY-DEPENDENT COMPLEMENT ACTIVATION AND DENV3 DISEASE SEVERITY

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Dengue Viruses (DENV), consists of a family with four serotypes. Dengue is a mosquito-borne agent responsible for causing Dengue Fever (DF), which in a small proportion of cases can further develop into a more severe disease, dengue hemorrhagic fever (DHF), characterized by blood plasma leakage, that can lead to cardiovascular shock and organ failure. Dengue disease overall is an inflammatory-driven pathology, with complement dysfunction having been implicated as playing a role in the progression to DHF. The extent to which antibody-dependent complement activation (ADCA) participates in this process is not fully known. To investigate the ability of dengue antibodies specific to DENV3 NS1 to perform ADCA using a novel bead-based complement assay. In this assay, DENV3 NS1 is bound to fluorescent beads to incubate with patient samples, allowing for the formation of immune complexes. The beads are then incubated with a complement source, allowing for immune complexes to perform ADCA. The beads are then stained with an anti-complement factor 3 (C3) fluorophore-conjugated antibody, and flow cytometry is used to quantify the deposition of C3 fragments. This assay was utilized to quantify ADCA in serial samples from acute and convalescent cases of primary and secondary DENV3 cases, from both DF and DHF. Additionally, antibody endpoint titers were measured by ELISA to determine correlation with ADCA. When comparing the ADCA capacity in the cohort, it is determined that secondary DENV3 infections have higher complement deposition than primary DENV3 infections and that secondary infections with DHF develop the most potent antibodies to activate complement. Antibody titer was shown to have a moderate correlation with C3 deposition and ADCA, as expected. Data utilizing the complement assay supports the hypothesis that secondary DENV3 infections show greater complement deposition, particularly in secondary DENV3 DHF cases during the onset of severe symptoms. Further work will be done with this assay to further explore the potential relationship between ADCA and severe outcomes of dengue.

6037

## STRUCTURE-GUIDED DENGUE VIRUS TYPE 2 SUBUNIT VACCINE DESIGN TO FOCUS ANTIBODY RESPONSE TO POTENT, NEUTRALIZING EPITOPES ON VIRAL ENVELOPE PROTEIN

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The four dengue virus (DV) serotypes are mosquito-borne flaviviruses responsible for dengue fever and dengue hemorrhagic disease. Leading vaccine candidates based on tetravalent live-attenuated DV formulations have had variable efficacy and safety due to an imbalanced response to the DV serotypes in the vaccine and dengue serostatus pre-vaccination. DV subunit vaccines based on recombinant envelope (E) proteins from the four serotypes have also performed poorly, partly because at physiological temperature the secreted wild-type E protein (WT rE) is mainly present as a monomer that does not display quaternary structure epitopes on the native E dimer recognized by potent neutralizing human antibodies (Ab). Using the molecular modeling software, Rosetta, and experimental approaches, we have previously defined a small number of mutations that stabilize DV rE dimer (SD rE) under physiological conditions of vaccination. Here, we report on studies to test if the SD rE vaccine antigen redirects the functionally-neutralizing Ab response to E dimer-dependent quaternary structure epitopes on the infectious virus. Since fusion loop (FL) Ab can cause antibody-dependent enhancement which increases disease severity upon heterologous DV infection, we also tested SD rE with mutated FL. To compare Ab properties induced by different antigens, we immunized mice with DV2 WT rE or SD rE variants. We found that SD-vaccinated mice had significantly higher DV2 IgG binding titer and more than 20-fold greater neutralizing Ab titer compared to WT-vaccinated mice. Using Ab depletion techniques to remove sub-populations of DV-specific Ab and recombinant E protein domain transplant viruses, we mapped the specificity of binding and neutralizing Ab induced by WT and SD rE. Studies are ongoing to compare levels of DV enhancing Ab by different vaccine constructs. Preliminary results indicate that neutralizing Ab from WT-vaccinated mice bind to simple epitopes on rE monomers, while those from SD-vaccinated mice are mostly dimer-specific. Our results demonstrate the promise of structure-guided design to preserve epitopes of interest on dengue subunit vaccines.

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## MOLECULAR ANALYSIS OF THE ANTIBODY REPERTOIRE ELICITED AFTER YELLOW FEVER VACCINATION

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Flavivirus co-circulation and outbreaks have been reported in the Americas during the last decade. Evidence indicates that pre-existing immunity to one virus can influence the disease course of a heterologous virus infection; however, little is understood about the molecular dynamics and prevalence of the cross-reactive antibodies in the repertoire of an endemic population vaccinated against yellow fever virus (YFV). Therefore, here we seek to determine the serological response among YFV, Dengue and Zika viruses following the YFV vaccination of healthy Brazilian donors. We performed YFV, DENV and ZIKV virus neutralization tests (PRNT) and indirect IgG ELISA in longitudinal samples obtained 0, 7, 14, 28 and 180 days after vaccination. Furthermore, we assessed the diversity and clonal expansion of the B-cell repertoire at seven and fourteen days after vaccination. All donors had anti-YFV antibodies that peaked between 14 and 28 days post vaccination. Results from both neutralization and ELISA tests revealed cross-reactive antibodies to ZIKV and DENV viruses before and 28 days after YFV vaccination, with NAb to DENV slightly higher (approximately 3-fold higher) than NAb to ZIKV among all individuals. The DENV and ZIKV seroprevalence in this cohort ranged from 75% and 50% (IgG ELISA) to 100% (PRNT), respectively. Analysis of the B cell clonotypes identified 600 shared clones among the individuals (75%). Most of these clonotypes were found expanded and presented greatest diversity 7 days after the vaccination. Taken together, we observed a variable degree of serological cross-reactivity across three flaviviruses, which we speculate is related to donor YFV vaccination status, endemic co-circulation of ZIKV and DENV, or both. These observations may have implications for accurate diagnostic testing, for vaccination strategy and for the study of host-virus interactions.

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## CONSERVED MONOCYTE RESPONSES TO ACUTE RNA VIRUS INFECTION

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The 21st century has seen five virus-driven pandemics. Despite emerging viruses constantly threatening global health, we remain largely unprepared for the next pandemic. Previous studies have shown that emergency myelopoiesis and monocyte dysregulation are associated with severe COVID-19. In this study, we aimed to identify conserved and

diverse features of antiviral immunity through single-cell proteomic and transcriptional profiling studies across acute RNA viral diseases. Our analysis revealed shifts in monocyte populations towards increased CD14+ monocytes across viral infections in humans and macaques caused by various viral species, including Lassa, Ebola, Marburg, influenza, Zika, and dengue. We integrated single-cell RNA sequencing (scRNA-seq) data from all publicly available datasets that profile non-COVID-19 viral diseases, including blood samples from 38 individuals infected with dengue, influenza, or RSV with an additional 49 samples from SARS-CoV-2-infected individuals. By using this integrated data, we found conserved shifts in monocyte phenotype that were correlated with disease severity and defined by genes that also mark myeloid-derived suppressor-like cells and bacterial sepsis-induced monocytes. This signature was observed across disease progression in Ebola-challenged rhesus macaques and could be detected across over 2000 bulk RNA-seq blood samples collected from virally-infected humans and macaques, allowing us to differentiate between infected and uninfected subjects. Our study demonstrates that integrative single-cell profiling can identify dysfunctional immune responses shared across different viral infections and yield detailed insights into the underlying monocyte-specific pathological processes that may be targeted in a broad-spectrum manner. We expect that our findings will contribute to the development of effective strategies for combating future viral pandemics.

**6040**

## ANTIBODIES AGAINST THE SARS-COV-2 DELTA VARIANT SHOWED CROSS-REACTIVITY TO INFLUENZA VIRUSES

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According to sparse information from various countries, the seasonal influenza virus circulation has drastically decreased during the COVID-19 pandemic. The S protein of SARS-CoV-2 is heavily glycosylated, much like type I fusion proteins of influenza virus hemagglutinin. These glycosylation patterns can either result in immune evasion or viral neutralization by generating cross-reactive antibodies. Here, we show the cross-reactivity of anti-SARS-CoV-2 antibodies against influenza viruses. Serum samples were collected from 311 SARS-CoV-2 infected individuals. The samples were tested for antibody titers against SARS-CoV-2 by ELISA &seasonal influenza virus strains (influenza A/H1N1, A/H3N2, B/Yamagata, &B/Victoria) using a Hemagglutination Inhibition Assay (HAI). In addition, SARS-CoV-2 antibody-positive but Influenza antibody-negative samples (n=16) were investigated to determine the SARS-CoV-2 antibody-neutralizing potential against influenza viruses by microneutralization (MN) assay. The SARS-CoV-2 genomes were sequenced using Illumina next-generation sequencing, &an in-silico protein structural analysis was performed to identify epitope &antibody binding similarities between SARS-CoV-2 &influenza viruses. Among 16 samples that didn't contain antibodies against Influenza A strains (H1N1 &H3N2), five showed high (MN titer≥20), &six showed moderate (MN titer≥10) capability to neutralize Influenza A. Subsequent in-silico analysis revealed that most efficient binding (>8 Kcal/mole) was found between the antibodies of SARS-CoV-2 delta variant (ΔG) with influenza A/H1N1 HA (Hemagglutinin), A/H3N2 HA, A/H1N1 NA (Neuraminidase), &A/H3N2 NA glycoproteins with -12.4, -9.3, -10.1, &-11.7 Kcal/mole, respectively. This investigation revealed that neutralizing antibodies of the delta variant cross-reacted with the Influenza A virus, which might protect against influenza viruses &reduce &shift the seasonal influenza circulation during the COVID-19 pandemic. Our findings warrant further study to explain the probable mechanisms of this cross-reactivity.

**6041**

## ROLE OF THE PLACENTA SPECIFIC CHROMOSOME 19 MICRORNA CLUSTER DURING ZIKA INFECTION

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The largest primate and placenta specific C19MC microRNA cluster has been implicated in the development and function of the placenta, and having an antiviral activity for specific viruses. The 2015 outbreak of the emergent flavivirus Zika in the Americas was associated with microcephalia and other congenital malformations. The risk of having congenital Zika was higher at the beginning of pregnancy, whereas the expression of C19MC miRNA cluster increases during pregnancy and is the highest at term. Thus, we hypothesized that, through its antiviral activity, C19MC miRNA could be implicated in the placenta protection of the foetus during pregnancy. The antiviral role of the C19MC miRNA cluster was demonstrated using a novel Knock-Out (KO) model. We generated an in vitro model from JAR human placental choriocarcinoma cell line using the CRISPR/Cas9 system. Through real time quantitative PCR, western blotting, flow cytometry, and immunofluorescence techniques to detect viral infected cells, we showed that C19MC KO cells infected with the Zika virus are more susceptible to viral infections than their wild-type counterparts. Additionally, the TCID50 assay and flow cytometry analysis showed a significantly higher viral infectivity of the supernatants containing virions released from infected KO cells than from WT cells. This antiviral effect of WT supernatants can be transferred to recipient cells (Vero cell line) by means of extracellular vesicles, suggesting a paracrine antiviral method of action. In conclusion, our study provides a novel KO-model to assess the complex role of C19MC miRNA cluster during viral congenital infections, and whether it can act, in part, via extracellular vesicles. Clear understanding of this cluster's antiviral role is key to the development of new and more reliable prognostic and therapeutic tools for viruses with a potential congenital effect.

**6042**

## USE OF A STABILIZED CONFORMATIONAL DENGUE VIRUS SEROTYPE 2 ENVELOPE ANTIGEN TO ISOLATE MEMORY-DERIVED NEUTRALIZING MONOCLONAL ANTIBODIES FROM A CONVALESCENT PATIENT.

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Dengue is a mosquito-borne disease caused by four serotypes of dengue virus (DENV), each of which can elicit serotype-specific or cross-reactive antibodies that target the envelope (E) structural glycoprotein. Understanding how DENV-specific B cell responses drive antibody-mediated protection is important to vaccine design. To directly study this, we used a recently developed recombinant E protein stablized dimer (recED) derived from DENV serotype 2 to probe DENV-specific memory B cells from a patient recovered from dengue. Complementary approaches including direct sorting of recED-binding memory B cells followed by antibody gene sequencing or culture and characterization of secreted antibody from recED-sorted cells yielded 25 novel lineages of DENV-reactive monoclonal antibodies (mAbs), at least three of which exhibited neutralizing activity against multiple serotypes including DENV2. Unlike other DENV cross-reactive mAbs, some of which show neutralizing activity against Zika virus, the characterized recED-binding mAbs recognized, but did not neutralize Zika virus. These results indicate that recED can be used

to tag DENV2-reactive surface immunoglobulin-positive memory B cells and isolate DENV-neutralizing antibodies from patients. These data extend in vivo studies showing that immunization of animals with recED generates DENV2-neutralizing responses. We hypothesize that this antigen can be used to define the landscape of DENV-specific B cell responses in defined vaccination and viral challenge settings.

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## TISSUE-SPECIFIC T-CELL RESPONSES AMONG 44 FATAL COVID-19 CASES

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T-cells play an essential role in recognizing and clearing viruses from infected tissues. The breadth and depth of circulating T-cell responses among patients with coronavirus disease 2019 (COVID-19) has in part been described. However, little is known about the distribution and evolution of T-cell responses within and across relevant tissues in patients with severe COVID-19. To fill this knowledge gap, we evaluated T cell clonality in lung, thoracic lymph node, and peripheral blood mononuclear cell (PBMC) samples collected from a cohort of 44 patients who died with or from COVID-19. We extracted DNA from formalin fixed paraffin embedded tissues collected at autopsy and from PBMCs collected perimortem. T-cell receptor beta sequencing was performed using the Adaptive ImmunoSeq platform. In preliminary analyses, significantly higher total unique T-cell rearrangements were observed in lymph node compared with lung tissues and PBMCs across all patients, indicative of highest T-cell diversity in lymph nodes. We observed evidence of clonal expansion (>1% of T-cell population) in 35 of 37 (95%) lung tissues, 14 of 37 (38%) lymph node tissues, and 35 of 41 (85%) PBMC samples. Additional analyses will characterize the impact of relevant metadata (e.g., demographic variables, viral load, interval from illness to death) on the breadth and depth of T-cell responses, including severe acute respiratory syndrome coronavirus-2 specific T-cell responses, within and across patients. This approach will provide unique insights into the evolution and specificity of T-cells responses among patients with severe and fatal COVID-19.

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## SEROPREVALENCE OF SARSCOV2 ANTIBODIES IN THE GENERAL POPULATION OF BAMAKO, MALI

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The coronavirus disease 2019 (COVID-19) pandemic is not yet known with certainty in terms of its major epidemiologic, clinical, and serologic characteristics, including its ability to spread and its severity in the general population. This study assesses the seroprevalence of total antibodies and understand associated factors for SARS-CoV-2 infection in the general population of Bamako, the capital and epicenter of COVID-19 in Mali, with ultimate goal of drawing conclusions on the magnitude of the pandemic and contributing to the improvement of control strategies. A cross-sectional survey was done in September 2022 to collect socio-demographic and clinical data, and blood samples. Seroprevalence of SARS-CoV-2 of antibodies anti-Spike and anti-RBD were determined by ELISA. RedCap system was used for data recording and analyzed with RStudio. A total of 3601 participants were enrolled, Mean age of participants was 33.5± 15.9-year-old; the sex ratio was 3.6 for female. Age groups such as 20-29 and 30-39 years were most representative with 28.9% (n=1043) and 26.9% (n=967), respectively. Overall, COVID-19 vaccine coverage among participants was 36%, consisting of Covidshield AstraZeneca (AZ), Johnson & Johnson (J&J), Sinovac, and BioNTech Pfizer vaccines. Overall, seroprevalence of SARS-Cov-2 antibodies to S and RBD were markedly high in the general population, with 98% and 97% respectively. Factor such as male sex earlier age (1-9 year-old were associated with lower antibody responses to S and RBD, whereas having previous contact with COVID-19 patient and receiving COVID-19 vaccine increased odds for antibody responses. This study showed high seroprevalences of antibodies anti-Spike and anti-RBD in general population of Bamako and identified some factors that may influence antibody responses. Our findings lead to conclude that SARS-CoV-2 exposure in the general population is much higher than indicated by case-based surveillance, and that the magnitude of the pandemic is underestimated in Mali.

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## IMPACT OF DENGUE VIRUS STRAIN AND MATURATION STATE ON DETECTION OF NEUTRALIZING ANTIBODIES INDUCED BY NATURAL INFECTION AND VACCINATION

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Dengue vaccine developers have relied on standard plaque or focus reduction neutralization tests (PRNT/FRNT) to assess immunogenicity of each of the 4 dengue virus (DENV) serotype components in tetravalent vaccines. However, leading vaccines have performed poorly in baseline dengue seronegative (SN) children despite stimulating neutralizing antibodies (NAbs) to DENV1-4 detected by standard PRNT/FRNT, which uses lab-adapted reference viruses. There are structural differences between DENVs produced in humans (predominantly mature) vs. cell lines (partially mature). The partially mature state of cell-culture produced virions may lead to neutralization in the standard assay by cross-reactive (CR) fusion loop (FL), potentially disease enhancing Abs. The goals of the current study were to compare the impacts of DENV strain (lab-adapted vs. low-passage) and maturation state on levels of NAbs to each serotype detected by the FRNT. We controlled for maturation state by growing virus stocks in regular Vero cells, producing partially mature virions, and Vero cells engineered to over-express furin (DENV processing protease), producing fully mature virions. We found that DENV CR human monoclonal Abs (MAbs) targeting the FL were maturation state sensitive, neutralizing partially mature virions better than fully mature virions. In contrast, neutralization by MAbs to serotype CR quaternary dimer dependent epitopes were maturation insensitive. Human sera from individuals recently exposed to a primary DENV infection also had higher titers of CR NAbs (vs. serotypes not responsible for primary infection) to partially mature lab strains compared to fully mature recent clinical isolates. Our results demonstrate that DENV NAbs detected using mature, low-passage clinical strains more accurately reflect the serotype responsible for infection compared to the current standard practice. We propose that using mature low passage clinical strains will also lead to more accurate determinations of the immunogenicity and protective potential of each serotype component in tetravalent DENV vaccines in comparison to the current standard.

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## MOLECULAR SURVEILLANCE OF SULFADOXINE-PYRIMETHAMINE AND AMODIAQUINE RESISTANCE MARKERS IN KARAMOJA REGION, AN AREA IMPLEMENTING SEASONAL MALARIA CHEMOPREVENTION IN NORTHEASTERN UGANDA

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The effectiveness of seasonal malaria chemoprevention (SMC) with sulfadoxine-pyrimethamine and amodiaquine (SPAQ) recommended for age groups at high risk of severe malaria, in areas where malaria transmission is highly seasonal, is threatened by widespread drug resistance in east and southern Africa. As part of a phased implementation study to evaluate the protective effectiveness of SMC with SPAQ in Karamoja region, we assessed the changes in the prevalence of resistance markers, over two annual rounds of SMC. Baseline and end-line health facility-based, cross-sectional surveys were conducted before and after SMC distribution for both rounds. In round one, 300 P. falciparum-infected dry blood spots were collected at each time point from symptomatic children aged 3 to 59 months from three districts while in round two, 750 samples using similar protocols were collected from five districts and analyzed for DNA purification using chelex method. Quintuple mutants (PfDHFR 59R, 51I, 108N, and PfDHPS 437G, 540E) that mediate moderate SP resistance are highly prevalent (range: 78-100%) and remained unchanged. The double mutants (PfDHFR 164L and PfDHPS 581G), the acquisition of which leads to significant pyrimethamine resistance, were rarely seen (prevalence range: <1%). This remains reassuring on the utility of pyrimethamine in SPAQ-SMC in Karamoja. The prevalence of PfCRT 76T, the principal mediator of 4-aminoquinoline (amodiaquine and chloroquine) resistance was low (~5-13%). Similarly, mutations in PfMDR1 (86Y and 1246Y) that moderate aminoquinoline resistance were low (<3%). 184F, a mutation with an unclear role in aminoquinoline resistance remains at a stable prevalence of up to 30%. While SP resistance is shown to be high, thus far SMC does not seem to impact the resistance profile across two annual rounds.

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## MOLECULAR MARKERS ASSOCIATED WITH ANTIMALARIAL DRUG RESISTANCE AND DISTRIBUTION OF MSP1 AND MSP2 ALLELIC FAMILIES IN RURAL ENDEMIC SETTINGS, NORTHWESTERN BURKINA FASO

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Antimalarial drug resistance surveillance through the characterization of associated genes is crucial for malaria control in areas of high endemicity and transmission. The high diversity of Plasmodium genotypes characterizes the dynamic of malaria transmission and demonstrates the complexity of malaria vaccine development. The aim of the present study was to evaluate the effect of Plasmodium genetic polymorphism on the markers associated with antimalarial resistance in endemic settings. Blood-thick smears and dried blood samples on the paper filters were collected from populations living in Nouna and Kodougou, two localities of the Demographic and Health Surveillance System of the Nouna Health Research Centre. The blood smears were used for malaria microscopic examination. Filter papers from malaria-positive samples were used for DNA extraction by Qiagen and amplified by nested PCR for the search of genetic polymorphism alleles. The PCR-RFLP was used to characterize the molecular markers associated with antimalarial drug resistance. The Spearman's correlation test was used to compare allelic frequencies, MOI and mutant pfcrt, pfmdr, dhfr and dhps genes prevalence. Out of the 285 samples positive for Plasmodium falciparum, 279 were successfully genotyped for markers associated with antimalarial drug resistance. Significant positive correlations were found between msp1 allele frequencies, mean multiplicity of infection (mMOI), and pfcrt mutant prevalence. No significant correlations were found between msp1 and msp2 alleles, mean multiplicity of infection (MOI), and the prevalence of pfmdr, dhfr, and dhps mutants. Overall, this study showed a weak relationship between the different parasitic clones of Plasmodium falciparum and the expression of mutant pfmdr, dhfr and dhps genes. The correlation with the chloroquine resistance gene is certainly due to the adaptation of the malaria parasite to this molecule, probably linked to its long-standing use as an antimalarial.

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## MOLECULAR MARKERS OF ANTIMALARIAL RESISTANCE, AN EXTENSION OF THERAPEUTIC EFFICACY MONITORING IN BURKINA FASO, 2021

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Data on molecular markers of resistance complement clinical data from therapeutic efficacy studies (TES). This study examined the prevalence of polymorphisms in genes associated with susceptibility to antimalarials used for treatment or chemoprevention using samples collected in a TES carried out in three sites in Burkina Faso: Niangoloko, Nanoro and Gourcy. Febrile children with uncomplicated P. falciparum monoinfection were recruited, treated with artemether-lumefantrine (AL), dihydroartemisinin-piperaquine or artesunate-pyronaridine, and followed for 28 or 42 days. Blood spots were collected on filter paper at Day 0 (D0) and follow up visits. All D0 samples and Day of Failure samples were included in molecular analyses, including 638 paired samples from treatment failures and 750 D0 samples from nonfailure patients combined into 152 pools for analysis. PCRs were performed to amplify full length pfk13, pfdhps, and pfmdr1 genes. Sequences were analyzed at loci representing the major reportable single nucleotide polymorphisms for each gene. There was no evidence of any pfk13 mutations associated with artemisinin resistance in the analyzed samples. The pfmdr1 Y184F mutation associated with reduced susceptibility to lumefantrine was found in the majority (59-64%) of samples. The pfdhps A437G mutation was present in all sites at rates ranging from 88-90%. The key K540E and A581G pfdhps mutations, defining the canonical "quintuple" and "sextuple" mutants associated with sulfadoxine-pyrimethamine (SP) resistance were present at low levels (<4%). The pfdhps haplotype VAGKGS, recently documented spreading westwards across the Sahel region of North Africa, was found in 7 (6%) samples from Nanoro, 2 (2%) samples from Gourcy, and 1 (1%) sample from Niangoloko. These results are consistent with continued high efficacy of artemisinin derivatives in Burkina Faso and provide insight to the suboptimal efficacy of AL. There is no indication of high-level SP resistance in any of the three sites. Continued monitoring, including molecular surveillance, is critical for decision-making on effective treatment and chemoprevention policy.

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## ASSESSMENT OF PLASMODIUM FALCIPARUM CLONALITY AND DRUG RESISTANCE IN AN ARTEMETHER-LUMEFANTRINE DRUG EFFICACY TRIAL IN NORTHWEST ETHIOPIA

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Despite recent progress in malaria elimination, 68% of Ethiopia's population still lives in malarious areas. High-transmission areas theoretically increase the likelihood of the spread of resistant strains that may compromise the therapeutic effect of artemisinin combination therapies (ACTs). To evaluate this, a total of 162 Plasmodium falciparum isolates from 54 patients across three time points (day-0, day-3, and day-7 of treatment) were identified from an Artemether-Lumefantrine (Co-Artem) drug efficacy trial conducted in Northwest Ethiopia in 2018. The samples were analyzed to determine the complexity of infection (COI) and haplotype profiles using the genetic markers msp2, ama1-d2, and ama1-d3, the presence of kelch13 mutants, and gene copy number (GCN) of pfmdr1 and pfplasmepsin2 associated with antimalarial resistance. Infections were polyclonal at day-0 based on the msp2 (3.93±0.24), ama1-d2 (3.13±0.23), ama1-d3 (1.70±0.16) haplotype profiles. An overall decrease in the COI was observed across all the haplotype markers at subsequent trial time points. A prevalence of 21.95% (3.27 times higher than the previous surveillance in 2014) was observed for the R622I kelch13 candidate SNP rendering partial artemisinin resistance. In addition, we detected the M476I (2.94%), Y493H (2.43%), P574L (2.43%), and C580Y (33.3%) validated SNPs for partial artemisinin resistance. An elevated copy number of the pfmdr1 gene was observed at day-0 (95% C.I.=[1.07,1.33]) but not for the plasmepsin2 gene (95% C.I.=[0.93, 1.02]). When comparing day-0 and day-7, the COI ($p<0.0001$), prevalence of kelch13 mutants ($p<0.0001$) and GCN of pfmdr1 and pfplasmepsin2 ($p<0.0001$) decrease following Co-Artem treatment. Our study confirms that Co-Artem remains effective in this region of Ethiopia. However, genetic analysis reveals a high COI and multiple kelch13 mutants, which have the potential to confer greater artemisinin resistance with time.

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## TEMPORAL GENOMIC ANALYSIS OF PLASMODIUM FALCIPARUM REVEALS INCREASED PREVALENCE OF PFAP2MU S160N AND PFMDR1 Y184F MUTATIONS ASSOCIATED WITH REDUCED PARASITE CLEARANCE OR SUSCEPTIBILITY TO LUMEFANTRINE IN CHOMA DISTRICT, SOUTHERN PROVINCE, ZAMBIA

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The emergence of anti-malarial drug resistance is a potential major impediment to achieving malaria control and elimination goals in Africa. The current intensive elimination activities, especially the use of antimalarial drugs such as artemisinin-based combination therapies (ACTs) in areas nearing pre-elimination like southern Zambia, is exerting significant selective pressure on malaria parasite populations that could facilitate the emergence of resistance strains. Analysis of temporal trends in drug resistance molecular markers can assist policy makers in choosing efficacious antimalarials and slow the emergence and spread of resistance. In a region of southern Zambia with low and seasonal transmission, we genotyped 441 Plasmodium falciparum samples collected from 2012 to 2018 from a cluster of 8 health centers using molecular inversion probes targeting 815 loci across 14 drug resistance genes. None of the isolates carried WHO-validated or candidate Kelch13 propeller mutations associated with artemisinin resistance, including recently reported mutations (R561H, A675V and C469Y) from Africa. In addition, none of the isolates carried known mutations associated with chloroquine (CQ) resistance at Pfcrt gene codons (72-76). However, 13% of isolates carried Pfap2mu S160N (a mutation associated with delayed ACT clearance in Africa), and 41% carried Pfmdr1 Y184F (a mutation associated with reduced susceptibility to lumefantrine), with increased prevalence between 2015-2018. More than 90% of sequenced samples carried N51I, C59R, and S108N (IRN triple mutants), and 81% carried A437G and K540E (GE double mutants) with less temporal variation. In conclusion, P. falciparum strains circulating in southern Zambia remain susceptible to ACTs and CQ, but the high prevalence of mutations associated with delayed ACT clearance or reduced susceptibility to lumefantrine warrants close monitoring of artemisinin and partner drug efficacy. Moreover, the high prevalence of mutations associated with SP suggests there may be a need to revise current treatment guidelines for intermittent preventive treatment of malaria in pregnancy.

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## THE IMPACT OF SMC ON PLASMODIUM FALCIPARUM RESISTANCE TO SULFADOXINE PYRIMETHAMINE (SP) AND AMODIAQUINE (AQ) OVER A 2 YEAR PERIOD OF SMC IMPLEMENTATION IN NORTHERN MOZAMBIQUE

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Malaria is a significant public health problem in Mozambique, with high incidence rates and a high number of cases leading to many deaths, particularly in children under 5 years old. Seasonal malaria chemoprevention (SMC) is recommended by WHO to prevent malaria, with Sulfadoxina-Pirymethamine (SP) plus Amodiaquine (AQ) being the approved treatment. SMC can reduce malaria cases by 75% and has been recommended in areas with high malaria incidence in children since 2012. However, resistance to SP and AQ has been found in Mozambique, and this study aims to determine the prevalence of resistance markers to SP and AQ before and after the first annual round of SMC implementation. Between November 2020 and February 2021, a survey was conducted before (baseline) and after one complete round of SMC (end line) to measure resistance to SPAQ in symptomatic children under five years with a positive RDT in selected health facilities in intervention and control areas. Four first-level health facilities were selected in each district of the intervention and control areas. Blood samples were collected prior to SMC delivery onto filter papers. Key markers monitored included dhfr, dhps, pfcrt, and pfmdr1. 1198 blood spot samples (598 at baseline, 600 at endline) were analysed for molecular markers of resistance to SP and AQ. The pfdhps gene showed SNPs in 99% of samples with sufficient deoxyribonucleic acid (DNA) and all pfdhfr samples had mutations. More than half of samples showed SNP at codon Y184F in pfmdr1, no SNP was found for Pfcrt. No significant differences were observed between groups for SNPs or mutation combinations. Pfcrt mutations were absent, suggesting amodiaquine resistance is not mediated by Pfcrt. SNP combinations of relevant Pfdhps-dhfr mutants were notable among the analysed samples. Long term implications of these results will be much better understood once chemoprevention efficacy study data will be available. Continuous monitoring of SP and AQ resistant markers is crucial as the country scale up the implementation of SMC and introduce other quimioprevention strategies using this drug.

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## ASSESSMENT OF QUANTITATIVE PCR FOR DETERMINATION OF DRUG RESPONSE OF PLASMODIUM FALCIPARUM IN THE ABSENCE OF DNA PURIFICATION

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Surveillance of antimalarial resistance in parasite populations is essential to identify and limit its spread in malaria-endemic communities. Genotyping of molecular markers, Therapeutic efficacy studies and in vitro/ex-vivo studies are commonly used to assess parasite sensitivity to drugs. However, in vivo efficacy studies are expensive and cumbersome, meanwhile, no single assay exists for both molecular genotyping and in vitro/ex vivo testing. Novel assays such as flow cytometry are accurate and fast, they require cumbersome downstream processing to obtain accurate results. Our goal is to develop a quantitative PCR-based molecular assay for determining growth, viability and drug resistance responses using validated molecular tools (qPCR). Parasite growth was assessed by deploying a direct PCR method targeting known parasite genomic regions (varATS & AMA-1). Laboratory-maintained Plasmodium falciparum strains were tested for 50% inhibitory concentrations of Chloroquine, Lumefantrine and dihydroartemisinin. These drugs were tested against laboratory-maintained P. falciparum strains 3D7(chloroquine-sensitive) and Dd2(chloroquine-resistant). Parasitemia was measured following post-drug exposure by flow cytometry and molecular assays. Differential growth relative to the drug-free assays was used to fit sigmoid curves of growth against drug concentration for IC50 determination in comparison to established methods. The IC50 values obtained from the direct PCR method correlated with results obtained from flow cytometry and fluorimetry with R2 values of 0.9996 and 0.9994 respectively. The bland-Altman analysis showed a better agreement between Direct PCR and Fluorimetry (Bias=3.676, Limit of agreement=4.09 - 11.40). Taken together, the direct PCR method allows for molecular detection of parasitaemia and parasite drug susceptibility in the absence of DNA purification. We conclude that upon further optimization this method can be successfully adapted into a handheld PCR-based assay that can be deployed in non-specialized settings for testing of field isolate sensitivity towards antimalarials.

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## COX UNIVARIATE AND MULTIVARIATE ANALYSIS OF THE DETERMINANTS OF PARASITE RECURRENCE BY DAY 28 AFTER REPETITIVE TREATMENT OF UNCOMPLICATED MALARIA WITH ARTEMETHER-LUMEFANTRINE DURING TWO YEARS IN MALI

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In malaria endemic countries, treatment and control of the disease is hampered by the recurrence of parasite. Some people may experience more than ten episodes of malaria by year, necessitating repetitive treatment. The determinants of these recurrences by day 28 after treatment with artemether-lumefantrine are not well characterized. The objective of this work was to investigate the determinants of parasite recurrence after repetitive treatment of uncomplicated malaria case with AL during two years in Mali. This work was part of the WANECAM study. Participants, from 6 months were followed for two years and received AL at the standard dose for each new episode of uncomplicated malaria. Social, demographic, clinical, parasitological and lumefantrine concentration at day 7 variables were used as covariates. Cox univariate and multivariate analysis were used to determine variables associated with the parasite recurrence by day 28 of follow-up. In total 671 participants were treated with AL for 1732 episodes during two years of follow-up. The overall recurrence prevalence was 14.13%. The recrudescence level was less than 1%. The Cox proportional-hazards regression analysis showed that lumefantrine day 7 concentration, age, the high transmission season, the study site, the day 0 parasitemia significantly predicted parasite recurrence by day 28 of follow-up. Our results showed that by day 28 each increase of the natural log of the lumefantrine day 7 concentration had about 40% lower hazard rate to have recurrence (HR = 0.607, (95% CI; 0.499 – 0.739)). Each increase of the age had about 5% lower hazard rate (HR = 0.946, (95% CI; 0.914 – 0.979)). While we found a strong relationship between the high transmission season (June to November) and parasite recurrence There was no recurrence during the low transmission season. These results showed the relationship between different factors that influence parasite recurrence. Understanding these relationships is critical for the development of strategies in malaria control and elimination.

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## HEALTH SEEKING BEHAVIORS AND BELIEFS SURROUNDING MALARIA IN THREE EAST AND SOUTHERN AFRICAN NEW GEOGRAPHIES PILOTING SEASONAL MALARIA CHEMOPREVENTION: A SECONDARY QUALITATIVE ANALYSIS

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Seasonal malaria chemoprevention (SMC) is a World Health Organization recommended intervention that consists of the intermittent administration of antimalarials to children in areas where transmission is highly seasonal. In 2021 and 2022, Malaria Consortium has been conducting hybrid SMC effectiveness-implementation studies to build the evidence base around new SMC in geographies in East and Southern Africa (ESA), and ensure that SMC is deployed safely, effectively, and sustainably in these settings; the approach used in the Sahel region may not be suited for ESA due to greater resistance profile, transmission, and immunity heterogeneities. Preliminary results from these studies show that SMC is an effective and highly acceptable strategy. However, one aspect that was not initially investigated in the qualitative component of these studies, but which provides insightful information on factors influencing interventions success, are the health seeking behaviours and beliefs surrounding malaria. To fill this research gap, an ongoing thematic analysis is being conducted on qualitative data collected between April and December 2022 in the Karamoja region of Uganda, Nampula province in Mozambique and Northern Bahr el Ghazal state in South Sudan. Qualitative methods employed included focus group discussions (FGDs) held with caregivers and community health workers in each setting, and semi-structured interviews held with key informants at various levels of the health systems in the three countries. A total of 41 FGDs and 51 interviews have been analysed. Perceptions, social practices, and religious beliefs surrounding malaria transmission, prevention, and health seeking behaviours have been described for each location and compared against each other, with a focus on identified similarities and differences. Results from this secondary qualitative analysis will contribute to the refinement of future SMC implementation campaigns with context-specific information for ESA geographies. Findings could be additionally used to generate hypothesis for further community-centred interventions.

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## RARE PLASMODIUM FALCIPARUM CORONIN GENE MUTATIONS FOLLOWING ARTEMISININ (ART) TREATMENT OF MALARIA IN SOUTH WESTERN NIGERIA

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Non-Pfkelch13 parasite protein variants have been implicated in artemisinin (ART) resistance, but not in Africa. Genetic markers underlying in vivo reduced ART efficacy among African P. falciparum populations are currently unclear. We investigated SNPs in Plasmodium falciparum actin-binding protein (Pfcoronin) associated with in vivo ART tolerance in Nigeria. Seven isolates showing parasitaemia after Day 3 in a 28-day therapeutic efficacy study of artemether-lumefantrine among 51 volunteers in Lagos, Nigeria were investigated. Molecular diagnosis was done by conventional and real-time PCR amplification of Pf18S rRNA gene, var acidic terminal sequence, telomere-associated repetitive elements-2 and coupled conventional and real-time Pf18S rRNA PCR. Twelve neutral P. falciparum microsatellite loci genotyping were analyzed to confirm recrudescence in comparison with msp2 genotyping. We genotyped drug resistance targets (DHFR_51, DHFR_59, DHFR_108, DHFR_164, MDR1_86, MDR1_184, DHPS_581 and DHPS_613), and sequenced PfCoronin bi-directionally for presence and association of mutations with ART tolerance. Molecular techniques employed detected P. falciparum infections. One infecttion was recrudescent by microsatellites analysis out of the four identified as recrudescent infections by msp2 genotyping. Presence of the drug resistance-associated haplotypes, pfdhfr/pfdhps/pfmdr1 (108T/N/51I/164L/59R/581G/86Y/184F) was observed in two samples. He, allelic diversity, for each microsatellite locus from pre- and post-drug administration, revealed no significant difference in the mean He (P = 0.19, Mann-Whitney test). Significant LD (IAS = 0.2865, P=0.02, Monte Carlo simulation) around the neutral microsatellite loci was observed. Seven new Pfcoronin SNPs (V55L, V67E, I68G, K69G, L77I, D154Y and E200Q) were found. SNPs here reported may guide investigations on mechanisms of emerging African ART resistance.

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## CHANGES IN SUSCEPTIBILITY OF PLASMODIUM FALCIPARUM TO LUMEFANTRINE IN EASTERN AND NORTHERN UGANDA OVER TIME

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Artemisinin-based combination therapies, the first line treatments for uncomplicated malaria, are threatened by emergence of artemisinin partial resistance in northern Uganda, where high prevalence of the PfK13 469Y and 675V mutations has been documented. Of additional concern, we recently reported decreased susceptibility to the key partner drug lumefantrine in northern, compared to eastern Uganda. To assess changes over time, we compared susceptibilities to 7 standard antimalarials of P. falciparum isolates collected from symptomatic patients in 2021 and 2022 in Patongo Health Centre in northern Uganda, and Tororo District Hospital and Busiu Health Centre, in eastern Uganda. We utilized 72-h growth inhibition assays with SYBR green detection and genotyped samples using targeted deep sequencing to identify linked genotypes of interest. With growth inhibition assays, median IC50s for lumefantrine were 14.7 (n=49) and 14.9 nM (n=94) in 2021 and 2022, respectively, in northern Uganda, compared to 3.0 (n=378), 5.1 (n=365), 6.2 (n=151) and 10.1 nM (n=144) in 2010-2013, 2016-2019, 2021, and 2022, respectively, in eastern Uganda. For 2021 and 2022 combined, median IC50s were significantly greater in northern vs. eastern Uganda (p =0.0001). Considering other standard components of artemisinin combination therapies (dihydroartemisinin, amodiaquine, piperaquine, pyronaridine, and mefloquine), IC50s were similar between northern and eastern Uganda. Preliminary molecular inversion probe sequencing of 70 genes of interest suggested that mutations in PfMDR1, PfK13, and falcipain cysteine proteases are associated with differences in lumefantrine susceptibility. The identified decreased susceptibility of P. falciparum to lumefantrine in northern Uganda is of great concern, as it suggests decreased antimalarial efficacy of artemether-lumefantrine, the first line antimalarial therapy in Uganda. Highlighting the need for continued surveillance of resistance patterns to inform on future trends, necessary for malaria control strategies.

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## EX VIVO SUSCEPTIBILITY OF PLASMODIUM FALCIPARUM ISOLATES TO STANDARD ANTIMALARIALS IN BOBO-DIOULASSO, BURKINA FASO

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Malaria remains the leading cause of morbidity and mortality in Burkina Faso. The country utilizes artemether-lumefantrine, artesunate-amodiaquine, and recently dihydroartemisinin-piperaquine as first line therapies for uncomplicated malaria. Seasonal malaria chemoprevention (SMC) with amodiaquine plus sulfadoxine-pyrimethamine is implemented country-wide in children 5 years and under during the transmission season. With concerns regarding potential resistance to many classes of antimalarials, monitoring the efficacy of available antimalarial drugs is a high priority. We measured ex vivo IC50s for 11 compounds utilizing 158 isolates collected from patients aged 3-60 years old presenting with uncomplicated Plasmodium falciparum malaria in Bobo-Dioulasso, Burkina Faso from July to December, 2021 and 2022. Assays utilized a standard 72 h growth inhibition microplate assay with SYBR Green detection, with results compared to those with laboratory-adapted control parasite strains. Isolates (median IC50 [range], nM) were generally highly sensitive to: chloroquine (9.9 [0.87 - 262.8]), monodesethylamodiaquine (21.5 [0.6 - 205.6]), piperaquine (6.3 [1.2 - 211.5]), lumefantrine (7.4 [0.5 - 78.2]), mefloquine (7 [0.2 - 29.9]), atovaquone (0.2 [0.01 - 1.0]), dihydroartemisinin (3.7 [0.5 - 24.7]), pyronaridine (3.0 [0.1 -36.7]) and quinine (49.8 [1.5 - 637.7]). With a few exceptions, parasites were resistant to pyrimethamine (32,649 [33.2 - 191,190]) and cycloguanil (844[3.6 - 8265]). All median IC50 values were similar to those reported from our recent ex vivo studies in eastern Uganda, and suggested good antimalarial efficacy of tested drugs except for pyrimethamine and cycloguanil. However, it is noteworthy that the median IC50 for monodesethylamodiaquine was greater than that recently reported in Uganda (7.1 [1.1 - 202]; p <0.0001), possibly related to widespread use of amodiaquine as part of SMC in Burkina Faso. Genotyping of isolates to characterize key mutations associated with antimalarial drug resistance is underway.

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## LACK OF CORRELATION BETWEEN IN VITRO POTENCY AND IN VIVO EFFICACY OF MADURAMICIN AGAINST PLASMODIUM LIVER STAGES

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Current strategies for anti-malarial drug development primarily focus on targeting the asexual and sexual stages of the parasite. Maduramicin, a polyether ionophore used as a coccidiostat in poultry has been shown to exhibit potent anti-plasmodial activity in vitro across Plasmodium species and stages with an IC50 of <14nM against both replicating (schizont) and dormant (hypnozoite) liver stages, and blood-stages of the parasite. In our efforts to further evaluate the efficacy and stage specific activity of maduramicin in vivo, wild type mice were infected with 2.5*104 P. berghei (Pb-GFP-luc) sporozoites and treated orally with maduramicin at timepoints corresponding to different stages of infection. Whole body in vivo imaging of mice was done at 24, 48 and 72 hours post infection, and thin blood smears stained with Giemsa were analyzed to determine blood parasitemia. While prophylactic administration of maduramicin prior to sporozoite infection fully blocked the formation of liver stages, delayed treatment after establishment of early or late liver-stages proved ineffective. Bioluminescence signal was detected in livers of the mice at 24 hours post treatment with an increase in signal at 48 hours, indicating a surprising lack of activity against liver-stages. Maduramicin was potent against blood-stages in vivo with animals being parasite free three days post drug administration with no recrudescence. Contrary to its high in vitro potency, a similar lack of activity against liver stages was observed in human liver-chimeric mice infected with P. cynomolgi, a strain that produces dormant hepatic hypnozoites. Parasite burden in treated livers could be quantified by qPCR with both schizonts and hypnozoites being detected by immunofluorescence assays four days post treatment, indicating the absence of liver-stage clearance. While further investigation is required to understand this disparity between in vitro and in vivo activity of maduramicin, our studies characterizing ionophores in murine malaria models highlight potential challenges in repurposing these molecules towards intervention strategies for malaria.

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## MALARIA DRUG RESISTANCE MARKERS MOLECULAR SURVEILLANCE USING ANOPHELES MOSQUITOES IN BURKINA FASO

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Plasmodium resistance to antimalarials is hampering the fight against malaria in endemic countries. Here, we screen multiple antimalarial drug resistance genes (pfmdr), sulfadoxine pyrimethamine resistance markers (dhfr and dhps) within Anopheles populations to highlight the potential dissemination of these drug resistance makers. Anopheles mosquitoes were collected by using CDC light traps and manual collections in the health district of Nouna in Burkina Faso. The heads/thoraxes were used for the detection of Plasmodium falciparum infection. Positive samples were subjected to PCR-RFLP to assess their drug resistance polymorphisms. Plasmodium infection rate in Anopheles vectors was 5.5% during this study. For the pfmdr genes, the prevalence was 7.4%, 83.4% and 9.2% for mutants, wild type and hybrid. For the dhfr gene, the prevalence of mutant alleles was 4.02%, 8.16% and 12.5% for codons 51, 59, 108 respectively. Mutations on codons 437 and 540 of the dhps genes were also observed during this study with a prevalence of 14.3% and 2%. These mutations have been notified both in An. gambiae, the major vectors of malaria, and in An. nili another important vector in this region. This study highlights the level of antimalarial resistance genes in mosquitoes. The presence of mutant alleles shows the need for regular monitoring of these molecular markers.

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## PFCORONIN MUTATIONS CONFER ARTEMISININ RESISTANCE IN PLASMODIUM FALCIPARUM BY ALTERING ACTIN HOMEOSTASIS: A POTENTIAL NEW PLAYER IN THE ENDOCYTIC AND VESICULAR TRANSPORT PATHWAY

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Pfcoronin mutations drive in vitro evolved artemisinin (ART) resistance in Senegalese P. falciparum isolates (coroninR100K/E107V). Coronin interacts with the cytoskeleton in other organisms, but its function in Plasmodium biology is not yet understood. To explore the role of Coronin, we immunoprecipitated (IP) Coronin and identified its interaction partners by mass spectrometry. In both CoroninWT and CoroninR100K/E107V parasites, Coronin's major interacting partner was actin, however, interactions with actin were substantially reduced in ring-stage CoroninR100K/E107V parasites. The second most enriched Coronin-interacting partner in CoroninWT rings was polyubiquitin (Ub), but like actin, Ub was significantly less enriched in CoroninR100K/E107V parasites. We hypothesized that ubiquitination might alter turnover or otherwise regulate actin behavior. Interestingly, western blot revealed that CoroninR100K/E107V rings contained 30% less actin than CoroninWT. To better understand which proteins might be ubiquitinated, we used IP with an anti-ubiquitin probe. Actin was highly enriched in CoroninWT line but not in the CoroninR100K/E107V parasites, consistent with the growing body of evidence that actin rearrangements involve regulation by ubiquitylation. We undertook ultrastructure expansion microscopy (UExM) to assess Coronin localization in rings. UExM revealed localization of Coronin at the parasite plasma/vacuolar membrane. Interestingly, we saw strong localization of Coronin at the food vacuole membrane and vesicular structures. To provide additional pharmacological evidence, treating CoroninR100K/E107V parasites with jasplakinolide, an actin depolymerization inhibitor, results in a reversal of the resistance phenotype, demonstrating the role of actin in coronin-mediated artemisinin resistance. These results demonstrate a role in endocytosis and vesicular transport—notably, actin is key to these processes. Altered vesicular transport has previously been implicated in kelch13-linked artemisinin resistance, and these results suggest that Coronin might work in the same pathway.

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## KELCH 13 AND NON-KELCH 13 MEDIATED ARTEMISININ DRUG RESISTANCE

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The emergence of ART resistance in the Greater Mekong subregion earlier and recently in African subcontinent has marked a major setback for malaria elimination worldwide. Although Kelch 13 has been proposed as the primary marker and determinant of artemisinin resistance in Plasmodium falciparum, many other genes and proteins with variable degrees of influence on resistance phenotype have been identified. Based on genome wide association studies, in vitro generated ART resistance parasites and literature review we identify and validate additional markers of ART drug resistance. We are currently characterizing Kelch 13 muations either emerging in South East Asia or specific to African parasites, additionally we are also validating polymorphisms found in other potential markers of ART

resistance like Falcipain 2a, DNA mismatch repair protein PMS1, putative, regulator of initiation factor 2 (eIF2) and Phosphatidylinositol 4-kinase, putative. We have generated genetically modified parasites with single nucleotide polymorphism or haplotypes found in each of these proteins and are currently performing drug assays and parasite fitness assays to study their effect on drug susceptibility and parasite growth. The introduction of these polymorphisms in different genetic backgrounds would reveal any variability present in their artemisinin resistance profiles, food vacoule morphologies and parasite progression. This work would help us elucidate the role of these markers in artemisinin resistance.

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## SUSCEPTIBILITY OF PLASMODIUM FALCIPARUM ISOLATES TO DIHYDROARTEMISININ IN NORTHERN AND EASTERN UGANDA IN 2021 AND 2022

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Artemisinin partial resistance, mediated primarily by mutations in the PfK13 propeller domain, was first reported in Southeast Asia, and has recently emerged in Africa, including Uganda and Rwanda, posing a great challenge to malaria control and elimination. We compared the prevalence of PfK13 mutations and ex vivo dihydroartemisinin (DHA) susceptibility of P. falciparum isolates collected in 2021 and 2022 from northern (N) Uganda, where resistance-associated PfK13 C469Y and A675V mutations have emerged, and eastern (E) Uganda, where such mutations have been rare. We collected whole blood samples from patients aged > 0.5 years with confirmed P. falciparum mono-infection attending Patongo Health Centre III in N. Uganda, and Tororo District Hospital and Busiu Health Centre IV in E. Uganda in May-August 2021 and March-July 2022. We evaluated 103 (N=65, E=38) and 160 (N = 110, E = 50) samples in 2021 and 2022, respectively. The pfK13 gene was sequenced using molecular inversion probe assays. Susceptibilities were determined with the ex vivo ring-stage survival assay (RSA; percentage survival, relative to controls, 66 h after a 6 h 700 nM pulse of DHA) and the standard 72 h microplate growth inhibition assay to estimate IC50 values. In N. Uganda, the prevalence of PfK13 mutations decreased (C469Y 34% to 10%, A675V 13% to 5%), RSA results were similar (median survival 3.5% to 3.1%) and IC50 values increased slightly (median IC50 2.3 nM to 3.5 nM) from 2021-22. In E. Uganda, the prevalence of PfK13 mutations was stable (C469Y 3% to 2%, A675V 3% to 4%), RSA survival increased (median survival 2.2% to 5.2%) and IC50 values increased (median IC50 1.5 nM to 2.5 nM) from 2021-22. The presence of C469Y or A675V mutations was associated with RSA values >5% in 2021, but not in 2022. Our results demonstrate persistent artemisinin partial resistance in northern and eastern Uganda, but with only a modest correlation between PfK13 mutation prevalence and drug susceptibility measures, highlighting the possible involvement of non-PfK13 mutations and the need for ongoing surveillance and monitoring of resistance patterns to inform malaria control strategies.

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## DECREASED EX VIVO SUSCEPTIBILITY OF PLASMODIUM VIVAX TO CHLOROQUINE IN NORTHWEAST COLOMBIA

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Plasmodium vivax was responsible for 247 million cases of malaria worldwide in 2021, including severe cases and deaths. This is due to the difficulty in controlling this species. In Colombia, chloroquine has been used as the first-line treatment for P. vivax. However, in 2001 therapeutic failure was reported in 11% of the patients evaluated, therefore, there is a need for surveillance to determine the in vitro susceptibility of P. vivax to chloroquine (CQ). In this study, an ex vivo short culture was established from clinical P. vivax isolates with a duration of 48 hours to evaluate the in vitro susceptibility to CQ against isolates of P. vivax obtained in the municipality of Tierralta, Córdoba, Colombia. The schizonts maturation method (WHO microtest) was used and compared with SYBR Green method for IC50 determination. Seven serial concentrations in triplicate were evaluated. The inhibitory concentrations of 50% were 37.15 nM for chloroquine. However, 2/19 had IC50 values greater between 100nM-200nM and only one showed a IC50 more than 200nM, in two isolates it was not possible to determine the IC50 value. The SYBR Green and maturing methods give similar results. In conclusion, an in vitro short-term culture was established, the clinical isolates evaluated were susceptible to CQ (66.6%), although 33.3% of the isolates showed very low susceptibility.

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## THE CONTINUED EXPANSION OF ARTEMISININ PARTIAL RESISTANCE MUTATION KELCH13 561H AND EMERGENCE OF 675V IN RUKARA IN 2021

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Plasmodium falciparum kelch13 (Pfk13) propeller-domain mutations that confer artemisinin partial resistance have emerged in Africa, with the first reported mutation being Pfk13-R561H in Rwanda. These mutations threaten control and elimination efforts and increase the likelihood of the emergence of partner drug resistance. Given this, it is vital to track their emergence and spread to inform modeling and public health efforts. In order to provide an update of the artemisinin resistance as well as potential RDT resistance we genotyped samples collected in 2021 from Rukara, Rwanda, the site where R561H was first detected in samples from 2014. Clinically validated artemisinin partial resistance mutations were found to be increasing in prevalence compared to previous reports from 2014. While the R561H mutation was observed at 23.5% (20/85 infections), A675V was seen at 8.5% (8/94). Additional nonsynonymous propeller mutations found were P574L at 2.4% (2/84), F699C at 1.1% (1/87), A575L at 1.1% (1/88), and P667R at 1.1% (1/94). In addition to these Pfkelch13 mutations, we also found that the Pfdhfr N164L mutation, associated with high grade Fansidar resistance, has increased in prevalence to 24.7% (19/77). We also investigated diagnostic resistance status and found 76.7% (102/133) contained intact HRP2/3. Overall, this investigation shows continued spread of artemisinin partial resistance within Rukara catchment area due to 561H, the concerning appearance of 675V previously found only at high prevalence in Uganda, and the emergence of dhfr 164L to a common level. Continued molecular surveillance in this region and surrounding areas is needed to monitor and understand these concerning antimalarial mutations which have significant potential impact on clinical and preventative therapy of malaria in east Africa.

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## COMPARISON OF STRENGTH OF SELECTION FOR PLASMODIUM FALCIPARUM ARTEMISININ RESISTANCE-ASSOCIATED MUTATIONS BETWEEN SOUTHEAST ASIA AND UGANDA

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Partial artemisinin resistance, mediated by mutations in the kelch13 protein (PfK13), emerged in Southeast (SE) Asia and, more recently, in east Africa (Ethiopia, Eritrea, Rwanda, and Uganda). We investigated the rates of increase in mutation frequencies by comparing annual changes in PfK13 prevalence in Uganda with historical estimates from five countries in SE Asia. We fitted a Bayesian mixed effects model to the annual prevalence by district for each recently identified mutation in Uganda with a random slope and intercept to estimate selection coefficients, which define the percent change in relative frequency of the mutant genotype per parasite generation. In Uganda, we estimate preliminary selection coefficients per year from 2016-2021 across 7 districts for the 469Y, 469F, and 675V mutations to have been s=0.33 (range 0.26-0.44; 95% CrI: 0.13-0.51), s=0.16 (range 0.09-0.24; 95% CrI: -0.42-0.79), s=0.20 (range -0.04-0.32; 95% CrI: -0.03-0.40), i.e. prevalences are estimated to have increased by 33%, 16%, and 20% respectively each year. The selection coefficient estimated for all three mutations combined across all sampled districts in Uganda is s=0.36 (95% CrI: 0.19-0.46). To compare our estimates to the spread of PfK13 mutations in SE Asia, we employed the same model on data from the MalariaGen Pf7k database using samples from 2003-2018 in Cambodia (4 districts), Laos (2 districts), Myanmar (1 district), Thailand (2 districts), and Vietnam (2 districts). Selection coefficients for the 580Y mutation, the dominant mutation in much of SE Asia, and for all validated PfK13 propeller domain mutations were estimated to be s=0.38 (95% CrI: 0.20-0.57) and s=0.25 (95% CrI: 0.08-0.42), respectively. Together, these findings suggest that the strength of selection of partial artemisinin resistance in Africa has been similar to that in SE Asia, where partial artemisinin resistance is now widespread.

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## INCREASED RATE OF ARTEMISININ-BASED COMBINATION TREATMENT FAILURE IN PATIENTS RETURNING FROM SUB-SAHARAN AFRICA WITH PLAMODIUM FALCIPARUM MALARIA; THE ROLE OF PFCORONIN GENE MUTATION

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Artemisinin-based combination therapies (ACTs) are recommended as first-line treatment against uncomplicated Plasmodium falciparum infection. However the emergence of mutations in the PfKelch13 propeller domain have resulted in resistance to artemisinin in Southeast-Asia. ACT treatment failures have been sporadically reported in Africa. Data of 15 Israeli travelers returning from Sub-Saharan Africa with P. falciparum malaria who showed ACT treatment failure were retrieved. Blood samples were tested for mutations in Pfkelch13 and Pfcoronin genes. Initial parasite load was evaluated through real-time PCR analysis of 18S rRNA and Pftubulin genes. Parameters were compared to well responders (n=55). During 2009-2020, 326 patients had P. falciparum malaria acquired in Africa. Of those, 15 (14 males, 24 to 69 years old) were clinically resistant to artemether-lumefantrine. Four had parasites in the blood after 3 days of treatment and 11 had recrudescence malaria 1-3 weeks later. No significant differences were found in average age and weight between the ACT-treatment failure and non-failure groups. Failure rate among ACT treated patients during 2009-2015 was 3% compared with 13% during 2015-2020. In all failures the Pfkelch13 propeller domain had wild type sequence. We did find the P76S mutation in the propeller domain of Pfcoronin in 4/15 (29%) of the treatment failure cases compared to only 3/55(5%) in the successfully treated patients (p=0.02). In conclusion, we observed an increasing rate of artemether-lumefantrine treatment failure in P. falciparum patients that could not be explained by patient characteristics, neither by a Pfkelch13 mutation. However, P76S mutation in the Pfcoronin gene was present more often in the treatment failure group and merits further investigation. The recent reports of increasing malaria incidence in Sub-Saharan-Africa partly attributed to COVID-19 related disturbances might also be a reflection of the wider spread of ACT resistance.

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## DETECTION OF PLASMODIUM FALCIPARUM KELCH 13 GENE MUTATIONS IN CLINICAL SAMPLES FROM FOUR SITES ACROSS KENYA REVEALS INTENSE GENOMIC EVENTS THAT COULD PURIFY RESISTANCE

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The emergence and spread of Artemisinin based treatment-resistant strains call for ongoing surveillance. Therapeutic efficacy studies are the gold standard methods for testing drug efficacy. Recently, parasites with mutations that cause delayed clearance by artemisinin-based combination therapy have been identified in Africa, but these parasites are rare. Clinical samples obtained from five geographically varied areas in Kenya identified the polymorphism of the Plasmodium falciparum Kelch 13 (PfK13) gene and other markers of malaria treatment resistance. Blood samples were tested for in vitro susceptibility to selected antimalarial drugs using malaria SYBR green 1 assay. Sanger sequencing was used to detect polymorphisms in PfK13. PfK13 polymorphisms identified twelve mutations at 5.58% comprising three nonsynonymous mutations at codons: P553L, E612D, and F491L, four synonymous mutations at codons V637V, A504A, L488L, and C469C, for the last 2; three mixed wildtype and synonymous mutations at codons A626A, K455E, and G497G and one mixture of wild type and nonsynonymous mutation at codon S600F. One sample included two P553L and G497G mutations. The mixed genotypes, synonymous mutation A504A, nonsynonymous mutations at E612D, and F491L, and all of the nonsynonymous mutations have not been previously described. Also, we reported the synonymous mutation C469C in two samples compared to the WHO-validated marker C469Y which has been linked to slow clearance of parasites in Uganda. Whereas Ugandan reports of the mutation C469Y are non-synonymous, mutation C469C is synonymous. Previously, Congo saw the presence of the mutation C469C. The Pfk13 propeller gene has significant genomic activity, suggesting that once the fitness of the novel variant is established, the mutant state will progress. Continuous surveillance with sustained tools is needed to detect resistance and prevent transmission.

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## IDENTIFICATION AND QUANTIFICATION OF PLASMODIUM FRAGILE IN AN IN VITRO CULTURE SYSTEM AND NON-HUMAN PRIMATE MODEL

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Zoonotic malaria infections are increasing in some areas of the world, posing a threat to malaria control efforts. Optimization of in vivo culturing of Plasmodium spp. that infect non-human hosts is critical for advancing our understanding of Plasmodium biology and identifying potential mechanisms that may underlie zoonotic infections. One such non-human primate (NHP) parasite is P. fragile, known to infect rhesus macaques (RMs) and induce clinical signs that mirror P. falciparum infection in humans. The aim of this work was to establish an in vitro culture system of P. fragile from the blood of an experimentally infected rhesus macaques (RM) and characterize P. fragile life stages during in vivo infection and in vitro culturing. An adult male RM (n=1) was intravenously inoculated with P. fragile (20x106 infected erythrocytes [iRBCs]). Peripheral parasitemia and parasite life stages were monitored throughout infection via Giemsa staining of thin and thick blood smears. Parasite life stages were counted in every 200 uninfected erythrocytes and reported as percentages. P. fragile cultures were started from 1mL of cryopreserved iRBCs, culture medium was changed daily, and fresh RM blood was added to restore 50% hematocrit. Cultures were monitored daily via Giemsa stain of thin smears, and life stages were quantified. Peripheral parasitemia was 15.8% at week 2 post-infection (p.i.) and 8.6% at necropsy (2.5 weeks p.i.). Giemsa staining of thin and thick blood smears collected throughout infection enabled identification of ring stages (5.9%) and trophozoites (6.6%), with no detection of schizonts or gametocytes. At necropsy, ring stage (1.1%), trophozoites (5.2%), schizonts (0.3%), and gametocytes (0.5%) were observed. P. fragile culture began at 2.9% parasitemia and reached 8.7% after 7 days of culturing. Establishing a continuous culture system of P. fragile is a critical step towards increasing understanding of the biology of P. fragile in vitro, providing needed tools for understand potential zoonotic malarias, and enabling translational research to facilitate therapeutic development.

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## LOW FREQUENCY OF HISTIDINE-RICH PROTEIN 2/3 (HRP2/3) AND FLANKING GENE DELETIONS CORRELATES WITH THE HIGH DIAGNOSTIC PERFORMANCE OF HRP2-BASED MALARIA RAPID DIAGNOSTIC TESTS IN CAMEROON

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Histidine-rich protein 2 (HRP2)-based malaria rapid diagnostic tests (RDTs) are widely used for the detection of naturally occurring Plasmodium falciparum infections. Despite evidence of studies reporting false negative HRP2 RDT results due to Pfhrp2 and Pfhrp3 gene deletions in Africa, there exists a paucity of data on the deletions of these genes in Cameroon. Furthermore, genetic polymorphism of the hrp2/3 genes has been shown to impact the performance of PfHRP2- based rapid diagnostic tests (RDTs). This study investigated the presence of deletions of the Pfhrp2, Pfhrp3, and the respective flanking genes and characterized the polymorphism spectrum of the the hrp2 gene in P. falciparum parasite isolates from two localities (Gounougou and Elende) in Cameroon. A community-based cross-sectional survey was conducted in two communities - Gounougou and Elende in 2021. Malaria diagnosis was performed on 400 samples using microscopy and RDT and Whatman paper dry blood spots were collected for molecular typing of 18srRNA, msp2, hrp2/3, and neighboring genes. Furthermore, sequencing of the hrp2 gene was performed on 45 P. falciparum isolates to score polymorphisms diversity. Polyclonal infection depicted by msp2 typing alongside genotyping of the Pfhrp2 and Pfhrp3 genes revealed 1.3% and 2.1% overall prevalence with no significant difference between both communities. PCR confirmed 04 hrp2-deleted false negative RDTs. A frequency of 5.3%, 7%, 4.1%, and 5.5% was recorded in the MAL7P1.230, MAL7P1.228, MAL13P1.475, and MAL13P1.485 respectively. Sequencing of the Pfhrp2 exon2 from 45 isolates revealed a high level of genetic diversity, marked by the presence of variable repeat types in parasite isolates. The immuno-dominant AHHAHHAAD (81%) amino-acid Baker repeat epitope was characteristic of circulating P. falciparum isolates agreeing with significant RDT performance. In conclusion, despite the presence of low-frequency gene deletions in the HRP2/3 backbones, HRP2-based RDTs still maintain a high performance capacity for detecting malaria in the field.

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## PRESENCE OF PFHRP2/3 DELETIONS INCLUDING POLYCLONAL INFECTIONS IN AN INTENSE MALARIA TRANSMISSION AREA OF SIAYA COUNTY, WESTERN KENYA

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Malaria remains endemic in western Kenya despite the various control interventions. Accurate diagnosis is key to the treatment and control of malaria. As such, the World Health Organization (WHO) recommends parasite-based confirmation of malaria prior to treatment. Plasmodium falciparum Histidine Rich Protein 2 (PfHRP2) based malaria rapid diagnostic tests (mRDTs) kits are commonly used throughout malaria endemic regions, including western Kenya, as an alternative to diagnosis with microscopy. However, the performance of the mRDT has been threatened by the emergence of the PfHRP2 deletion. In Siaya, western Kenya, an intense malaria transmission area, PfHRP2/3 deletions could be present but not detected due to polyclonal infections where the wild-type gene is predominant. As such, we investigated the presence of the PfHRP2/3 deletion using one-step multiplex qPCR in a pediatric cohort in Siaya (n=206). PfHRP2/3 deletions were detected in 12 parasite isolates (5.8%, 95% CI 1.9-8.7%). The PfHRP2 monoclonal deleted strains were present in 2 isolates (1%, 95% CI 0-2.4%), while no parasite isolates had PfHRP3 single deletion. Further, 9 isolates (4.4%, 95% CI 1.9-7.3%) had deletions for PfHRP2 and 1 isolate (0.5%, 95% CI 0.0-0.8%) had PfHRP3 deleted but were masked by polyclonal infection. The average relative abundance of PfHRP2 deleted parasites was 9.6%, (95% CI 4.8-14.5), while wildtype was 90.4% (95% CI 85.4-95.4) in polyclonal infections. Further analysis revealed that false negative mRDT results were associated with low parasite densities that were beyond the detection threshold (P≤0.001). The study provides evidence of PfHRP2-deleted strains, including polyclonal infections and their relative abundance in Siaya County. These results underscore the need for an active systematic surveillance program within the lake endemic

region. Moreover, our results are important in informing the Division of National Malaria Control Program (DNMCP) in establishing the profiles of PfHRP2/3 deletions and decisions making on the use of PfHRP2-based mRDTs in Kenya.

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## PREVALENCE OF PFHRP2 AND PFHRP3 DELETIONS, AND PFKELCH13 MUTATIONS ASSOCIATED WITH PARTIAL RESISTANCE TO ARTEMISININ DERIVATIVES IN SOMALILAND

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Countries in the Horn of Africa Network for Monitoring Antimalarial Treatment (HANMAT) have reported high rates of Plasmodium falciparum (Pf) with hrp2 and hrp3 deletions, and sporadic pfhrp2/3 deletions have also been reported in Somaliland and in UK travellers returning from the region. In addition, pfkelch13 mutations associated with partial resistance to artemisinin derivatives have been reported in east Africa, further potentially threatening malaria control efforts in the region. The existence and extent of such Pf variants in Somaliland is unknown. We conducted systematic surveillance of pfhrp2/3 deletions and pfkelch13 mutations in five districts of Somaliland in 2021 and 2022. From suspected malaria cases, a total of 943 samples were collected, of which 231 were RDT positive and 238 microscopy positive, with a total of 33 discordant samples (HRP2-RDT negative/microscopy positive). We conducted molecular analysis on all the samples, and 195 were qPCR positive for Pf. Amongst these pfhrp2/3 deletions were confirmed in half of the districts with varying prevalence and in both mono and multiclonal infections. The detailed analysis and prevalence of the pfhrp2/3 deletions as well as the percentage of the deletions causing false-negative RDT results will be presented. We will also present analysis of pfkelch13 variants among those 195 qPCR-positive samples and the relationship with pfhrp2/3 deletion, and discuss implications of the findings for malaria control programme in Somaliland.

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## ONE-STEP MULTIPLEX DIGITAL PCR FOR THE DETECTION OF PFHRP2 AND PFHRP3 DELETIONS IN POLYCLONAL INFECTIONS

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Early detection of malaria cases is critical for malaria control and elimination strategies. The majority of Rapid Diagnostic Tests (RDT) used for point-of-care diagnostics in malaria-endemic countries target the HRP proteins coded by Plasmodium falciparum histidine-rich proteins 2 and 3 genes (pfhrp2/3). Due to the global emergence of P. falciparum (Pf) parasites lacking pfHRP2/3 protein, the performance of HRP-based RDTs has been compromised. Lately, there has been an increase in pfhrp2 and pfhrp3 deletion reports in Africa, threatening malaria control efforts. Pfhrp2/3 deletion in polyclonal infections, common in high transmission settings have been shown to give false-negative RDT results, particularly at low parasitaemia (close to detection limit of RDTs). Therefore, there is an emerging demand to develop a rapid molecular tool capable of detecting those parasite clones with greater accuracy. In this study, we report the development of a one-step rapid digital PCR assay based on microfluidic nanoplate technology that measures the fluorescence released by each partition in a 3-target multiplex PCR. This assay is able to accurately detect pfhrp2 and pfhrp3 gene deletions and the ratio of parasites carrying deletions in an Pf infection. We optimized the assay using culture-adapted laboratory lines with different pfhrp2 and pfhrp3 status. Different mixtures and parasitaemia were tested to assess the limit of detection, improving the results from a previously published multiplex qPCR in our lab. Further validation was performed using field samples. This digital PCR assay has significantly improved the accuracy of detecting pfhrp2/3 deletions in multiclonal infections, common in high-malaria transmission settings. Moreover, the high sensitivity of this assay to capture the true clonal diversity of Pf infections can be applied to other malaria targets for other studies.

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## CHANGES IN MALARIA TEST POSITIVITY RATE FOLLOWING SCALE UP OF LIFE-SAVING MALARIA CONTROL INTERVENTION IN EBONYI STATE, SOUTH EAST NIGERIA

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Nigeria accounts for 26.6% of global malaria cases according to the 2022 World Malaria Report. Surveillance is paramount to track malaria morbidity and guide decision-making for appropriate transmission-reducing responses. One tracking measure is the malaria test positivity rate (TPR) indicator which is used as an alternate indicator of malaria morbidity since it is based on parasitological confirmation. Over the years, TPR remained high regardless of season possibly due to poor quality of testing and documentation. This study investigates the correlation between the observed positive change in TPR and the interventions implemented to support the Ebonyi State Malaria Elimination Program (SMEP). These efforts include case and data management training, implementation of Behavior Economics (BE) prototypes to improve fever case management, supportive supervision and archiving of used rapid diagnostic test (RDT) cassettes to increase health provider adherence to national guidelines for fever management. This analysis consisted of a pre-post intervention comparison of TPR in similar periods over a 4-year period using secondary routine data from the National Health Management Information System across the 762 health facilities of the state. The data found that the pre-intervention TPR from January-March 2019 was 71% and remained high at 68% from January to March 2020. It is worth noting that COVID did not affect the TPR as only a total of 2,064 confirmed cases were reported in the state from 2020 to 2023. The intervention commenced in July 2020 and six -month post-intervention (January- March 2021), the TPR declined to 54% and further reduced to 50% from January to March 2022. While the reduction in TPR is promising, other factors such as bed net use can highly affect it. Nevertheless, it remains one measure of the effectiveness of the support to Ebonyi SMEP on the implementation of malaria program and suggests that training, supervision, archiving of RDT cassettes and BE prototypes implementation can influence the quality of malaria case management. These findings strongly present the opportunity to scale up our efforts in other states.

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## IMPROVING QUALITY OF MALARIA MICROSCOPY THROUGH ONSITE COACHING AND MENTORSHIP TO HEALTH FACILITIES IN TANZANIA

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Quality malaria microscopy relies on the skills of technicians to prepare and examine blood films, high quality reagents, and efficient internal and external quality assurance. Capacity development is necessary to improve quality of diagnosis; however, scarce resources limit the number of technicians able to participate in training. From 2021 to 2022, the U.S. President's Malaria Initiative (PMI) Impact Malaria project, in collaboration with the National Malaria Control Program and health management teams, conducted Malaria Services and Data Quality Improvement (MSDQI) supportive supervision visits for microscopy at 55 health facilities in four project-supported regions. We categorized an average score <50% in key indicators as poor and unacceptable, 50-75% moderate and needs improvement, and >75% as good performance. In 2021, 16 (29%) facilities scored below 50% in preparation, staining, and examination of malaria slides. Nine mentors who had previously successfully completed advanced Malaria Diagnostics Refresher Training (MDRT) provided on-the-job training to staff at these 16 facility laboratories on sample collection, blood film preparation, and examination. A follow-up MSDQI visit conducted in 2022 to assess the quality of microscopy services found that these 16 facilities scored above 75% in specimen collection after receiving the mentorship. The proportion of facilities with poor performance (<50%) in fixing and staining of blood films decreased from 20% to 0%, and the proportion of facilities with moderate scores (50-75%) improved from 20% to 50%. The proportion of facilities with good performance scores (>75%) in blood film examination improved from 60% to 100%. The onsite mentorship may have improved the quality of malaria microscopy among laboratory technicians at health facilities. The use of mentors from local health management teams can bridge the gap of limited resources to reach a larger group of laboratory staff who may not have access to formal MDRT.

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## EXTERNAL VALIDATION OF THE WORLD HEALTH ORGANIZATION INTEGRATED MANAGEMENT OF CHILDHOOD ILLNESS (IMCI) PROTOCOL FOR MALARIA TESTING IN LOW MALARIA RISK AREAS

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In low malaria risk areas, the WHO Integrated Management of Childhood Illness (IMCI) chart booklet recommends testing for malaria only in febrile sick children with no obvious cause of fever. The safety of this approach is unclear since the clinical presentation of malaria is unspecific. We performed an external validation study to assess the predictive performance of identifying malaria in febrile children using the IMCI protocol in low malaria risk areas. We used data from ePOCT+, a digital clinical decision support algorithm that helps primary care health workers in the management of sick children in Rwanda and Tanzania. Sick children aged 2-59 months, presenting with fever or a history of fever to a health facility located in a low malaria risk area (i.e. malaria positivity in <5% of febrile children) were included. Follow-up visits and presence of IMCI danger signs made up exclusion criteria. Primary outcome was a positive malaria rapid diagnostic test (mRDT) or the detection of Plasmodium spp by microscopy (thick blood smear). Two diagnostic strategies were evaluated: 1) malaria test only in patients with no obvious cause of fever, 2) malaria test in patients with no obvious cause of fever or with recent travel history. Between December 2021 and January 2023, 7'209 patients from 11 Tanzanian and 15 Rwandan health facilities tested for malaria were included. 126 (1.8%) cases had a positive malaria test. The first strategy had a sensitivity of 24.6% (95%CI 23.6-25.6%) and a specificity of 87.0% (86.2-87.8%). If health workers had adopted this testing strategy, 75.4% (95/126) of febrile children with malaria would not have been identified and thus probably not treated (symptomatic and incidental parasitaemia cases combined). When adding travel history, sensitivity rose to 27.0% (26.0-28.0%) and specificity stayed stable at 86.3% (85.5-87.1%). The sensitivity of the IMCI chart booklet was low and the inclusion of travel history did not increase it significantly. Given the results of this study and considering the trade-off between the need to detect malaria cases and the avoidance of wasted tests, alternatives to the IMCI approach should be explored.

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## IMPROVING MALARIA DIAGNOSIS THROUGH QUALITY ASSURANCE IN RWANDA FY 2021-2022

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Malaria remains a leading cause of morbidity and mortality in Rwanda, with almost 1 million malaria cases and 71 deaths reported in 2021-2022. Significant progress in malaria control has been made through the implementation of various interventions, including the scale-up of community-based management of malaria to include adults. However, the diagnosis of malaria continues to be a challenge, with the accuracy of diagnosis at health facilities (microscopy), and community level (malaria rapid diagnostic tests (mRDT)) needing improvement. To improve the quality of malaria diagnosis, an external quality assessment/quality control (EQA/QC) was conducted in selected health facilities and at the community level in all the 30 Rwanda districts. At the community level, the EQA/QC of mRDT was performed by direct observation of community health workers (CHWs) performing the test. At the hospital level, assessment of the laboratory settings and microscopy procedure was done. Fifteen slides per hospital visited were selected and retested by eight WHO accredited malaria microscopists from Rwanda National Reference Laboratory. A total of 704 CHWs were observed. 92% of these were trained on mRDT testing, and the main steps of performing mRDTs were correctly followed in 95% of cases. A total of 42 hospitals were visited and 630 slides retested. Discrepancy from two observers for positive and negative results was 1.5%. Two hospitals exceeded the acceptable range of discrepancy. Over 80% of the laboratories assessed had adequate laboratory space, infrastructure and good quality supplies to undertake microscopy work.

The staining SOPs were followed in 88% of cases. Improvements are needed in accurately recording the opening date and validation of new Giemsa solution, regularly undertaking QC of health centers' blood films, and reporting parasites density for positive blood films. Overall, the EQA/QC identified areas that need improvement. Performance of mRDT by CHWs was good. However, continuous monitoring and evaluation of malaria diagnosis is crucial for appropriate treatment and referrals.

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## THE TESTSMART TRIAL: RESULTS FROM A CLUSTER-RANDOMIZED TRIAL OF MALARIA DIAGNOSTIC TESTING AND CONDITIONAL SUBSIDIES TO TARGET ACTS IN THE RETAIL SECTOR IN KENYA AND NIGERIA

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We present the results from an innovative, multi-country cluster-randomized trial (CRT) designed to improve targeting of artemisinin combination therapies (ACT) to individuals with confirmed malaria infection who seek treatment in private medicine retailers (PMR). The majority of ACTs in sub-Saharan Africa are distributed through PMRs. Unnecessary consumption of ACTs purchased over the counter in PMRs is widespread due to their low price, high perceived efficacy, and absence of diagnostic tools to guide drug use. We hypothesized that creating price differentials through targeted ACT subsidies dependent on malaria testing status (untested, negative, positive) could improve targeting and use of ACTs among clients of PMRs. From January 2021 to February 2023, we tested a client-directed intervention in the form of a diagnosis-dependent ACT subsidy combined with a provider-directed incentive for testing against a comparison arm in 48 PMRs in Lagos, Nigeria and 39 in Bungoma and Transnzoia counties, Kenya. All PMRs were provided access to low-cost malaria rapid diagnostic tests (RDT) which they sold to clients who wished to purchase one. PMRs in the intervention arm received 0.1 USD for performing a RDT and were instructed to give a free ACT to any client with a positive test for which they were reimbursed. Information from 5695 clients in Kenya and 3879 in Nigeria was collected through exit interviews.Preliminary results show that the program substantially increased testing uptake in both arms relative to baseline, which in turn improved targeting of ACTs. For example, in Kenya, 67.8% of untested clients received an ACT, compared to 24.1% of malaria negative clients. However, the price differentials did not have an additional impact on test uptake or ACT consumption in the intervention arm compared to the control. These findings provide additional insight into point-of-care decision making for fevers and suggest that although RDTs can be integrated into PMR practices, client level subsidies may not be optimal for modifying purchasing decisions. We plan to present final and detailed findings from Kenya and Nigeria during the scientific session.

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## IMPLEMENTATION OF TWO-STEP MALARIA RDT DETECTIONPFHRP2/PLDH COMBINING WITH POINT-OF-CARE TESTS FOR BACTERIAL INFECTIONS IN THE MANAGEMENT OF FEBRILE DISEASES IN CHILDREN UNDER-5 YEARS IN BURKINA FASO

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In low and middle incomes countries (LMICs) such as sub-Saharan Africa (SSA), the management of febrile diseases remains challenging given the lack of practical diagnostic tools to screen the real cause of fever and the limits of malaria rapid diagnostic tests. In order to improve the management of febrile diseases in children under 5 years, this study has been conducted. The study was conducted at the Field Station of Sigle, set-up by the Clinical Research Unit of Nanoro (CRUN). All patients from 6-59 months attending the outpatient clinic of the health facility of Bologho in the health district of Nanoro (Burkina Faso), with documented fever or history of fever within the pass 7 days were invited to participate to the study. Participants were randomized either the intervention package (e-Algorithm or RDT-decisional algorithm arm(RDT-DA)) or routine system. The intervention package was constituted by the following PoC tests: two-step malaria RDT detection PfHRP2 and pLDH, CRP, white blood cells (WBC) count, oximetry, Group A Streptococcus, and Salmonella/Shigella. Antimalarial prescription was 42.05% (164/390) in e-Algorithm arm, 43.65% (172/394) in RDT-DA and 52.30% (232/392) in standard practice system [risk difference (RD): -10.25% (p p<0.001) for e-Algorithm and -8.65% (p<0.001) for RDT-DA). Antibiotics were prescribed in 46.92% (183/390) in e-Algorithm arm, 50.25% (198/394) in RDT-DA arm and 76.28% (299/392) in routine system [RD: -29.36% (p<0.001) for e-Algorithm and -26.03% (p<0.001) for RDT-DA]. The reduction of antibiotic prescription greater in children without malaria [RD:-64.79% (p<0.001) for e-Algorithm arm and -61.62% (p<0.001) for RDT-DA algorithm arm. In conclusion, implementation of two-step malaria RDT and PoC tests for bacterial infections has potential to improve the management of febrile diseases in children under 5 years and reduce inappropriate prescription of antibiotics. Nevertheless, the use of CRP test is not suitable differentiate bacterial to non-bacterial infections in children with malaria.

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## SEROLOGICAL MARKERS PREDICT PLASMODIUM VIVAX RELAPSES IN A RETURNING INDONESIAN SOLDIER COHORT

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Relapses from latent liver-stage parasites (hypnozoites) are a key challenge for Plasmodium vivax elimination. Relapses are responsible for >80% of blood-stage infections. There are currently no tools to detect hypnozoites, but P. vivax blood-stage infections induce strong antibody responses arising quickly after infection and decaying slowly over time. Antibodies are thus markers of both current and recent past infections, allowing identification of people recently exposed to P. vivax and thus at risk of carrying hypnozoites. This study aimed to test the ability of antibody signatures to a validated set of 13 antigens to predict relapse risk in two cohorts of soldiers (n =

592) who returned to a malaria-free area from a 9-month deployment to a malaria-endemic area between 2018 and 2022. All soldiers were assessed by Luminex serology on the day of recruitment and followed actively every two weeks and at time of febrile symptoms until first recurrent P. vivax parasitaemia, for up to 6 months. An optimised machine learning Random Forest classification algorithm was used to classify soldiers as exposed during the previous 9 months. Over 110 soldiers experienced relapses during follow-up with significant heterogeneity in relapse risk between the two cohorts (Cohort 1: 25, Cohort 2: >85). Soldiers who relapsed had higher median antibody titers for most of the biomarkers compared to those who did not relapse. In cohort 1, our diagnostic tool had 75% sensitivity and 93% specificity at identifying future relapses using blood samples from recruitment day. Recurrent infections strongly boosted antibody titres both in sero-positive and initially sero-negative soldiers. Analyses of the 2nd cohort are ongoing but preliminary results indicate a comparable performance. This demonstrates the ability of serological markers to identify people at risk of relapse with high accuracy. In our presentation, we will present the final results across both cohorts and discuss the implications of these results for the development of a novel public health intervention P. vivax serological testing and treatment (PvSeroTAT) for relapse prevention.

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## THRESHOLD LIMITS OF DETECTION AND QUANTIFICATION OF MALARIA PARASITES IN DRIED BLOOD SPOT: A COMBINED APPROACH OF MID-INFRARED SPECTROSCOPY AND MACHINE LEARNING

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Mid-infrared spectroscopy (MIRS) combined with machine learning (ML) have shown potential in detecting malaria infections. The technique is reagent-free, simple to use and cost-effective, but its malaria parasite detection thresholds remain unknown. This study aims to investigate the lower limits of detection and quantification of MIRS-ML approaches under different hematocrit levels, including anemia, to further evaluate its potential for malaria control. The study was conducted at the Ifakara Health Institute laboratory in Tanzania. Blood samples were obtained from PCR-tested malaria-free individuals, and parasite cultures were performed using Plasmodium falciparum strains of NF54 and FCR3. Hematocrit ratios (50%, 25%, and 12.5%) and malaria parasitemia levels (6%, 0.1%, 0.002%, 0.00003%, and 0%) were created through two-way matrix serial dilutions. Dried blood spots were analysed using MIRS and ML classifiers and regressors to detect and quantify malaria parasites at different parasitemia levels and in the presence of anemia.We created two logistic regression models, one including only the highest parasitemia (6%) and negative control, and a second model that included all parasitemia levels. The first detected malaria infections at accuracy of 97%, 90%, and 90% for Normal (50%), mild (25%), and severe (12.5%) anemia, respectively. The second model was 100% accurate in detecting malaria infections at 6%, 0.00003% and negative control, but less accurate in detecting of 0.1% and 0.002% (>80% and >50%, respectively). When Support vector machine regressor were used in the analysis, this model outperformed other regression ML models, quantifying five-class malaria parasitemia with a Root Mean Square Error, RMSE = 0. MIRS-ML approaches can detect and quantify at low parasitemia (1-10 malaria parasites/µl of blood) with > 95% accuracy. Importantly, malaria parasite detection and quantification by MIRS-ML approaches were not affected by anemia. These initial findings indicate that MIRS-ML approaches could be valuable for detecting and quantifying malaria infections, particularly in resource-limited settings.

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## DETECTION OF PLASMODIUM MALARIAE AND PLASMODIUM KNOWLESI THROUGH IMPROVEMENTS IN MICROSCOPY SERVICES IN CAMBODIA

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The primary method of malaria diagnosis in Cambodia is the use of Rapid Diagnostic Tests (RDTs), particularly outside hospitals. Currently available RDTs only detect Plasmodium falciparum (Pf) and P. vivax (Pv). P. malariae (Pm) and P. knowlesi (Pk) are detected primarily in research studies using molecular diagnostic tools. Training and support for microscopy diagnosis has recently taken place in six provinces managed by the Ministry of Health, with financial and technical support from USAID/PMI through the Cambodia Malaria Elimination Projects (CMEP and CMEP2) and the World Health Organization. This includes 5-day training courses on microscopy diagnosis and organization of national competency assessments for malaria microscopists to ensure quality diagnosis at the point of care. If patients test negative by RDT but malaria symptoms continue, then a blood smear is taken for microscopy. If the blood smear is positive for Pm/Pk, a blood smear and dried blood spot are taken for confirmation at the National Center for Parasitology, Entomology and Malaria Control (CNM) through both microscopy and polymerase chain reaction (PCR). This investment has led to a corresponding rise in Pm and Pk cases diagnosed through routine CMEP2-supported activities from zero to 58 (Jan 2021-Feb 2023). Slide confirmation by CNM through microscopy examination classified 50 cases as Pm, six as Pk, and two were not definitive. PCR is used as it can be difficult to differentiate between Pm/Pk by microscopy. The PCR results showed that 42 cases were confirmed as Pm, nine as Pk, one as Pf/Pm and six were not definitive. The majority (79%, n=58) of these cases occurred during the rainy season (May-Oct 2022). These results suggest there is likely ongoing Pm/Pk transmission that is undetected in areas without microscopy services in Cambodia. Microscopy services at health centers remain limited and updates to the suspected case definition may be needed to ensure all Pm/Pk infected patents are tested. As Cambodia is aiming for the elimination of all human malaria species by 2025, introducing and sustaining quality microscopy will be essential to detect all forms of malaria species.

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## LOW PREVALENCE OF PLASMODIUM FALCIPARUM HISTIDINE-RICH PROTEIN 2 AND 3 GENE DELETIONS—A MULTIREGIONAL STUDY IN CENTRAL AND WEST AFRICA

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Treatment of malaria parasite-infected individuals and the effectiveness of malarial control efforts may be compromised by Plasmodium falciparum parasites with deletions of P. falciparum histidine-rich protein 2 (PFHRP2) and pfhrp3 genes because such parasites may not be detected by PfHRP2-based rapid diagnostic tests (RDTs). Here, we assessed the frequency of parasites with pfhrp2 and pfhrp3 deletions at four different

study sites: Gabon and Republic of Congo in Central Africa and Nigeria and Benin in West Africa. Using a highly sensitive multiplex qPCR (4plex qPCR), we analysed 534 samples from Gabon, 917 from the Republic of Congo, 466 from Nigeria, and 120 from Benin. Low prevalences of single deletions in pfhrp2 (ranging from 0% to 1%) and pfhrp3 (ranging from 0% to 0.03%) were observed across all study sites, namely Gabon, Republic of Congo, Nigeria, and Benin. In addition, P. falciparum parasites with two deletions were found exclusively in Nigeria, accounting for 1.6% of all samples that were analysed. Thus, our pilot investigation suggests that, currently in Central and West Africa, there is a low risk for false-negative RDT results caused by deleted pfhrp2/pfhrp3 genes. However, continuous, and comprehensive monitoring is warranted to detect any (potentially fast) changes in this status and to make sure that RDTs continue to be an appropriate method for monitoring and diagnosing malaria in these regions.

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## HIGH PREVALENCE OF PLASMODIUM FALCIPARUM HRPII-DELETED VARIANTS ASSOCIATED WITH LOW RAPID DIAGNOSTIC EFFICACY 13 YEARS AFTER INTRODUCTION OF MALARIA RDTS IN EASTERN ZIMBABWE

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Malaria has receded from most of Zimbabwe over the past decade following scaled-up vector control and artemisinin-based combination therapy (ACT) interventions for possible elimination of the disease. The advent of Plasmodium falciparum histidine-rich protein II (PfHRPII)-based rapid diagnostic tests (RDTs) and ACTs at inception of the 21st millennium ushered in a new era of complementary test-and-treat campaigns to accelerate towards malaria elimination, including mandatory RDT confirmation of clinical cases before the administration of ACT. However, the emergence of PfHRPII-deleted parasite variants is undermining RDT accuracy and posing a growing threat to prompt/effective testing and treatment for malaria control and elimination. We examined P. falciparum HRP-II RDT diagnostic performance on 1,125 presenting febrile suspected malaria cases using microscopy as gold standard. We report poor RDT efficacy on the presenting febrile cases, at 49% sensitivity, highly associated with rampant (61%) PfHRPII-deleted malaria parasite variants (OR [95%CI]: 14 [6.6 - 29.6], n = 331) 13 years after introduction of RDTs in Mutasa, Zimbabwe. Our data show ten-fold higher odds (OR [95%CI]: 10 [3.2 - 31.0]) of PfHRPII-deleted variants among malaria cases of 2021 compared to 2004 (pre-RDT introduction). RDT false-negative rate correspondingly increased from baseline 9% to 51%, suggesting formidable parasite adaptation to RDT use. Akin to the Horn of Africa our study is concerning for emergence of diagnostic resistant parasites in Southern Africa that may undermine regional elimination efforts.

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## A DIGITAL MICROSCOPE FOR THE DIAGNOSIS OF PLASMODIUM FALCIPARUM PARASITES WITH HRP2 AND HRP3 DELETION AND P. VIVAX

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The most frequent and most sensitive class of rapid tests for Plasmodium falciparum diagnosis rely on detection of the HRP2 and HRP3 proteins. Deletions of the hrp2/3 genes are a major concern and where the frequency of the deletion is high, alternative diagnostic tools are needed. The Noul miLab is a portable digital microscope for malaria diagnosis. The miLab conducts smear preparation from a droplet of blood, staining, and imaging. An algorithm detects infected RBCs, and displays them on a screen. Time-to-result is approximately 20 minutes, with less than two minutes hands-on time. We evaluated the miLab among 659 febrile patients in Gondar, Ethiopia, where co-transmission of P. falciparum and P. vivax, and high frequency of hrp2/3 deletions make diagnosis challenging. By qPCR, 76.2% of patients tested positive; 40.4% for P. falciparum, 12.3% for P. vivax, and 23.8% with mixed infection. 34.7% of P. falciparum infections carried hrp2 deletion, and 91.5% hrp3 deletion. The miLab diagnosed 51/52 (98%) of P. falciparum infections with hrp2 deletion at densities >20 parasites/µL. The sensitivity of the miLab for P. falciparum at densities >200 parasites/µL (as determined by qPCR) was 93.6%, and 90.2% at densities >20 parasites/µL. The miLab was more sensitive than an LDH-based RDT and local microscopy with sensitivities of 80.3% and 82.1% at densities >20 parasites/µL. For P. vivax, the sensitivity of the miLab was 83.9% at densities >200 parasites/µL, and 82.7% at densities >20 parasites/µL (RDT: 64.4%, microscopy: 56.7%). Specificity of the miLab was 84.5%. At densities >20 parasites/µL, the miLab misclassified 4/100 P. falciparum mono-infections as P. vivax, and 4/36 P. vivax mono-infections as P. falciparum. Further, it identified only 5/23 mixed infections correctly. In conclusion, the Noul miLab is more sensitive than microscopy and thus a valuable addition to the toolkit for malaria diagnosis in particular in areas with high frequencies of hrp2/3 deletions. Incorrect diagnosis of species and low sensitivity for mixed-species infections are currently being addressed through updates to the algorithm.

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## FITNESS COST OF PFHRP2/3 GENE DELETION & K13 R622I MUTATION IN NATURAL INFECTIONS IN ETHIOPIA: TRANSMISSION POTENTIAL OF PARASITES EVALUATED BY DIRECT MEMBRANE FEEDING ASSAYS

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Parasites with pfhrp2/3 gene deletions are fast spreading, especially in the Horn of Africa. Its unique heterogeneous spatial distribution is also evident at lower scales. In our previous studies, we confirmed its co-occurrence with parasites carrying the R622I kelch mutation. From Sept 2022 - March 2023, we examined the transmissibility of P. falciparum parasites with & without pfhrp2/3 gene deletion to colony-maintained An. arabiensis mosquitoes (n=5290) in direct membrane feeding assays (DMFA) in Ethiopia. A total of 182 microscopy positive patients were screened & 53% had negative result using HRP2-based RDT. They had pfhrp2 (88%, 83/94) & pfhrp3 (95%, 89/94) gene deletion by digital PCR. The median HRP2 antigen concentration (multiplex bead-based assay) was higher in the RDT positive (1,060pg/mL; IQR 675-1284; p=0.001) than RDT negative samples (10; IQR 9-16). Transmissibility of parasites with pfhrp2 deletion was higher (80%, 20/25) than without deletion (70%, 19/27). Overall, 47% of mosquitoes were infected with no difference between the two groups. The oocyst density in infected mosquitoes was higher in parasite without deletion (median 13, IQR 4-53) than with gene deletion (4, 2-17; p=0.082). Infectivity to mosquitoes was strongly associated with total parasite by 18S based qPCR (ρ=0.376, p=0.006) & male (ρ=0.381, p=0.006) & female (ρ=0.375, p=0.007) gametocyte densities measured by sex specific RT-qPCR. These were not different between pfhrp2 deleted & undeleted parasites. In conclusion, the overall prevalence of pfhrp2 (50%, 89/179) & pfhrp3 (90%, 161/179) deletion substantially increased in this study compared to our previous study in 2021. Parasites with pfhrp2 deletion were as transmissible to mosquitoes as wild type parasites. Currently, we are analyzing sequence results from the amplicon NGS to examine the association of drug resistance markers such as R622I with the above

outcomes. Based on our previous study, the frequency of R622I mutation was high in the same area (49%). We are also collecting more samples & doing extra DMFAs to boost the observation in the short transmission season (April-May 2023).

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## PERCEPTIONS OF FACILITY-BASED AND COMMUNITY HEALTH WORKERS IN KENYA: IMPLICATIONS FOR PROGRAMS BASED ON FINDINGS FROM THE KENYA MALARIA BEHAVIOR SURVEY

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Facility-based health workers and community health volunteers (CHVs) play essential roles in malaria prevention and treatment. Community perceptions of health workers, however, remain under-examined in Kenya. In 2022, Kenya's Division of National Malaria Program (DNMP) and Breakthrough ACTION Kenya implemented the Malaria Behavior Survey in the malaria-endemic Lake region of Western Kenya. 1,787 women and 466 male partners participated from 1,456 households. Descriptive and bivariate analyses assessed differences in community perceptions of health workers by socio-demographic characteristics. Perceptions of health workers were also key covariates in multivariable logistic regression models of prompt and appropriate care-seeking for fever. Favorable perceptions were based on agreement with statements about facility-based health workers and CHVs overall and for case management. Scores were summarized and dichotomized for analysis. Most respondents reported overall favorable perceptions of facility-based health workers (95%) and CHVs (82%). Favorable perceptions of both types of health workers were significantly higher among female than male respondents. 61% of respondents had favorable perceptions of CHVs regarding malaria care-seeking and treatment in particular, and this varied significantly by respondent sex (female: 64% vs. men: 57%; p<0.01). 43% and 50%, respectively, of respondents perceived that CHVs always have 1) medication to treat malaria and 2) rapid diagnostic test kits to see if a person has malaria. Caregivers reporting favorable perceptions of CHVs had 1.9 times increased odds of reporting prompt and appropriate care-seeking for a child under five who had a fever in the past two weeks (AOR: 1.9; 95% CI: 1.1-3.4). The associations between CHV perceptions and care-seeking are cross-sectional yet suggest that service delivery and social and behavior change programs can improve perceptions of the quality of care and trustworthiness of CHVs. Perceptions that CHVs do not have supplies they need could be addressed by correcting supply issues and subsequently assuring communities that supplies are available.

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## A NOVEL HUMANIZED MURINE MODEL TO ASSESS PRIMAQUINE-INDUCED HEMOLYSIS IN G6PD DEFICIENCY

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Approximately half a billion humans have some form of glucose-6-phosphate dehydrogenase deficiency (G6PDd), limiting the ability to safely achieve radical cure of Plasmodium vivax using primaquine or tafenoquine due to risk of hemolysis. Because most G6PDd is caused by an unstable enzyme variant, very young RBCs have increased G6PD activity and are resistant to hemolysis. As such, primaquine can have a "self-limiting" character if given in regimens that promote controlled early hemolysis and reticulocytosis. Accordingly, modified primaquine regimens have the potential to be both safe and effective in G6PDd patients. Historically, there has been no mouse model of G6PDd that recapitulates an RBC age-dependent gradient of G6PD activity, limiting testing of primaquine dosing strategies. We report the generation of two new strains of mice in which the murine G6PD gene was replaced with either the (A-) deficient or non-deficient (ND) human genomic DNA. RBC G6PD activity and protein levels were measured by spectrophotometric assay and Western blot, respectively. In vivo biotinylation pulse chase was used to visualize "young RBCs" from 1-6 days of age, which were isolated by streptavidin-coated magnetic bead depletion. "All age" RBCs from A- mice had 12.8% normal G6PD activity (p<0.0001) and only trace amounts of G6PD protein. In contrast, 1-6 day old A- RBCs had 56% normal G6DP activity and easily detectable G6PD protein. qPCR showed no difference in G6PD mRNA in bone marrow from A- and ND mice. After 4 days of primaquine challenge (50mg/kg/day), A- mice had a 17% drop in hematocrit compared with ND mice (p<0.001). A- mice also had increased reticulocytes (14.5%) compared to ND mice (4.9%) (n=12, p=<0.0001). Essentially 100% of cleared RBCs were older than 6 days. RBCs from ND mice had normal G6PD activity and protein, with only subtle effects of primaquine challenge. 3 out of 3 primaquine challenge experiments had similar results. While great care must be taken in translation of therapeutic details from mice to humans, the newly described mice serve as a tractable platform to test general primaquine dosing strategies.

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## PREDICTING OPTIMAL ANTIMALARIAL DRUG COMBINATIONS FROM A STANDARDIZED PLASMODIUM FALCIPARUM HUMANIZED MOUSE MODEL

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The development of new combinations of antimalarial drugs is urgently needed to prevent the spread of parasites resistant to drugs in clinical use and contribute to the control and eradication of malaria. In this work, we evaluated a standardized humanized mouse model of erythrocyte asexual stages of Plasmodium falciparum (PfalcHuMouse) for the selection of optimal drug combinations. Firstly, we showed that the replication of P. falciparum was robust and highly reproducible in the PfalcHuMouse model by retrospective analysis of historical data. Secondly, we compared the relative value of parasite clearance from blood, parasite regrowth after suboptimal treatment (recrudescence), and cure as variables of therapeutic response to measure the contribution of partner drugs to combinations in vivo. To address the comparison, we first formalized and validated the day of recrudescence (DoR) as a new variable and found that there was a log linear relationship with the number of 46 viable parasites per mouse. Then, using historical data on monotherapy and two small cohorts of PfalcHuMice evaluated with ferroquine plus artefenomel or piperaquine plus artefenomel, we found that only measurements of parasite killing, (i.e., cure of mice) as a function of drug exposure in blood allowed direct estimation of the individual drug contribution to efficacy by using multivariate statistical modelling and intuitive graphic displays. Overall, the analysis of parasite killing in the PfalcHuMouse model is a unique and robust experimental in vivo tool to inform the selection of optimal combinations by pharmacometric PK/PD modelling.

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## MODELLING THE HAEM DETOXIFICATION PATHWAY IN PLASMODIUM FALCIPARUM TO AID IN TARGET DECONVOLUTION AND MECHANISM OF ACTION STUDIES

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The parasite Plasmodium falciparum (Pf) causes the deadliest form of malaria. Critical to parasite survival is the haem detoxification pathway. This biochemical pathway, which occurs in the parasite digestive vacuole (DV), results in the digestion of haemoglobin (Hb) and subsequent formation of an inert crystal, haemozoin (Hz). Disruption of this pathway is an attractive antimalarial target as it includes numerous biophysical and biochemical targets such as protease enzymes, endocytosis mediators, transporters and molecules required for Hz. Incorporating these parameters and others into a mathematical model would aid in mode of action studies and may inform rational drug design. Numerous parameters are imperative for model development. In the current study, the volume of the DV lumen and the rate of Hb uptake were found to be crucial to the model, and were studied herein using confocal microscopy techniques. Validation of the model, by way of pathway perturbation, was studied using a cellular fractionation assay that assessed the impact of inhibitory chemotypes on the levels of Hb, free haem and Hz. To study the lumen volume, red blood cells pre-loaded with pHrodo™ dextran beads were incubated with Pf trophozoites to allow reinvasion. Following incubation, individual parasites were visualised with an Airyscan LSM 980 confocal microscope and image processing was carried out using ImageJ. These studies revealed that lumen growth in NF54 followed a Gompertz growth curve, while Dd2 followed a sigmoidal growth trend. We also examined differences in lumen volume of other Pf strains with a varying degree of sensitivity to piperaquine. Finally, parasites were treated with a set of endocytosis and protease inhibitors to perturb the pathway. Using cellular fractionation assays, treatment with butanedione-2,3 monoxime (an endocytosis inhibitor) caused a decrease in the levels of haem and Hz; whereas E-64 (cysteine protease inhibitor) caused a dramatic increase in the levels of undigested Hb and a subsequent decrease in the levels of Hz. These mechanistic studies help define haem perturbation properties characteristic of various inhibitor classes.

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## THE ACTIVITY OF NOVEL SLOW-ACTION ANTIPLASMODIAL 1,3,4-OXADIAZOLES IS ASSOCIATED WITH A PLASMODIUM FALCIPARUM PALMITOYLTRANSFERASE

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New chemoprevention drugs are needed to help protect vulnerable populations in areas of high malaria endemicity as we move towards eradication. We have identified novel N,N-dialkyl-5-alkylsulfonyl-1,3,4-oxadiazol-2-amines as a new antiplasmodial chemotype with a different action to delayed-death slow-action drugs like clindamycin. Structure activity relationship analysis of >60 analogues identified multiple compounds with potent activity against drug-sensitive and drug resistant asexual stage Plasmodium falciparum parasites (IC50 <40 nM) and >2,500 selectivity for P. falciparum versus human cells. Genome sequencing of P. falciparum parasites selected for in vitro resistance to representative members of this series identified mutations in a palmitoyltransferase gene. This phenotype was confirmed using CRISPR/Cas9 mediated gene editing. Further studies are underway to investigate whether this P. falciparum palmitoyltransferase is the target of slow action 1,3,4-oxadiazoles and thus a new chemoprotection drug target, or alternatively, a mechanism of resistance to these compounds.

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## A SYSTEMS BIOLOGY APPROACH TO UNDERSTAND THE MECHANISMS OF ACTION OF KALIHINOL, A POTENT NEW ANTIMALARIAL

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The Kalihinol analogues belong to the Isocyanoterpenes (ICT) family of chemical compounds. ICTs have been shown to have potent activity against multiple microbial pathogens including the human malaria parasite Plasmodium falciparum. Our work shows that the kalihinol derivative retains its potency against both drug-sensitive and -resistant parasites with IC50 values in the low nM range. This activity translates into transmission-blocking potential, as the compound inhibit sexual differentiation in vitro. Through phenotypic analyses and cell biological assays we demonstrate that the apicoplast is one of the sites of action of the drug in the P. falciparum-infected red blood cell. Drug-drug interaction studies as well as chemical rescue using isopentenyl pyrophosphate (IPP) confirmed that one Kalihinol analogue exerts its antimalarial activity through alteration of apicoplast metabolic processes. We also confirm, through metabolomic profiling and drug-protein interactions assays, that our analogue interacts with several proteins involved in apicoplast membrane biogenesis and lipid trafficking. Prolonged drug pressure assay followed by whole genome sequencing and CRISPR-cas9 genome editing, identify the mode of resistance acquired via the vesicular trafficking system. In vivo studies in humanized mice model demonstrate that Kalihinol analogues suppress P. falciparum growth with good pharmacokinetic and safety properties. We also confirmed the efficacy of the compound against P. knowlesi growth. Altogether our multi-omics approach not only unraveled a novel of mode of action for an antimalarial, but also mechanism involved in drug resistance. The unique properties of this class of antimalarials, their high potency, excellent therapeutic profile, and the limited capacity of the parasite to mount resistance make them ideal compounds to further develop as a potential next generation of drugs for the treatment and elimination of malaria infection.

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## EVALUATION OF THE IN VITRO GAMETOCYTOCIDAL ACTIVITY OF TAFENOQUINE IN COMBINATIONS WITH METHYLENE BLUE AND OTHER ANTIMALARIAL COMPOUNDSTAFENOQUINE IN COMBINATIONS WITH METHYLENE BLUE AND OTHER ANTIMALARIAL COMPOUNDS

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Resistance to artemisinin-based combinations in Southeast Asia is jeopardizing malaria control and elimination efforts. There is an urgent need for new antimalarial drugs, especially those with potent gametocytocidal properties to reduce malaria transmission. Tafenoquine (TQ) possesses hypnozoitocidal, but poor blood schizontocidal and gametocytocidal properties. Ideally, TQ should be used in combination with a partner drug with strong schizontocidal and gametocytocidal activities. We have

previously shown MB to synergise the in vitro blood schizontocidal activity of TQ. In the present study, we evaluated in vitro gametocytocidal activity of TQ in combinations with antimalarial drugs including chloroquine (CQ), dihydroartemisinin (DHA), lumefantrine (LUM), piperaquine (PPQ), pyronaridine (PRN), methylene blue (MB) and 10-aminoartemisinins compounds, artemiside and artemisone, with potent blood schizontocidal and gametocytocidal activities. Plasmodium falciparum 3D7c gametocyte viability was assessed by measuring adenosine triphosphate (ATP) production. TQ inhibited ATP production in stage II-III and stage IV-V gametocytes with IC50 values of 2,453 ± 1,054 nM and 3,616 ± 1,360 nM, respectively, whereas MB was highly active against stage II-III and stage IV-V gametocytes with IC50 values of 27.7 ± 6.5 nM and 50.9 ± 24.5 nM, respectively. Addition of MB synergised the gametocytocidal activity of TQ against stage II-III gametocytes with summary fractional inhibitory concentrations (ΣFIC) of 0.52-0.73, but was mildly antagonistic against stage IV-V gametocytes with ΣFIC of 1.37-1.60. TQ-DHA and TQ-artemisone combinations were additive against early and late stage gametocytes, while TQ-artemiside was mildly antagonistic with ΣFIC of 1.09-1.33 and 1.99-2.30 against stage early and late stage gametocytes, respectively. Addition of CQ, PPQ, LUM and PRN at fixed concentrations corresponding to their maximum physiologically achievable concentrations to TQ had no significant effect on gametocytocidal activity of TQ. Our data suggest that MB can be considered as a potential partner drug with TQ for malaria elimination.

**6093**

## A FIRST-IN-HUMAN SAFETY, TOLERABILITY, AND PHARMACOKINETICS STUDY OF MMV367, A NEW CANDIDATE ANTI-MALARIAL AGENT FOR ACUTE UNCOMPLICATED MALARIA

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MMV367 is a first in class, fast acting, pyrrolidinamide blood stage inhibitor of Plasmodium falciparum co-developed by MMV and GSK with an anticipated novel antimalarial mechanism for treatment of acute uncomplicated P. falciparum malaria. It is highly active against mutant and resistant strains including field isolates, demonstrating absence of cross resistance. This FIH study sponsored by MMV (NCT05507970) evaluated the safety, tolerability, and PK of MMV367 in 47 healthy male and female volunteers. Part 1 was double-blind randomised, placebo (Pbo)-controlled with 4 sequential fasted cohorts (6 active and 2 Pbo, each) receiving a single, ascending dose of 100, 300, 750 and 1500 mg. Part 2 was an open-label, crossover, fed-fasted cohort (n=8) dosed with 440 mg. 400 mg QD for 3 days was tested in Part 3 (6 active and 2 Pbo). At the writing of this abstract, the study is completed, and data are still blinded. Unblinded study results will be presented. No serious or severe adverse events were reported. 14 treatment emergent adverse events (TEAEs) - all causality - were observed. 2 mild TEAEs (abdominal pain - epigastric pain) reported by the same participant in the 750 mg cohort, were judged as IMP-related. No other IMP-related TEAEs, clinically relevant ECG, vital signs or laboratory tests changes were reported. Cmax values were achieved 2 - 4 h post-dose in Part 1. MMV367 mean t½ values across cohorts ranged from 16.3 to17.5 h. Compared to the fasted state, geometric mean relative bioavailability after a high fat meal based on Cmax, AUC0-last and AUC0-inf were 161, 133 and 133%, respectively. Median Tmax were 3.5h (fasted) and 5h (fed) post-dose. Accumulation ratio after 3-day QD dosing was 2-fold. MMV367 demonstrated safety, tolerability and PK warranting further clinical development. This new candidate will be further tested against induced blood-stage malaria in healthy participants to provide a PK-PD model supporting Phase 2 study design.

**6094**

## EFFICACY OF PRAZIQUANTEL FOR TREATMENT OF PLASMODIUM FALCIPARUM INFECTION IN ASYMPTOMATIC GABONESE ADULTS

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To date praziquantel (PZQ) is the only licensed drug for the treatment of schistosomiasis. Also, there is preliminary evidence that PZQ may have an effect on Plasmodium parasites. This potential effect was assessed by the CORMA-MAL study, a phase 2a, single-center, randomized, blinded, placebo-controlled trial recruiting semi-immune, Gabonese adults with an asymptomatic Plasmodium falciparum parasitemia ranging from 200 to 5000 parasites/µl. PZQ was administered once daily as 40mg/kg dosage for three days (D0, D1, D2); Placebo was administered also once daily for three days. An effective antimalarial treatment was given to all participants at the end of study on D7, as well as to those reaching pre-defined rescue treatment criteria. Recruitment and follow-up of all 44 study participants was completed in February 2023 and the first preliminary results are presented here. 14% (3/22) of participants in the Placebo arm needed to receive the rescue treatment versus 5% (1/22) in the PZQ arm (p=0.29). The median (IQR) parasite reduction in the PZQ arm was 87% (0% to 99%) on D3 compared with 36% (-193% to 58%) in the Placebo arm (p=0.037) and 93% (45% to 100%) versus 68% (-228% to 100%), respectively on D7 (p=0.2). Linear regression models indicate a significant hourly decrease of log10(parasitemia) between D0 and D7 in the PZQ arm (y= -0.006*x + 2.4; p<0.0001), while there was a non-significant trend of decreasing log10(parasitemia) in the Placebo arm (y= -0.0018*x + 2.3; p=0.16). Out of 22 participants in the Placebo arm there were 3 (14%) participants with microscopic parasite clearance, compared with 7 (32%) out of 22 in the PZQ arm (log-rank test p=0.15). Computing the area under the curve (AUC) of log10(parasitemia) between D0 and D7 indicated that total parasite mass was lower in the PZQ arm (Mean AUC: 335) than in the Placebo arm (Mean AUC: 415), although not significantly (p=0.12). Concluding, preliminary data indicate some anti-plasmodial activity of PZQ in comparison to Placebo, however, further research is needed.

**6095**

## ETHIOPIAN PLASMODIUM VIVAX HYPNOZOITES FOR MATION DYNAMICS AND THEIR SUSCEPTIBILITY TO REFERENCE ANTIMALARIAL DRUGS

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One of the key obstacles to malaria elimination is largely attributed to Plasmodium vivax's ability to form resilient hypnozoites in the host liver that cause relapsing infections. As a result, interruption of P. vivax transmission is difficult. P. vivax transmission occurs in Duffy-positive individuals and have been mainly thought to be absent in Africa. However, increasing studies using molecular tools detected P. vivax among Duffy-negative individuals in various African countries. Studies on the African P. vivax has been severely limited because most of malaria control program focus mainly on falciparum malaria. In addition, there is a scarcity of laboratory infrastructures to overcome the biological obstacles posed by P. vivax. Herein, we established field transmission of Ethiopian P. vivax for routine sporozoite supply followed by liver stage infection in Mali. Furthermore, we evaluated local P. vivax hypnozoites and schizonts susceptibilities to reference antimalarial drugs. The study enabled the assessment of local African P. vivax hypnozoite production dynamics. Our data displayed the ability of the African P. vivax to produce hypnozoite forms ex vivo at different rates per field isolate. We report that while tafenoquine (1µM) potently inhibited both hypnozoites and

schizont forms; atovaquone (0.25μM) and the phosphatidylinositol-4-OH kinase (PI4K)-specific inhibitor KDU691 (0.5μM) showed no activity against hypnozoites forms. Unlike hypnozoites forms, P. vivax schizont stages were fully susceptible to both atovaquone (0.25μM) and the (PI4K)-specific inhibitor KDU691 (0.5μM). Together, the data revealed the importance of the local platform for further biological investigation and implementation of drug discovery program on the African P. vivax clinical isolates

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## VIRTUAL SCREENING OF THE NATURAL COMPOUNDS LIBRARY IDENTIFIES NATURE IDENTICAL SYNTHETIC COMPOUND METHYL GREVILLATE AS A NOVEL PFHDAC1 INHIBITOR WITH STEREOSPECIFIC MULTISTAGE ANTIMALARIAL ACTIVITY AND IN VIVO EFFICACY

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Malaria control relies on treatment with effective antimalarials. Although artemisinin (ART) combination therapies (ACTs) are currently the first line of treatment for Plasmodium falciparum (P.f.). As part of identifying specific inhibitors and novel targets essential for P.f survival, we focus on histone deacetylase 1 protein (PF3D7_0925700, PfHDAC-1) as a potential protein target. To identify hits from natural compound libraries potentially able to block PfHDAC1, we performed CADD based screening of n=135,335. These screening efforts identified commonly found tricyclic diterpenes/ terpenoid compounds. The hit library was negatively screened against human HDAC proteins. Methyl grevillate (MetG) among others was found to the top interacting compound with PfHDAC1 and had no binding affinity with human orthologue. MD simulation studies were conducted to validate the top hits, MetG had an energy score of -56.32. The MetG along with other top compounds were tested against erythrocytic stages which showed IC50 of 35.298±2.63 nM. Enzymatic validation assay revealed that MetG specifically blocks with inhibition curve fitting on competitive inhibitor models. In-vitro antiplasmodial efficacy testing of MetG in asexual stages confirmed its potent activity against both PfD6 (CQ-sensitive) and PfW2 (CQ-resistant) parasites. Anti-gametocidal evaluation showed distorted morphological changes in the nucleus of the RBC-dwelling parasites. Further testing in mice models of P. berghei blood stage and liver-stage malaria, showed in-vivo inhibitory activities. Most importantly, the evaluation using field isolates from clinical samples showed equally effective inhibition with IC50 of 198.45±6.1 nM. In addition, cytotoxicity testing showed negligible toxicity with 100x the IC50 in PBMCs, HEK293 and Huh 7.1. Notably, MetG was active against the pathogenic liver stage of the malaria parasite with IC50 of 78.91 nm. Taken together, these results suggest that MetG is a potential target-specific multi-stage inhibitor of P.f. malaria.

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## POPULATION PK ANALYSIS OF CHLOROQUINE IN A HUMANIZED MOUSE MODEL OF PLASMODIUM FALCIPARUM MALARIA

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The Plasmodium falciparum humanized mouse model is a validated tool to predict the efficacy of new drugs in humans. The aim of this work was to develop a population PK model of chloroquine in a P. falciparum humanized mouse model to investigate the relationship between model PK parameters and evolution of parasitaemia. Thus, human erythrocytes were engrafted into 60 immunodeficient female NODscidIL2Rgammanull (NSG) mice and they were infected with P. falciparum intravenously. The pharmacological treatment started when the percentage of parasitaemia in mice reached either 1 or 10 %. Single and repeated doses of chloroquine from 2.5 to 300 mg/kg were administered orally once daily for 1 to 6 days. Drug concentration data from all dosing schedules were modelled using Phoenix® NLMETM 8.3 and FOCE method. The relationship between physiological or pathological covariates and parameter estimates were explored. Since the animals were examined at different occasions, the inter occasion variability in individual parameters was also evaluated. Model parameters, diagnostic plots and internal validation techniques (VPC and bootstrap) were evaluated for model performance. Overall, 466 chloroquine concentration data were available and were successfully characterized by a 1-compartment model with linear elimination associated to an additive error model. Of the covariables tested, concentration of parasitized erythrocytes significantly influenced volume of distribution. The volume of distribution was lower when the concentration of parasitized erythrocytes was higher. Our results demonstrate that the PK of chloroquine in this model is highly dependent on the concentration of P. falciparum in peripheral blood, suggesting that this fact should be taken into account to understand and model the PK behavior of chloroquine.

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## WHOLE GENOME SEQUENCE ANALYSIS OF CANDIDATE GENES IDENTIFIED THREE LOCI POTENTIALLY RELATED TO MEFLOQUINE NEUROPSYCHIATRIC SIDE EFFECTS

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Despite effective once-weekly antimalarial prophylaxis, mefloquine has fallen out of favor due to neuropsychiatric effects. While genetic susceptibilities have been identified, pharmacogenomic testing guidance is not currently available for mefloquine. Volunteers with a history of exposure to mefloquine with or without adverse neuropsychiatric symptoms were invited to participate in a cross-sectional case-control study based on medical review of past mefloquine exposures. Pharmacogenomic analysis was performed on previously suspected 7 genes with 15 associated variants including ORM1 (S, F1, F2); MTHFR (A1298C, C677T); MDR1 (C1236T, G2677T, C3435T); PYK2 (rs2883490); HT2A (rs7997012, rs1928040, rs6311, rs6313); ADA (G22A); and ADORA2A (A2A) (T1976C, C2592T). There were 50 participants enrolled into one of four groups: those who had 1) mefloquine exposure and long-term adverse effects (AEs) greater than 6 months (n = 23); 2) exposure with subsequent AEs less than 6 months (n = 12); 3) exposure and no AEs (n = 8); and 4) a control group with a history of post-traumatic stress disorder (PTSD) but no mefloquine exposure or

traumatic brain injury (n = 7). Among volunteers exposed to mefloquine, the rs141942830 ADORA2A variant was potentially over-expressed among volunteers who had either long-term or short-term AEs compared to those who did not. Two additional variants were under- or over-represented relative to the comparable gnomAD population frequency, suggesting differences from reference controls. MTFHR was enriched for variation for volunteers who had long-term side effects compared to those with short-term or no side effects. A pharmacogenomics approach may help develop a mechanism to integrate test data and clinical findings to guide safer mefloquine use. These non-silent variants may serve as mediators to alternate pathways for signal transduction or drug metabolism, which may be future routes of research.

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## OPTIMAL DOSING OF SINGLE LOW DOSE PRIMAQUINE FOR TRANSMISSION BLOCKING OF P. FALCIPARUM IN CHILDREN.

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Single low dose primaquine (SLDPQ) to block the transmission of artemisinin-resistant Plasmodium falciparum and curtail its spread was first recommended in 2012 but insufficient data at that time precluded the WHO from suggesting age- and weight based dosing tables. Dosing in young children is challenging because limited if any suitable paediatric forms are available at the right tablet strength and a lack of pharmacokinetic data mean that approximation methods used for children, notably, allometric scaling are used to derive regimens. However, such regimens may not be optimal and require tablet fractions. Our group is developing child-friendly paediatric primaquine, in the form of flavoured uncoated tablets of 2.5, 3.75, 5, 7.5 & 15 mg, for optimal dosing regimens for transmission blocking and radical cure. Pharmacokinetic data from age dosed SLDPQ were analysed and models developed to predict primaquine maximum concentrations and exposures as surrogates of the bioactive oxidative metabolites that are gametocytocidal and haemolytic in G6PD deficiency. Analysis is ongoing and we will present optimal weight-based SLDPQ regimens for stand-alone use and adapted to the different dosing bands of the artemisinin based combination therapies in common use.

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## ASSESSMENT OF PLASMODIUM VIVAX BURDEN IN A MALARIA PRE-ELIMINATION CONTEXT AMONG HARD-TO-REACH POPULATIONS: P. VIVAX SEROLOGY AND PCR AMONG CLANDESTINE GOLD-MINERS IN THE GUIANA SHIELD (2015-2019)

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In a context of malaria pre-elimination in Suriname and French Guiana, residual transmission persists among hard-to-reach and mobile populations such as illegal gold miners. Plasmodium falciparum has been adressed by specific strategies but P. vivax (Pv) is particularly challenging, due to high proportion of asymptomatic carriers and low access to radical treatment leading to relapse. Our work aims to estimate the burden of Pv in the gold miner population in this region and its evolution between 2015 and 2019. Data derived from two cross-sectional surveys, conducted in 2015 and 2019 at the border between Suriname and French Guiana. Illegal gold miners having returned for less than 7 days from a gold mining site located in French Guiana were enrolled (questionnaire and collection of a venous blood sample). Samples were analyzed by qualitative polymerase chain reaction (PCR) according to the Snounou method. A serology specfic for Pv (described by Longley et al. In 2020) was used to identify individuals who had probably presented with Pv infection during the last 9 months. A total of 411 individuals were included in 2015, 378 in 2019. Among them, 36 (8.7%) had PCR detected Pv parasitemia in 2015 and 15 (3.9%) in 2019. For serology, a threshold corresponding to 63% sensitivity and 90% specificity was identified as the most appropriate for the study population: under these conditions, 45% of the participants in 2015 and 28% in 2019 had probably had a recent acute Pv infection, and were therefore potentially carriers of hypnozoites. In total, 40 (13.9%) of the 288 seropositive individuals had a positive PvPCR. Among the 51 positive PvPCR, 78% were Pv seropositive. In a context of low malaria prevalence, the proportion of individuals with serological markers of recent exposure to Pv remains significant. This species represents a major challenge for malaria elimination, and must targeted by tailored interventions. Moreover, these biological methods could be used as evaluation methods for new strategies for Pv elimination.

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## IMPLEMENTATION OF MALARIA COMMUNITY CASE MANAGEMENT (MCCM) IN TANZANIA: SUCCESSES, CHALLENGES, AND WAY FORWARD

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The World Health Organization recommends community case management of malaria to improve access to prompt, effective testing and treatment by trained community members living in proximity to patients. In Tanzania, many rural areas with high malaria endemicity are difficult-to-reach with malaria health services; however, Tanzania's new 2021 policy restricts community-based malaria testing and treatment only to licensed medical personnel called Community-Owned Resource Persons (CORPS), not Community Health Workers (CHWs). To evaluate the implications of this policy, the National Malaria Control Program conducted a pilot of malaria community case management (mCCM) between June and December 2022 in 311 villages across 10 councils with high malaria burden and low access to health services. One CORPS per village was recruited; following placement, CORPS conducted malaria rapid diagnostic testing (mRDT) for patients with suspected malaria and provided treatment for uncomplicated malaria following national guidelines. Cases exhibiting severe malaria were referred to the nearest health facility. During the pilot, 35,409 patients sought health services from the CORPS. Of these, 33,030 (93.3%) were tested by mRDT, and 10,631 (32.2%) were positive. Initially, only 104 CORPS could be recruited because of the licensing requirement, and only 77 (74.0%) of these CORPS were retained during the pilot, serving only 24.8% of the 311 villages. The most cited reason for leaving CORPS duties was other employment opportunities. Of the 77 CORPS, 23 (29.9%) were not residents of their assigned village, and among these 8 (34.8%) faced poor acceptability from the community. Meanwhile, there were 434 CHWs who also resided in the 311 villages. Studies in Tanzania have shown CHWs are widely accepted within their communities. Coverage of malaria diagnosis and treatment achieved by relying on CORPS was lower than expected. Further evaluation might identify successes in the provision of services and reasons for limited coverage and attrition of CORPS to help expand access to community case management.