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ABSTRACT BOOK

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0001

PHARMACOKINETIC PROPERTIES OF THE IRRUA-RIBAVIRIN-REGIMEN IN THE TREATMENT OF LASSA FEVER IN NIGERIA - A PROSPECTIVE OBSERVATIONAL STUDY

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Lassa fever is an acute infection with variable severity caused by the Lassa virus. Lassa virus causes annual outbreaks in many regions of West Africa with a marked surge in case numbers since 2018. The recommended antiviral therapy is ribavirin, the currently accepted regimens are the so-called McCormick regimen and the Irrua-ribavirin-regimen. However, evidence for ribavirin safety and efficacy in Lassa fever patients is poor and pharmacokinetic data for said regimens are not available. The Irrua-ribavirin-regimen is based on once daily dosing and thus became the standard of care in adult Lassa fever patients in Nigeria. The main objective of this research was to characterize the pharmacokinetic properties of the Irrua-ribavirin-regimen. A prospective, observational study was conducted at the Irrua Specialist Teaching Hospital in Edo State, Nigeria. PCR confirmed Lassa fever patients with mild to moderate disease severity that were to be treated according to the local standard of care were invited to participate. Blood samples for pharmacokinetic analyses were obtained 0.5, 1, 3, 5, 8, 12 and 24 hours after the 1st, 4th and 10th dose. Lassa signs and symptoms, hematology and biochemistry parameters were assessed every other day. During the 2020 and 2021 transmission seasons 20 patients were included. Plasma concentration time profiles of ribavirin were analyzed. The population pharmacokinetic analysis of the first 13 participants indicated that the PK was best described by a two-compartment model. The typical PK parameters were 10.6 L/h for clearance, 29.7 L/h for distribution clearance, 68.5 L for central and 644 L for peripheral volume of distribution. A low inter-patient variability of 25 %CV on clearance and 28.8 %CV on peripheral volume of distribution was found. This is the first clinical study describing the pharmacokinetics of intravenous ribavirin therapy of Lassa fever providing important insights into the potential mode of action of ribavirin in the treatment of Lassa fever. Despite the severity of the disease, drug exposure was remarkably consistent between subjects and PK of ribavirin was similar compared to other populations.

0002

EBOLA TRANSMISSION INITIATED BY RELAPSE OF SYSTEMIC EBOLA VIRUS DISEASE

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During the 2018-2020 Ebola virus disease (EVD) outbreak in North Kivu province in the Democratic Republic of Congo, EVD was diagnosed in a patient who had received the recombinant vesicular stomatitis virus-based vaccine expressing a ZEBOV glycoprotein (rVSV-ZEBOV) (Merck). His treatment included an Ebola virus (EBOV)-specific monoclonal antibody (mAb114), and he recovered within 14 days. However, 6 months later, he presented again with severe EVD-like illness and EBOV viremia, and he died. We initiated epidemiologic and genomic investigations that showed that the patient had had a relapse of acute EVD that led to a transmission chain resulting in 91 cases across six health zones over 4 months.

0003

EBOLA VIRUS INFECTIONS FROM PERSISTENTLY INFECTED SOURCES DURING THE NORD KIVU 2018-2021 EBOLA VIRUS DISEASE OUTBREAK IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Persistently infected Ebola virus disease (EVD) survivors were first identified during the 2013-2016 West Africa EVD outbreak. Investigations into flare-up events after the end of active transmission between symptomatic individuals identified a role for sexual transmission from survivors. A further two cases expatriated to their home countries experienced recrudescence manifesting as detectable virus in ocular fluid or cerebrospinal fluid (CSF). The Democratic Republic of the Congo (DRC) has experienced multiple

EVD outbreaks, with the most recent occurring between 2018 and 2021 in the Nord Kivu and Ituri provinces. The National Biomedical Research Institute (INRB) in the DRC has sequenced over 24% of Ebola virus (EBOV)-positive samples taken during the outbreak. During the latter stages of the outbreak, four cases of infection from a persistent source were identified: a case of EVD relapse causing onwards transmission to 91 others, two cases of meningitis with EBOV positive CSF, and the resurgence of the outbreak in 2021, eight months after it was declared over, by a suspected sexual transmission of the virus from a survivor. However, during the peak of the outbreak, there were likely other cases of transmission from persistently infected sources that went unnoticed due to the overwhelming numbers of cases at the time. We undertook genomic and epidemiologic case investigations to identify and characterize probable cases infected from persistently infected sources throughout the epidemic. Phylogenetic analysis showed a reduced evolutionary rate on the branches leading to the cases, which acts as a hallmark of infection from a persistent source. We identified four further probable cases of EBOV infection from persistently infected sources, from four different health zones, and discuss the case reports for each one.

0004

INSIGHTS INTO FILOVIRUS GLYCOPROTEIN FUNCTION FROM EPITOPE MAPPING AND INFECTIVITY ANALYSES

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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 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. We also mapped GP binding activity in sera of mice injected with DNA encoding MAbs (DMAbs). 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 identify GP residues important for EBOV infectivity, we performed infectivity assays with the full GP mutation library, using a lentivirus pseudotype system. We identified critical residues whose mutation abrogated infectivity without affecting GP conformational integrity. Their locations suggest crucial roles in GP conformational changes that cause virus-host membrane fusion. Additionally, to identify uncharacterized EBOV cellular receptors, we assayed wild-type GP infectivity in non-permissive cells individually expressing 6,000 unique human membrane proteins of our membrane proteome array (MPA). This has identified candidate membrane proteins that enable EBOV infectivity.

0005

RIFT VALLEY FEVER VIRUS REASSORTMENT IN THE CULEX TARSALIS MOSQUITO VECTOR

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Rift Valley Fever Virus (RVFV) is a zoonotic mosquito-borne pathogen endemic to sub-Saharan Africa and the Arabian Peninsula which causes Rift Valley Fever in ruminant livestock and humans. RVFV is a tri-segmented (L, M and S segment), negative-/ambi-sense, single-stranded RNA virus, which can reassort with other RVFV strains during co-infection of a susceptible host. Reassortment (RA) events have the potential to lead to novel viruses with altered virulence, transmissibility, and/or host range. This is especially of concern in RVFV endemic regions that frequently use live attenuated RVFV vaccines for livestock vaccination. Previously, we evaluated the frequency of RA in mammalian cells and sheep using the following three strains of RVFV: the vaccine strain MP-12 and two wild-type strains, one from Kenya (128B-15) and one from Saudi Arabia (SA01-1322). Our results in mammalian hosts indicated that the frequency of RA is rather low. In the current study, we evaluated the RA frequency of RVFV in *Culex tarsalis* mosquitoes. Briefly, two groups of mosquitoes (n=200 per group) were co-infected via blood meals containing an equal titer of approximately 10^7 pfu/ml of MP-12 and Kenya 128B-15 strain (group 1), or $10^{6.5}$ pfu/ml of Kenya 128B-15 and SA01-1322 (group 2), respectively. Midguts (MGs; n=30) and salivary glands (SGs; n=30) were dissected from fully engorged mosquitoes 14 days post exposure. MGs and SGs were homogenized, and virus plaques were isolated. The three genomic segments of fifty plaque-purified viruses from each tissue of group 1 and 2 were genotyped using strain-specific RT-qPCR genotyping (GT) assays. Reassortant viruses (RAVs) identified by GT assays were confirmed by Sanger sequencing. Our preliminary results show that >80% of plaque-purified viruses from the MGs of groups 1 and 2, and >60% of SGs plaque- purified viruses from group 1 are RAVs. Our current findings suggest a higher frequency of RA occurs in the mosquito vector when compared to the mammalian host.

0006

CREATING A PEDIATRIC PREDICTIVE DIAGNOSTIC TOOL FOR EBOLA VIRUS DISEASE

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Ebola virus disease (EVD) has caused several hemorrhagic fever outbreaks. Prior investigations have shown young children to be especially vulnerable, with high mortality rates. As such, there is a critical need for the rapid diagnosis of children so they can appropriately isolate and begin treatment. This study aimed to address knowledge gaps with the development of a predictive model to diagnose EVD in children at risk.

We analyzed retrospective data from patients presenting at International Medical Corps' Ebola Treatment Units in Liberia and Sierra Leone from 2014-2015. Candidate predictors, which included age, sex and 10 other variables from the current World Health Organization (WHO) guideline for identifying suspect Ebola cases, were entered into a logistic regression model to predict EVD diagnosis using forward stepwise regression with 10-fold cross validation. A 7-point risk score system was developed by converting the regression coefficient of each predictor in the final model to an integer. We assessed the discrimination and calibration using the Area Under the Receiver Operating Characteristic curve (AUC) and calibration-in-the-large respectively, for the derived model and risk score system. Of the 12 candidate predictors included in the analyses, 3 variables were positively associated with an EVD diagnosis: any bleeding (OR 7.5; 95% CI 3.7-16.0), a confirmed Ebola contact (OR 34.9; 95% CI 16.1-89.2) and unknown Ebola contact (OR 6.56; 95% CI 2.24-20.0). Abdominal pain (OR 0.30; 95% CI 0.17-0.53) was negatively associated with an EVD diagnosis. Model discrimination, measured using the AUC, was 0.87 (95% CI = 0.83 - 0.90). Both the model and the risk score system demonstrated a greater net benefit at risk score of 1 or higher (equivalent to 8% risk probabilities) compared to the WHO case definition for EVD diagnosis. Prompt diagnosis of pediatric EVD is challenging but this predictive model and risk score is more beneficial than the WHO case definition. To establish the utility of these tools, external validation is currently underway using data from the current EVD outbreak in the Democratic Republic of the Congo.

0007

HORIZONTAL AND VERTICAL TRANSMISSION OF HEPATITIS B IN THE DRC: EARLY FINDINGS FROM THE HOVER-HBV STUDY

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Hepatitis B virus (HBV) remains endemic in sub-Saharan Africa, despite an effective vaccine. Limited evidence suggests horizontal transmission within households or communities is an important driver of transmission and continued endemicity in sub-Saharan Africa, but the relative impact of horizontal versus vertical (mother-to-infant) transmission remains unknown. We are conducting a prospective, cross-sectional study to understand drivers of household transmission of HBV in urban Kinshasa, DRC. Households of HBV-positive (exposed households) and HBV-negative (unexposed households) mothers identified by screening during antenatal care at two high-volume maternity centers are eligible for enrollment. Household members undergo point-of-care HBV surface antigen (HBsAg) testing, provide blood samples for serological analysis and sequencing, complete questionnaires, and are offered HBV vaccine, if HBsAg-negative and exposed to HBV in the household. Since February 2021, 47 (24 HBV-exposed and 23 HBV-unexposed, by index mother status) of a target 200 households have been enrolled, totaling 296 individuals, including 185 children <18 years of age. Beyond known HBV+ index mothers, four additional household members tested HBsAg+, three from an HBV-exposed and one from an HBV-unexposed household. Of 172 individuals eligible for vaccination, only 39 have been vaccinated and 32 refused vaccination. The most common potential HBV exposures reported to-date are use of street salons (46% vs. 55% among HBV-exposed and unexposed households) and sharing nail clippers in the household (49% vs. 46%). Additional household and community exposures assessed include shared razors, traditional scarring, blood transfusions, sexual activity, and premasticated food. Spatial and phylogenetic analyses will complement epidemiological survey data to identify risk factors for HBV and transmission networks in urban Kinshasa. This study of HBV transmission will shed light on primary transmission modes in the DRC, where HBV is endemic and healthcare infrastructure is limited.

0008

EFFECTIVENESS OF THREE TYPES OF DUAL ACTIVE INGREDIENT TREATED NETS COMPARED TO STANDARD LONG-LASTING INSECTICIDAL NETS AGAINST MALARIA OUTCOMES: RESULT FROM A CLUSTER RANDOMIZED CONTROLLED TRIAL IN TANZANIA

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The massive scale-up of long-lasting insecticidal nets (LLINs) has led to major reductions in malaria burden in many sub-Saharan African countries. This progress is threatened by widespread insecticide resistance among malaria vectors. Next generation LLINs are urgently needed to reverse this trend. This trial evaluates the most promising LLINs combining two active ingredients (AI) with different mode of actions against malaria outcomes. A four parallel-arm, single-blind, cluster-randomized trial was conducted in 84 clusters in Misungwi district, North West Tanzania between October 2019 to February 2021. Interventions were assigned to 21 clusters each, the study arms were 1) Royal Guard®, combining two insecticides, pyriproxyfen (PPF) and the pyrethroid alpha-cypermethrin; 2) Interceptor® G2, combining chlorfenapyr (CFP) and alpha-cypermethrin; C3) Olyset® Plus, a LLIN combining a synergist, piperonyl butoxide (PBO) and the pyrethroid permethrin, and the reference arm 4) Interceptor®, a standard LLIN containing the pyrethroid alpha-cypermethrin only. Each household were allocated one net for every two people. Malaria infection prevalence was assessed in 50 children (aged 6 months to 14 years) per cluster at 12, 18 and 24 months post intervention. A cohort of 35 children (aged 6 months to 10 years) in each cluster was followed over 2 years to assess malaria cases incidence. Infection prevalence and case incidence were analysed using intention to treat comparing each of the dual AI LLIN to standard LLIN. Findings of these analysis will be presented. This is the first evaluation of Royal Guard and Interceptor G2 in a RCT and findings will constitute the body of evidence required by the WHO for a potential recommendation.

0009

EIGHT IS ENOUGH? ADDRESSING THE DESIGN AND ANALYSIS OF CLUSTERED TRIALS AIMED AT CONTROLLING VECTOR-BORNE DISEASES WITH LIMITED ACCESS TO LARGE NUMBERS CLUSTERS

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Cluster randomized trials (CRTs) allow for the evaluation of a community or group (cluster) level intervention. For studies that require a CRT design

to evaluate cluster-level interventions aimed at controlling vector-borne diseases, it may be difficult to assess a large number of clusters while simultaneously performing the additional work needed to monitor participants, vectors, and environmental factors associated with the disease. One example of a CRT with few clusters was the "efficacy and risk of harms of repeated ivermectin mass drug administrations for control of malaria (RIMDAMAL)" trial. Although previous work has provided recommendations for analyzing trials like RIMDAMAL, additional evaluation is needed for study designs with count outcomes. We used a simulation study to apply two analysis frameworks (individual level and cluster level analyses) to three CRT designs (single-year, two-year parallel and two-year crossover) in the context of RIMDAMAL II, a two-year follow-up of RIMDAMAL. Both individual level (with adjustments for small sample sizes) and cluster level analyses yielded reliable results across different study designs. The crossover design generally yielded higher power relative to the parallel equivalent with only one model not achieving 100% power. For RIMDAMAL II, we recommend an individual level analysis that accounts for the small number of clusters because it can simultaneously yield high power (98.6%) and control the type-I error rate (4.91%) while providing greater flexibility relative to the cluster level analyses. Although RIMDAMAL II is already underway as a parallel trial, applying the simulation parameters to a crossover design yielded improved power, suggesting that crossover designs may be valuable in settings where the number of available clusters is limited. For studies aiming to control vector-borne diseases, when interventions must be randomized at the village level, it is vital to find a balance between the often-limited number of clusters and the often-conservative recommended statistical approaches. Through our work, we have shown that multiple analysis approaches can be reliable.

0010

COMMUNITY KNOWLEDGE AND PERCEPTION ON MALARIA PREVENTION AFTER INTRODUCTION OF NEW, DUAL ACTIVE INGREDIENT INSECTICIDE-TREATED NETS (ITNS) IN THE HEALTH DISTRICTS OF BANFORA, GAOUA, AND ORODARA IN BURKINA FASO

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Malaria is a parasitic infection that is transmitted to humans through the bite of female *Anopheles* mosquitoes. Considering the emergence and intensification of insecticide resistance in key mosquito populations, dual active ingredient insecticide-treated nets (ITNs) that are effective against insecticide resistant mosquitoes have been developed. In 2019, standard pyrethroid, pyrethroid and piperonyl butoxide (PBO), and Interceptor® G2 (BASF) ITNs were deployed in Gaoua, Orodara, and Banfora districts, respectively. As part of the New Nets Project, we conducted a study to collect anthropological data and assess factors that could influence ITN uptake and usage. Anthropological data were collected in the three health districts from July to September 2019. We used a combination of in-depth interviews (n=146), structured observations (n=217), participant observations (n=469), and focus groups discussions (n=36). The study participants were mothers of children under the age of five, pregnant women, heads of households from different socio-professional backgrounds, and community leaders. A thematic analysis was conducted. All interviews were transcribed and analysed using NVivo software. Most respondents believe that mosquitoes transmit malaria. However, the sun, eating mangoes, going out in the rain, eating fatty foods, and excessive consumption of sugar and oil were also identified as factors that cause malaria. The best protection against mosquitoes is consistent use of an ITN. However, poor conditions of use of an ITN in some places limits the optimization of protection against mosquitoes. Raising awareness on the primary cause of malaria and better use of nets following distribution campaigns could contribute to reduce malaria transmission.

0011

INVESTIGATING THE DURABILITY OF THE BIOEFFICACY OF NEXT GENERATION NETS FROM COMMUNITIES IN BENIN

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A new generation of insecticide treated nets have been developed for malaria vector control and are being evaluated in communities across Africa. Clear guidelines to investigate the durability of the bio-efficacy of these nets in the field are however lacking. We performed a series of laboratory studies and experimental hut trials to help identify suitable procedures for investigating the durability of the entomological efficacy of pyrethroid-PBO nets (PermaNet 3.0), pyrethroid-chlorfenapyr nets (Interceptor G2) and pyrethroid-pyriproxyfen nets (Royal Guard) collected from communities in Benin at baseline and 6-12 months post distribution. To identify a suitable test method, we tested each net type in cone bioassays, tunnels tests and experimental huts. To identify an appropriate mosquito strain for monitoring each active ingredient, we tested susceptible and resistant mosquito strains with varying levels of resistance. For Royal Guard, we compared the use of mosquito egg laying to dissection of ovaries for monitoring pyriproxyfen in survivors. With PermaNet 3.0 and Royal Guard, cone bioassay mortality rates at baseline were >80% with the pyrethroid susceptible strain but reduced with Royal Guard as the level of pyrethroid resistance increased allowing for more survivors to assess the reproductive effects of pyriproxyfen. Full efficacy (>80% mortality) was achieved with Interceptor G2 using a pyrethroid-resistant strain in tunnel tests but not in cone bioassays. Bioassays results corroborated findings in experimental hut trials. The overall reduction in the fertility of mosquitoes which survived exposure to Royal Guard did not differ substantially between mosquito's egg laying and dissection methods. The results show that cone bioassays may be suitable for monitoring the bioefficacy of pyrethroids, pyriproxyfen and PBO in nets while tunnel tests will be more suitable for chlorfenapyr. Pyrethroid-resistant strains will be more suitable compared to pyrethroid susceptible strains for monitoring the bio-efficacy of all three non-pyrethroid active ingredients (PBO, Pyriproxyfen and chlorfenapyr).

0012

USE OF A PORTABLE FIELD-ADAPTED LIQUID CHROMATOGRAPHIC SYSTEM (C-VUE MACHINE) TO DETERMINE THE QUANTITY OF DELTAMETHRIN ON INSECTICIDE-TREATED NETS PAIRED WITH WHO CONE BIOASSAYS TO DETERMINE ITN BIOEFFICACY AS PART OF A THREE-YEAR DURABILITY MONITORING IN MALI

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Monitoring insecticide levels and physical integrity over time is essential for assessing the durability of insecticide-treated bednets (ITNs), which largely depends on the net handling habits of users. The objective of this study was to assess the insecticide content of ITNs (Yorkool and Permanet 2.0) at 6, 12, 24 and 36 months after a mass net distribution

carried out in Mali in December 2017. At each time point, 30 nets were randomly collected from households in the districts of Kenieba and Kita, together with information about ITN washing practices. The insecticidal effectiveness of the ITNs was assessed with the World Health Organization (WHO) cone test using a laboratory-reared susceptible colony of *Anopheles gambiae* (Ngousso) and the residual insecticide content measured by a non-destructive sampling technique with a portable field-adapted liquid chromatographic system (C-Vue ®) that was validated running samples in parallel with standardized WHO - HPLC methods. At 12, 24 and 36 months post distribution, nets had been washed an average of three times over the previous 6 months, most commonly using detergent or bleach. For Yorkool nets, the average deltamethrin concentration was 0.69 mg/m^2 at 6 months and gradually decreased to 0.08 mg/m^2 at 36 months. The values for Permanet 2.0 nets were 0.46 mg/m^2 at 6 months and a final measure of 0.06 mg/m^2 at 36 months.Until the 24 months evaluation, the proportion of nets with minimal effectiveness (at least 75% Knock Down "KD" or 50% 24h mortality) was greater than 80% for both net types and both sites, however, the proportion of nets with optimal effectiveness (at least 95% KD or 80% mortality) was less than 80%. The WHO standardized cone test and C-Vue evaluation respectively demonstrated that the effectiveness and insecticide content of both net types were consistently lower than expected at 3 years . The C-Vue was used successfully for the first time in Mali to measure the insecticide concentration of ITNs and produced results that were consistent with cone bioassays. This new technology allows for an affordable and locally available method to perform ITN durability monitoring in malaria endemic countries.

0013

(HALF) LIFE OF THE PARTY: EXPLORING THE IMPACT OF BEDNET DURABILITY AND RETENTION ON MALARIA BURDEN

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Insecticide-treated nets (ITNs) are one of the most widespread and cost-effective malaria prevention tools in sub-Saharan Africa, but maintaining high net coverage has proven challenging in many settings. Shorter-than-expected retention times are a major driver of low net coverage, as many households discard nets well before they are scheduled to receive new ones. The primary reason for net disposal is physical damage, which suggests that manufacturing more durable nets might lead to longer retention times and higher intervention efficacy. This study uses a microsimulation model to explore the potential impact of increased net retention on malaria incidence across a range of transmission intensities and seasonalities. We also explore these effects across different ITN coverage levels and insecticide efficacies. We find that increasing median ITN lifespans by one year could lead to significant reductions in malaria incidence, with stronger effects in high-transmission areas and at medium levels of net coverage. With a unit costing approach, we show the implications of longer net retention on cost per case averted. These results contribute to arguments for the value of including net durability as an official standard in ITN manufacturing.

0014

A NOVEL ALGORITHM TO DEFINE ADEQUATE PROTECTION OF LONG-LASTING INSECTICIDAL NETS: INSIGHTS FROM LATIN AMERICA

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The life of a long-lasting insecticidal net (LLIN) is measured by estimating its durability, physical integrity, and bio-efficacy. However, there is a need to integrate these parameters in a way that allows public health workers to estimate the level of protection provided by LLINs in a community. Here we present a novel algorithm to integrate these various types of data from durability surveys, using the results of a cross-sectional survey conducted at 32 months after a Permanent 2.0 distribution in a malaria focus in Guatemala. In this survey, the durability, usage, physical integrity and insecticide content determined by X-ray fluorescence were measured following WHO guidelines. We developed a working definition of a LLIN providing adequate protection, which integrates these measurements, following a cascading sequence: if the net was received by the household, if it was still present in the household, if it was used at least once, if it was used the night before the survey, if it was in serviceable condition (based on physical integrity) and if the concentration of deltamethrin was above 10 mg/m^2. Nets that met all these criteria were considered to be providing adequate protection. At 32 months, 66% of the nets were still present. Of these, 95% had been used at least once, 71% had been used the night before, 84% were in serviceable condition and 65% of the nets had a concentration above 10 mg/m^2. However, the overall proportion of LLINs providing adequate protection based on our algorithm was estimated to be just 21%. The provision of LLINs is a cornerstone of global efforts for the control and potential elimination of malaria, which is now a near-term goal in several Latin American countries. Our algorithm is the first attempt to integrate durability, usage, physical integrity, and bio-efficacy data to estimate the protective effect of the nets in a community. From a programmatic point of view, if validated, this algorithm could be useful to guide LLIN replacement strategies and increase the impact of this critical intervention.

0015

BIOMARKERS OF INTESTINAL INJURY ARE ELEVATED IN SEVERE MALARIA AND PREDICT IN-HOSPITAL AND POST-DISCHARGE MORTALITY

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Malaria continues to be a significant cause of illness and mortality in children. Severe malaria increases the risk of invasive bacterial infections, and there is evidence suggesting increased gastrointestinal permeability in severe malaria (SM). Sequestration of malaria parasites is well described and *in vivo* assessment of microcirculatory blood flow in the rectal mucosa demonstrates marked disruptions. However, the extent of intestinal injury in SM is not well characterized. We analyzed biomarkers of intestinal damage in children with SM, and their association with clinical complications and mortality, in a prospective multi-site observational cohort study. We enrolled children 6 months to 4 years of age with SM (n=600) and asymptomatic community children (CC, n=120) and followed them for 12 months to assess post-discharge mortality and morbidity. Serum samples were analyzed for two markers of intestinal injury, trefoil factor 3 (TFF3) and intestinal fatty acid binding protein (iFABP). Both TFF3 and iFABP were significantly higher in children with SM compared to the CC (p=0.0006 and p=0.0001 for TFF3 and iFABP, respectively), and were markedly increased in children with acute kidney injury (AKI) and acidosis (p<0.0001 for all). TFF3 and iFABP both predicted in-hospital mortality (odds ratio (OR) [95% confidence interval (CI)], 4.6 [2.8, 7.7] and 2.3 [1.7, 3.1] for a natural log increase in TFF3 and iFABP, respectively). TFF3, but not iFABP, was associated with increased post-discharge mortality (OR 2.5, 95% CI 1.2, 5.2). At 1 month follow up, iFABP levels had returned to community levels, while TFF3 levels remained elevated. Our results suggest that intestinal injury is common in pediatric severe malaria, and is

associated with increased in-hospital and post-discharge mortality. Studies are ongoing to determine the contributions of the parasite and host to intestinal injury in severe malaria.

0016

A COORDINATED TRANSCRIPTIONAL SWITCHING NETWORK MEDIATES ANTIGENIC VARIATION OF HUMAN MALARIA PARASITES

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Malaria continues to cause substantial morbidity and mortality throughout the developing world. A key trait of malaria parasites is their ability to systematically alter the antigens they expose to the immune system, thereby circumventing adapting immunity. This requires tightly regulated transcriptional activation and silencing of individual members of large, multicopy gene families that encode variant surface antigens. Expression of these genes is mutually exclusive and controlled epigenetically, however how transcriptional switching is coordinated is unknown. Here we describe an unusual genetic network in which switching events are coordinated by a unique "node" within the network. Deletion of this single, highly conserved locus disrupts switching, resulting in parasites that have a drastically reduced ability to change *var* gene expression, thus disabling the process of antigenic variation that is required to maintain a chronic infection. The discovery of this network provides an explanation for how parasites possessing a relatively small repertoire of variant antigen encoding genes can limit antigen exposure, thereby maintaining chronic infections.

0017

TRANSCRIPTOMICS REVEALS PERTURBATIONS IN AUTOPHAGY AS A CENTRAL PROCESS IN KENYAN CHILDREN WITH SEVERE MALARIAL ANEMIA

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Severe malaria anemia (SMA, Hb<5.0g/dL and any density parasitemia) is an immune-mediated inflammatory disease with complex etiology and acute progression, predominantly witnessed in children under 5 years residing in holoendemic *Plasmodium falciparum* malaria transmission regions, such as western Kenya. The pathogenesis of SMA remains only partially defined, creating barriers for clinical management. To provide insight into the molecular "signature pathways" that characterize the pathogenesis of SMA, differentially expressed genes (DEGs) were identified through transcriptomic analysis (mRNA-seq) using Next-Generation Sequencing on blood samples collected from children with acute malaria: non-SMA (Hb>5.0 g/dL, n=41) and SMA (n=29). Total RNA was Isolated for library preparation by NEBNext Ultra II with PolyA+ Selection. RNA-seq was performed on an Illumina® NovaSeq S4 (Illumina, California, USA). Enrichment analysis of functional gene sets was performed using KEGG and Reactome with significance defined as *P*-value adjusted (*Padj*)<0.05. Additional enrichment analysis was conducted using MetaCore™ with DEGs (log_2) and a false discovery rate (*FDR*)-adjusted *P*< 0.05. There were 6,862 DEGs with SMA defined by 1,420 up-regulated and 3,442 down-regulated genes. Autophagy was among the top-ranked pathways in KEGG (n=59, *Padj*=1.58x10^{-4}) and Reatome (n=57, *Padj*=1.58x10^{-3}). Analysis with MetaCore™ identified 17 protein coding genes in the autophagy pathway associated with SMA, with 13 genes (MTOR, UVRAG, BCL2, DAPK1, GABARAP, RB1CC1/FIP200, BECN1, AMBRA1, ATG13, ULK1, GABARAPL2/GATE-16, BCL2L1/Bcl-XL, and ATG14/Barkor) up-regulated and 4 genes (ATG3, ATG5, ATG7 and ATG10) down-regulated. Collectively, these results reveal, for the first time, that perturbations in autophagy pathways and processes are central to the pathogenesis of SMA.

0018

DRAMATIC CONSEQUENCES OF DISRUPTING LIPID HOMEOSTASIS IN PLASMODIUM FALCIPARUM

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We have recently shown that a wide range of antimalarials targeting two different transmembrane proteins (PfATP4 and PfNCR1) cause rapid disruption of cholesterol homeostasis in *P. falciparum*. Neither the parasite nor its host RBC is capable of cholesterol synthesis, and thus proper dispensation of this important lipid requires redistribution of cholesterol that was endowed to the RBC at the time of its maturation. Here we report that depletion of accessible cholesterol from the RBC plasma membrane by methyl-β-cyclodextrin (MBCD) has dramatic consequences, resulting in an inability of the parasite to invade RBC as well as in inhibition of the parasite growth. These defects were complemented by reconstitution with cholesterol or epicholesterol but not with desmosterol. These results suggest an important role for the aliphatic portion of the sterol, but not the polar group, in parasite invasion and growth. Using live time-lapse videography of fluorescently tagged trophozoite stage parasites, we detected rapid expulsion of the parasite when exposed to MBCD for just 30 min. The propelled trophozoites were still surrounded by parasitophorous vacuolar membrane (PVM) while remaining tethered to intact RBCs. Electron microscopy revealed the PVM to be compromised in the extruded parasites. Remarkably, prior 2 h treatment with PfATP4 or PfNCR1 inhibitors prevented the extrusion of trophozoites when exposed to MBCD. Overall, these findings suggest a dynamic movement of accessible cholesterol within *P. falciparum*-infected RBC that is critical for parasite survival. It would be fruitful to explore molecular players participating in this hitherto unknown aspect of parasite physiology.

0019

ADMISSION LACTATE DEHYDROGENASE PREDICTS RISK OF BLACK WATER FEVER IN CHILDREN WITH SEVERE MALARIA

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Black water fever (BWF) is a form of severe malaria that is increasingly reported among children in malaria endemic countries. However, there is limited information on predictors of BWF in children with severe malaria. The aim of this study was to evaluate clinical and laboratory predictors of BWF in children surviving severe malaria. We conducted a prospective observational study enrolling 600 children with severe malaria (SM) and 120 community children (CC), aged 0.5-4 years, between 2014 and 2017 at two hospitals in Kampala and Jinja, Uganda. All children with SM received intravenous artesunate followed by oral artemisinin-combination therapy and were asked to return to the study hospital when sick over one year of follow-up. On admission, 23.6% (n=141) of children with SM had BWF. Over one year follow-up 70/556 survivors of SM (13%) had at least one sick visit for BWF, while only 1% (1/120) of CC had a sick visit for BWF. 75% of children with BWF were from the Jinja site. Of the 70 children with SM who had BWF during follow up, 26 (37%) had

multiple visits for BWF. Children with SM who had BWF during follow-up had higher initial admission white blood cell count, total bilirubin, and lactate dehydrogenase (LDH) levels compared to children without BWF over follow-up (mean (SD), WBC, 21.4 (16.7) vs. 14.9 (10.3) x10^3/uL; total bilirubin, 2.2 (4.2) vs.1.1 (2.1) mg/d; LDH, 1526 (1365) vs. 848 (801) U/L, p<0.001 for all). There was no difference in age, sex or sickle cell status between children who had ≥1 episode of BWF during follow up compared to children who had none. Elevated admission levels of LDH, a marker of hemolysis and cellular injury, predicted risk of BWF in children with SM (incidence rate ratio 2.1 [95% CI 1.2, 3.7], p=0.01) after adjustment for child age, sex, and study site. Measurement of plasma LDH in children with severe malaria may help to identify children at risk of BWF after the episode of severe malaria.

0020

A NOVEL AMINO ACID SUBSTITUTION INAP2-G BLOCKS SEXUAL COMMITMENT IN PLASMODIUM FALCIPARUM

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The dramatic transition from asexual reproduction to sexual differentiation in *Plasmodium* is essential for malaria transmission in the field. In *P. falciparum,* the expression of two genes has been shown to be critical for the initial switch, *gametocyte development 1* (*gdv1)* and an *aptella 2* transcription factor (*ap2-g)*. In this pathway, GDV1 protein is required to increase *ap2-g* expression levels and initiate gametocytogenesis. To further interrogate the underlying regulation, we targeted an *in silico* predicted serine/threonine kinase (*STPK*: PFB0665w) which has an expression profile similar to *gdv1*. The resulting *P. falciparum* KO line (Δ*STPK-3D7*) showed a complete loss of gametocyte production. However, this phenotype was not reproduced in a subsequent independent KO attempt suggesting another unknown genetic event underlying gametocyte deficiency. Ring stage transcriptome analysis from Δ*STPK-3D7* parasites revealed a downregulation of *ap2-g* as well as AP2-G dependent gene transcripts. This expression profile is consistent with an alteration in the pathway before or, possibly, at *ap2-g* since AP2-G is known to augment its own expression. Whole genome sequencing of Δ*STPK-3D7* revealed a non-synonymous mutation in *ap2-g* in which valine (aa 2163, V_{2163}) in the AP2 domain (aa 2160-2210) is replaced with leucine (L). Although V to L is a conservative substitution, it was not observed in any of the many field isolate sequences listed in PlasmoDB.org, consistent with a potentially essential function. To test directly whether the V_{2163}L mutation inhibits AP2-G function, we introduced the L_{2163} mutation into *ap2-g* in gametocyte competent NF54 parasites, producing *NF54.ap2-g-L* and *NF54.ap2-g-L-GFP.FKBP* lines. Both L_{2163} lines showed a complete loss of gametocytes. Moreover, switching L_{2163} to the wild type allele (V) in Δ*STPK-3D7* and *NF54.ap2-g-L-GFP.FKBP* lines, restored gametocyte production. This work clearly demonstrates the key role of a single aa change in the function of AP2-G providing a target for further drug development.

0021

PERIPHERAL PLASMODIUM FALCIPARUM INFECTION IN EARLY PREGNANCY IS ASSOCIATED WITH INCREASED MATERNAL MICROCHIMERISM IN THE OFFSPRING

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Rare maternal cells traffic into the fetus during human pregnancy and are maintained into adulthood, a phenomenon known as maternal microchimerism (MMc). Placental malaria has been associated with increased cord blood MMc, however, the impact of maternal peripheral malaria on MMc is unknown. Further, maternal cells acquired in the setting of pregnancy malaria may be functionally active in the offspring. We therefore sought to determine the impact of maternal peripheral *P. falciparum* parasitemia during pregnancy on MMc and to determine whether maternal cells expand during primary parasitemia in the offspring. We conducted a nested cohort study of maternal-infant pairs from a prior pregnancy malaria chemoprevention study. MMc was measured by quantitative PCR targeting a maternal-specific marker in genomic DNA from cord blood, first *P. falciparum* parasitemia, and pre-parasitemia. Logistic and negative binomial regression were used to assess the impact of maternal peripheral parasitemia, symptomatic malaria, and placental malaria on cord blood MMc. Generalized estimating equations were used to assess predictors of MMc during infancy. Maternal parasitemia before 20 weeks gestation was associated with increased detection of cord blood MMc (AOR=3.91, p=0.03), whereas late parasitemia, symptomatic malaria, and placental malaria were not. The first parasitemia episode in the infant was not associated with increased MMc relative to pre-parasitemia. Maternal parasitemia early in pregnancy may increase the amount of MMc acquired by the fetus. Future work should investigate the impact of this MMc on immune responses in the offspring.

0022

SARS-COV-2 INFECTIONS DURING THE FIRST EIGHT MONTHS OF THE EPIDEMIC IN NIGERIA: SEROPREVALENCE ESTIMATES FROM A HOUSEHOLD SURVEY IN THREE STATES

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Population-based serological testing can generate more accurate estimates of prior SARS-CoV-2 infections than routine surveillance data, which depend on testing rates and symptoms. From February 27—October 1, 2020, Nigeria reported 59,001 COVID-19 cases, though molecular testing rates were low. From September–October 2020, we conducted a household (HH) survey to assess SARS-CoV-2 seroprevalence in three states, Enugu (south), Gombe (north), and Nasarawa (central), which reported 1,234, 888, and 453 confirmed COVID-19 cases, respectively, by October 1, 2020. Two-stage cluster sampling was used, with 34 enumeration areas (EAs) sampled per state and 20 HHs per EA. All HH members were eligible. Participants were asked about behaviors and COVID-19 symptom history since March 2020; oral and nasopharyngeal swabs were taken for PCR and blood sampled for SARS-CoV-2 antibody and malaria rapid diagnostic testing. Abbott Architect IgG and Euroimmun NCP IgG assays were used for SARS-CoV-2 serology screening; samples positive on either assay were tested on the multi-antigen target Luminex xMAP assay. Luminex-positive samples were considered seropositive. Of 1,931 occupied HHs, 1,827 (94.6%) consented; of 8,823 eligible HH members, 8,356 (94.7%) consented. PCR detected 20 active SARS-CoV-2 infections in total. SARS-CoV-2 seroprevalence was 23.4% (95% CI: 18.1,

28.8) in Enugu, 8.8% (95% CI: 6.4, 11.2) in Gombe, and 18.7% (95% CI: 14.6, 22.7) in Nasarawa, with no significant differences by sex or urban/rural location. In all states, children <10 years had lower seroprevalence than those 10+ years (Enugu: 17.3% vs. 25.4%, p=0.0296; Gombe: 4.7% vs. 10.9%, p=0.003; Nasarawa: 12.7% vs. 21.4%, p=0.0002). More than half (52.1%) of infections were asymptomatic. Prevalence of *P. falciparum* malaria was 21.0% in Enugu, 45.4% in Gombe, and 38.6% in Nasarawa; malaria was more prevalent among seropositive individuals in Enugu (24.5% vs. 19.9%, p=0.0184), but not in other states. About 1 in 10 Nigerians in Gombe and 1 in 5 in Enugu and Nasarawa had evidence of prior SARS-CoV-2 infection as of October 2020; most of the population remains susceptible to COVID-19.

0023

ESTABLISHING A SENTINEL SURVEILLANCE FOR THE NOVEL CORONAVIRUS DISEASE 2019 (COVID-19) IN BANGLADESH

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icddr,b, in collaboration with the Institute of Epidemiology, Disease Control and Research (IEDCR), Government of Bangladesh, established a hospital-based surveillance platform for screening suspected COVID-19 patients to understand the COVID-19 situation in different regions where nearby testing facility (reverse transcription polymerase chain reaction, RT-PCR) was unavailable. We conducted the surveillance at three secondary level public and one tertiary level private hospital in different regions. Study staff enrolled suspected COVID-19 patients with any of the symptoms within the last 7 days- fever, cough, sore throat, and respiratory distress. They recorded clinical and epidemiological data, collected and transported nasopharyngeal swabs to icddr,b, Dhaka for SARS-CoV-2 test using RT-PCR. Findings were reported to the authorities over email and to the patients over short message service within 36 hours. Staff followed up with all patients after 30 days for the outcome of the illness over telephone. From 10th June to 31st August 2020, COVID-19 was detected in 39% (922/2345) enrolled patients. It was more common in outpatients with a peak positivity in July (54%). The median age of the confirmed COVID-19 cases was 38 years (IQR: 30-50), 71% were male, and 9% were healthcare workers. Among them, cough (67%) was the most common symptom followed by fever (53%). Diabetic patients were more likely to get COVID-19 than non-diabetic (48% vs. 38%, p<0.05). The death rate among COVID-19 cases was 2.3% (21/922). Death was associated with age ≥ 60 years (OR:13.5; 95% CI: 5.4-33), shortness of breath (OR:14.4; 95% CI: 4.8-43), co-morbidity (OR:13.9; 95% CI: 3.2-60), smoking history (OR: 3.9, 95% CI: 1.5-9.8), hospital admission (OR:13.3; 95% CI: 5.3-33) and health care seeking in <2 days due to critical illness (OR: 5.4; 95% CI: 1.8-17). More than one-third of suspected patients attending surveillance hospitals had COVID-19. Our effort strengthened the government's capacity for rapid case detection, reporting and quick containment efforts. This surveillance should be continued to monitor the trends of COVID-19 over time.

0024

PERSONAL PROTECTIVE EQUIPMENT (PPE) SEES THE LIGHT: LOW-COST METHYLENE BLUE PLUS LIGHT INACTIVATES SARS-COV-2 ON PPE INCLUDING MASKS AND RESPIRATORS

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The COVID-19 pandemic has resulted in severe shortages of personal protective equipment (PPE) necessary to protect front-line healthcare personnel. Shortages are critical, especially in low-resource settings, and underscore the urgent need for simple, efficient, and inexpensive methods to decontaminate SARS-CoV-2-exposed masks and respirators, enabling reuse when using crisis capacity strategies. We hypothesized that methylene blue (MB) photochemical treatment, which is used in multiple clinical applications including inactivation of virus in plasma, could decontaminate PPE exposed to coronavirus. We tested the ability of MB with light (MBL) treatment to inactivate coronaviruses on three N95 filtering facepiece respirator (FFR) and two medical mask (MM) models. We inoculated FFR and MM materials with three different coronaviruses, including two separate isolates of SARS-CoV-2, and treated the materials with 10-μM MB followed by exposure to 50,000 lux of broad spectrum light (~1/3 the brightness of sunlight) or 12,500 lux of red light for 30 minutes. In parallel, we subjected intact FFRs/MMs to five cycles of MBL decontamination (5CD) to assess the impact on integrity using multiple standard test methods and compared the results to the FDA-authorized vaporized hydrogen peroxide plus ozone (VHP+O_3) method. Overall, MBL robustly and consistently inactivated all three coronaviruses with 99.8 to >99.9% virus inactivation across all FFRs and MMs tested. FFR and MM integrity was maintained after 5CD of MBL treatment, whereas one FFR model failed after 5CD with VHP+O_3. MB plus ambient light and pretreatment of FFRs with MB also resulted in complete inactivation of SARS-CoV-2. MBL decontaminated FFRs and MMs by inactivating three coronaviruses without compromising integrity through 5CD. MBL decontamination of FFRs and MMs is effective and low-cost, thus making it applicable in *all resource settings*. These attractive features support the utilization and continued development of this novel PPE decontamination method.

0025

NON-ADHERENCE TO INFECTION PREVENTION AND CONTROL MEASURES FOR COVID-19 AMONG COMMUNITY HEALTH WORKERS: A POTENTIAL THREAT TO COMMUNITY HEALTH PROGRAMS IN UGANDA, 2020

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Community healthcare workers (CHWs) in Uganda provide integrated community case management (iCCM) of fever, cough, and diarrhea to children 2-59 months. COVID-19 has threatened the safety and continuity of these services. To assess COVID-19 perceptions and preparedness among CHWs, we conducted a cross-sectional survey in Moyo and Adjumani Districts in December 2020. We interviewed and observed randomly selected CHWs affiliated with each of 10 randomly sampled health facilities per district and collected data on CHWs' perceived COVID-19 risk, access to infection prevention and control (IPC) supplies, adherence to IPC measures when providing community-based services, and COVID-19 training received. We used logistic regression to assess associations between CHW characteristics and risk perception.We interviewed 231 CHWs (43% female). Median age was 38 (IQR: 32-46) years and median CHW tenure was 9 (IQR: 4-15) years; 66 had completed secondary education or higher. Most (92%) had attended training on COVID-19. Nearly half (44%) did not have access to a functional handwashing station (with soap and water); 37% did not have enough medical masks in the last month. Among 130 CHWs with access to functional handwashing stations, 31 (24%) did not adhere to proper handwashing practices. Among 145 CHWs with adequate masks in the last month, 104 (72%) did not regularly wear them. Most (89%) reported changing gloves between patients and maintaining social distancing at work (82%). Only 20% reported adhering to all four assessed IPC measures (i.e., wearing masks, washing hands, using/changing gloves between patients, maintaining social distance) and 24% perceived COVID-19 as a serious risk. Longer service as a CHW (21-30 vs. <10 years) was associated with higher perceived risk of COVID-19 (OR 3.4, 95% CI 1.2-10.1).While nearly all CHWs had COVID-19 training, there is a need to improve access to IPC supplies and adherence to all IPC measures, particularly use of masks and handwashing. Supportive supervision and behavior change approaches such as peer mentorship by more experienced CHWs, may help address these gaps and minimize disruption to iCCM.

0026

COVID-19 VACCINATION INTENTION IN AN ARBOVIRAL COMMUNITY COHORT IN PONCE, PUERTO RICO

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As of April 11th, 2021, more than 103,000 confirmed COVID-19 cases and 2,100 deaths have been reported in Puerto Rico, where COVID-19 vaccination started on December 15, 2020. In the Communities Organized for the Prevention of Arboviruses (COPA) study, a community-based cohort in southern Puerto Rico, we interviewed participants aged ≥14 years about COVID-19 vaccine intention from November 12, 2020 to March 31, 2021. We calculated frequencies and evaluated associations between COVID-19 vaccine intention and participant demographics using Pearson $\chi 2$, Fisher exact tests, one-way ANOVA, and unadjusted relative risks. No significant changes were found after adjusting to account for sample size and age and sex, using a log-binomial model. Among 1,036 participants interviewed, 934 (90%) answered the vaccine intention questions, median age was 37 years (range 14-80), 539 (58%) were female, 870 (93%) identified as Latino, and 61 (7%) reported having been previously diagnosed with COVID-19. Most participants (79%) reported that they would be willing to receive a COVID-19 vaccine,12% wouldn't receive it, and 9% were not sure. The most frequently reported reason for not intending to receive or not being sure about receiving a COVID-19 vaccine was concern about safety or side effects (61%). People previously diagnosed with COVID-19 (RR 1.3, 95%CI 1.2-1.3), who had contact with a COVID-19 case (RR 1.3, 95%CI 1.2-1.3), who believed vaccines are important to prevent diseases (RR 2.9, 95%CI 1.8-4.7), and who would accept a dengue vaccine if available (RR 3.1, 95%CI 2.4-4.0) were more likely to report they would receive a COVID-19 vaccine. Participants aged 31-40 were less likely to report they would receive a COVID-19 vaccine when compared to those aged 14-20 (RR 0.9, 95%CI 0.8-0.9). No differences were found by sex, race, or education. COVID-19 vaccine intention is high among COPA participants and is associated with a previous COVID-19 diagnosis or exposure and general positive attitudes towards vaccines. Public health messaging in Puerto Rico should address concerns regarding COVID-19 vaccine safety and side effects tailored to the local context.

0027

DEVELOPING EVIDENCE-BASED RECOMMENDATIONS FOR FULLY VACCINATED TRAVELERS TO REDUCE RISK OF SARS-COV-2 SPREAD, MARCH 2021

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Travel is an important factor in the spread of SARS-CoV-2 including variants of concern (VOC). Recommendations for travelers during the pandemic require measures that protect individuals during the journey and in the communities where travelers visit and live. Travel risk reduction strategies have focused on a combination of pre- and post-travel testing and self-quarantine that can impose significant burden on both travelers and public health resources. During April 2021, with the increasing availability of SARS-CoV-2 vaccines, CDC re-examined the scientific rationale for US travel recommendations. We reviewed literature from studies that evaluated vaccine effectiveness (VE) against infection for FDA-authorized vaccines through March 17, 2021. To estimate the impact of vaccination combined with other prevention measures, we used vaccine effectiveness ranges of 60-90% in models previously developed to assess impact of testing and quarantine on travel-associated transmission risk. Models were also developed to assess travelers' risk of SARS-CoV-2 infection at travel origin and destination using vaccination coverage rates. At the time of our analysis, estimated VE for FDA-authorized vaccines was reported as 64%-92%. Models using a 90% effective vaccine showed that adding pre-and post-travel testing and a 7-day self-quarantine provided a median estimated 9.7% (range 8.6, 9.9) risk reduction beyond the impact of vaccination for vaccinated travelers. Models also showed that vaccination coverage impacts risk; with increasing vaccination coverage at origins and destinations, risk of introduction by a traveler were reduced proportionally. Our models showed that with an effective SARS-CoV-2 vaccine testing and quarantine provide only marginal additional risk reduction for the fully vaccinated traveler. Vaccination coverage and vaccine performance against emerging variants are important considerations, particularly for international travelers from locations where SARS-CoV-2 burden is high. As such, testing of international travelers continues to be a valuable public health tool.

0028

STRUCTURAL RACISM AND COVID-19 RESPONSE: HIGHER RISK OF EXPOSURE DRIVES DISPARATE COVID-19 DEATHS AMONG BLACK AND HISPANIC/LATINX RESIDENTS OF ILLINOIS, USA

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Structural racism drives the social, economic, and community factors leading to decades of policies and conditions that have resulted in residential segregation, lack of employment opportunities that limit economic mobility, and poorer population health for minoritized populations. In 2020, Black and Hispanic/Latinx communities across the United States, including the state of Illinois, experienced disproportionately high rates of COVID-19 cases and deaths. Public health officials in Illinois implemented targeted programs at state and local levels to increase intervention access and reduce disparities. To quantify how disparities evolved through the epidemic, data on SARS-CoV-2 diagnostic tests, COVID-19 cases, and COVID-19 deaths were obtained from the Illinois National Electronic Disease Surveillance System for the period from March 1 to December 31, 2020. Relative risks of COVID-19 cases and deaths were calculated for Black and Hispanic/Latinx vs. White residents, stratified by age group and epidemic interval. Deaths attributable to racial/ethnic disparities in incidence and case fatality were estimated with counterfactual simulations. From March to May of 2020, the risk of a COVID-19 case for Black and Hispanic/Latinx populations was more than twice that of Whites across all age groups. The relative risk of COVID-19 death reached above 10 for Black and Hispanic/Latinx individuals under 50 years of age compared to age-matched Whites in the early epidemic. In all Illinois counties, the relative risk of a COVID-19 case was greater or not significantly different for minoritized populations compared to White. 79.3% and 86.7% of disparities in deaths among Black and Hispanic/Latinx populations respectively were attributable to differences in age-adjusted incidence compared to White populations rather than differences in case fatality ratios. Relative lack of access to health care, crowded living conditions, and high-risk occupations are the result of structural racism, which placed Black and Hispanic/Latinx populations at higher risk of exposure to SARS-CoV-2 and thus higher COVID-19 mortality rates.

0029

IMPACT OF CURRENT MALARIA INFECTION AND PREVIOUS MALARIA EXPOSURE ON THE CLINICAL PROFILES AND OUTCOME OF COVID-19 IN A HIGH MALARIA TRANSMISSION SETTING: A PROSPECTIVE COHORT STUDY

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Our understanding of the potential impact of SARS-CoV-2 and malaria co-infection on host susceptibility and pathogenesis remains unclear. We determined the prevalence of malaria and describe the consequences of SARS-CoV-2 and malaria co-infection in a high burden malaria setting. This was a prospective cohort study of hospitalized Covid-19 patients in Uganda. Malaria diagnosis was done using rapid diagnostic tests, microcopy and molecular methods. Previous *P. falciparum* exposure was assessed using serologic responses to a panel of *P. falciparum* antigens using a multiplex bead assay. Additional evaluations included complete blood count, markers of inflammation and serum biochemistries. Of 597 PCR confirmed Covid-19 cases enrolled between 16th April and 30th October 2020, 500 (84.1%) were male and median age (1QR) was 36 (28-47) years. Overall prevalence of *P. falciparum* infection was 11.7% (70/597, 95% CI 9.4 to 14.6), with highest prevalence in the 0-20 years (21.7%, 5/23, 95% CI 8.7-44.8) and > 60 years (19.6%, 9/46, 95% CI 10.2-34.1) age groups. Confusion [5.7% (4/70) vs. 1.5%, (8/527), p=0.04] and vomiting [5.7% (5/70) vs.1.0%, 5/527), p=0.007] were more frequent among patients with *P. falciparum* infection. Patients with low previous P.falciparum exposure had a higher frequency of severe/critical Covid-19 cases (30.2%, 16/53, p=0.001), a higher burden of comorbidities [hypertension (30.2%, 16/53, p=0.02) and diabetes (22.6%, 12/53, p=0.003)] and more deaths (3.8%, 2/53, p=0.01). Among patients with no comorbidities, those with low previous exposure still had a higher proportion of severe/critical Covid-19 cases (18.2%, 6/53 vs. 2.0%, 1/56, p=0.01) compared to those with high exposure. Prevalence of *P. falciparum* infection among Covid-19 patients was relatively high. Though Covid-19 patients with *P. falciparum* infection had a higher frequency of confusion and vomiting, co-infection with malaria did not seem deleterious. Low previous malaria exposure was associated with severe/critical Covid-19 and adverse outcomes.

0030

IMPACT OF COVID-19 ON PEOPLE LIVING WITH HIV/AIDS IN THAILAND: A PROSPECTIVE STUDY

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The pandemic of COVID-19 has effect to healthcare system particularly people living with HIV/AIDS (PLWHA). In Thailand, there are limited data regarding the effect of this emerging infectious disease with PLWHA. We hypothesized that COVID-19 would have substantial effect on PLWHA also using telemedicine may increase the quality of life and decrease the risk of contracting COVID-19 infection. Methods: The prospective telephone-based questionnaire study conducted from July 2020 to January 2021 in Ramathibodi hospital, Thailand. All PLWHIV over 18 years old seen in ID outpatient clinic were eligible. The prospective telephone-based questionnaire study conducted from July 2020 to January 2021 in Ramathibodi hospital Mahidol University, Bangkok, Thailand. All PLWHIV aged over 18 years old who are following-up in outpatient clinic were eligible. One thousand nine hundred and fourteen (74%) of 2,597 PLWHA participated in the study. Median age was 48 years old (IQR 40-55). Thirty eight percent were female, 62% male. Most people lived in Bangkok (52%) and others from 70 provinces across Thailand and other countries in Asia. Half were treated over 10 years. Fifty eight percent of participants who missed appointments reported to be due to COVID-19. Participants were asked to reschedule appointments were more from Bangkok (p =.0044). Seven percent of participants using telemedicine with 89% reported high satisfaction. Ninety two percent preferred to use telemedicine if possible. Nine percent received ART from the mailing service with high satisfaction rate of 83%. Twenty-two participants had privacy concern without significant correlation in educational level (p=.22). Thirty one percent preferred to receive medication by themselves at the clinic. Two percent missed their ART and it was unrelated to educational level (p=.14). There were challenge in the care of PLWHA during COVID-19 pandemic. The concern for privacy was still present. With ongoing epidemic of COVID-19 and possible future epidemic, understanding the effect and possible solutions will enhance the best possible care for PLWHA.

0031

ATYPICAL PRESENTATION OF LEPROSY IN AN HIV POSITIVE PATIENT

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Leprosy (Hansen's disease) is one of the neglected tropical diseases which is a curable, chronic infectious disease caused by bacteria called *Mycobacterium leprae* transmitted from person to person through respiratory droplets. It affects mainly nerves, skin, eyes, and nasal mucosa. Diagnosis of leprosy is most commonly based on clinical signs and symptoms. Atypical presentations like our case, calcinosis cutis-like presentation may be diagnosed with histopathology. Management is multi-drug treatment (MDT). A fifty-year-old known human immunodeficiency virus, a positive male patient, on antiretroviral medication, presented with a skin rash of one week duration. It began as a small raised painless swollen lesion on the forearm bilaterally and progressively involve the lower leg associated with occasional burning sensation and numbness on hand and foot. Upon examination, firm to hard nodule and papule on dorsal distal for-arm and hand, and shine. He had also an enlarged non-tender right and left ulnar nerve. Biopsy was done from the raised skin lesion showed diffuse foamy macrophage and around nerves which harbour numerous acid-fast bacilli. The patient managed multi-drug treatment with a good outcome.

0032

EARLY-LIFE ENTERIC INFECTION AND ENTEROPATHY MARKERS ARE ASSOCIATED WITH CHANGES IN ADIPOKINE, APOLIPOPROTEIN AND CYTOKINE PROFILES LATER IN CHILDHOOD CONSISTENT WITH THOSE OF AN ADVERSE CARDIOMETABOLIC DISEASE RISK PROFILE IN A PERUVIAN BIRTH COHORT

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Metabolic syndrome is a cluster of risk factors for cardiovascular disease that afflicts over a billion people worldwide and is increasingly seen in younger age groups and low-resource settings in the global south. Enteropathogen exposure and environmental enteropathy in infancy may lead to metabolic syndrome by disrupting the metabolic profile in ways that are detectable in cardiometabolic markers later in childhood. 217 subjects previously enrolled in a birth cohort in Amazonian Peru were followed up annually from ages 2 to 5 years. Blood samples collected in later childhood were analyzed for 37 cardiometabolic biomarkers, including adipokines, apolipoproteins, cytokines, and other analytes. These were matched to extant early-life markers of enteropathy ascertained between birth and 2 years of age. Multivariate and multivariable regression models were fitted to test for associations. Fecal and urinary markers of intestinal permeability and inflammation measured from birth to 2 years of age were independently associated with later serum concentrations of soluble CD40-ligand, a proinflammatory cytokine correlated with adverse metabolic outcomes. Fecal myeloperoxidase was also strongly, directly associated with later levels of the anti-inflammatory adipocytokine omentin-1. Cumulative enteric protozoa exposure before 2 years of age showed stronger associations with later cardiometabolic markers than enteric viruses and bacteria and overall diarrheal episodes. Early-life markers of enteric infection and enteropathy were associated with numerous changes in adipokine, apolipoprotein and cytokine profiles later in childhood consistent with those of an adverse cardiometabolic disease risk profile in this Peruvian birth cohort. Markers of intestinal permeability and inflammation measured in urine and stool during infancy, may predict disruptions to cytokine and adipocytokine production in later childhood that are precursors to metabolic syndrome in adulthood. Chronic enteric infections, such as by protozoan pathogens, may be more important drivers of these changes than symptomatic diarrhea or growth faltering.

0033

CHARACTERIZATION OF TYPHOID INTESTINAL PERFORATION IN AFRICA: RESULTS FROM THE SEVERE TYPHOID FEVER SURVEILLANCE IN AFRICA (SETA) PROGRAM

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Typhoid intestinal perforation (TIP) is a severe, life-threatening complication of untreated or improperly treated typhoid fever. Mortality rates range from <1% in high-income countries to >30% in parts of sub-Saharan Africa. A lack of blood culture surveillance contributes to delays in diagnosis and management of typhoid fever, allowing progression to complications including TIP. Diagnostic challenges also limit understanding of incidence and mortality rates of TIP. The Severe Typhoid Fever Surveillance in Africa (SETA) program, a multi-country surveillance study, addresses these knowledge gaps by systematically collecting data on culture positive typhoid cases and characterizing the frequency, risk factors, and complications associated with typhoid fever, including epidemiology, presentation, and time trends of TIP. Patients with clinical suspicion of TIP, reporting abdominal pain with or without objective fever or history of fever, were enrolled. A venous blood sample was taken for culture investigation of *Salmonella* Typhi, and when surgery was performed, a sterile surgical site culture was obtained. Based on a combination of typhoid culture positivity and surgical verification of intestinal perforation, we classified TIP as confirmed, probable, possible, or suspect cases. We assessed 608 patients (353 males, 255 females) with clinically suspected TIP diagnoses from 6 African countries. Twenty of these were confirmed TIP cases, 9 probable, 195 possible, and 384 suspect cases. Ages ranged from 0-85 years, with the most seen between 5-10 years of age. Results from a generalized linear model indicate monthly typhoid cases were predictive of monthly TIP cases in the Democratic Republic of the Congo, Ethiopia, Ghana, and Nigeria, however, not in Burkina Faso or Madagascar. The overall mortality rate ranged from 2.7% (Ghana) to 27.3% (Madagascar). TIP mortality remains high in sub-Saharan Africa, with our data suggesting the burden is likely underestimated. A coordinated approach that includes vaccination with typhoid conjugate vaccine, access to care, and investments in infrastructure can help prevent this deadly disease.

0034

NITROIMIDAZOLE VERSUS PAROMOMYCIN IN TREATMENT OF DIENTAMOEBA FRAGILIS INFECTION; CLINICAL AND MOLECULAR EVALUATION

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Dientamoeba fragilis (DF), is a common gastrointestinal protozoan (second only to *Blastocystis* spp.). This parasite is associated with gastrointestinal symptoms, yet its pathogenicity is still controversial. To assess pathogenicity and treatment response, we prospectively evaluated all patients with chronic gastrointestinal symptoms and positive stool PCR for DF only (or co-infected with *Blastocystis* spp). Treatment courses, clinical response and post-treatment molecular results were recorded. Clinical cure was defined as resolution of symptoms as reported by the patient and molecular response was defined as negative PCR for DF following treatment course. During 2 years of study period 46 patients

were eligible, 47.8% were male, median age 36.5 (range 3-83) years, and 75% had travel history prior to symptoms onset. The most common symptoms were loose stool (87%) and abdominal pain (85%) for an average period of 8 months before presenting at our clinic. Altogether, 74 treatment courses with clinical response were recorded. Treatment courses included mainly nitroimidazoles based regiments (54%) and paromomycin (31%) with clinical cure rates of 27% and 82% respectively (p<0.0001). Molecular response was evaluated in 39 samples with molecular response documented by negative stool in 20% of samples of patients with nitroimidazoles-based regiments while after paromomycin courses in 73.3% (p=0.0024). A high correlation was found between molecular cure and clinical response. In conclusion, our results support the notion that DF should be considered as a pathogenic protozoon since there was a correlation between DF eradication and clinical cure. Paromomycin should be the preferred treatment option.

0035

VALIDATION OF A MOBILE HEALTH TOOL FOR VIRAL DIARRHEA ETIOLOGY PREDICTION IN YOUNG CHILDREN: A PROSPECTIVE MULTICENTER STUDY IN BANGLADESH AND MALI

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Diarrheal diseases are a leading cause of morbidity and mortality in children under five years in low- and middle-income countries. Determination of diarrhea etiology at the point-of-care is crucial for patient management especially appropriate antibiotic use. In this prospective observational study, our objective was to externally validate the accuracy of a new mobile health (mHealth) tool ("App") for predicting viral etiology of acute diarrhea in children 0-59 months. Patients were enrolled at sites in Dhaka, Bangladesh and Bamako, Mali. The App used previously-derived and internally validated models for the prediction of viral diarrhea using patient data entered by a nurse and external data including climate and seasonal viral data. Models included 1) current patient data only (age, bloody stool, vomiting, breastfeeding status, and mid-upper arm circumference) 2) current patient data + seasonality 3) current patient + local weather data 4) current patient + historical pre-test odds (historical rates of viral diarrhea by site and date) 5) current patient + recent patient pre-test odds. A stool sample was collected from patients for Taqman Array Card multiplex molecular diagnostic testing for 32 enteric pathogens for assignment of diarrhea etiology using an episode-specific attributable fraction (AFe) threshold of >0.5. Model performance was assessed with area under the receiver operating characteristic curve (AUC), calibration-in-the-large, and calibration slope. The current patient + seasonal model had an AUC of 0.754 (0.665 - 0.754) for both study sites combined, calibration-in-the-large of α=-0.393 (-0.455- -0.331) and calibration slope of β=1.287 (1.207 - 1.367). Stratified by site, the current patient + pre-test odds model performed best with an AUC of 0.783 (0.705 - 0.86) in Mali, while the current patient + seasonal model performed best with an AUC of 0.71 (0.595 - 0.825) in Bangladesh. MHealth tools may adequately identify patients with high likelihood of viral-only etiology of diarrhea who do not warrant antibiotic use. Further studies are underway to evaluate the impact of this tool on antibiotic prescribing behaviors.

0036

INVESTIGATING MASS DRUG ADMINISTRATION COVERAGE AND CONTRIBUTORS TO PERSISTENT TRACHOMA TRANSMISSION IN MOROTO DISTRICT, UGANDA

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In Moroto District, Uganda baseline trachomatous inflammation-follicular (TF) prevalence was 57.1%. After nine mass drug administrations (MDAs), surveys show reduced TF prevalence, but it was still above the stop-MDA threshold (<5%). In December 2020, the Ministry of Health (MOH) conducted a coverage evaluation survey (CES) to validate reported coverage for a September 2020 MDA and to investigate potential contributors to persistent transmission in Moroto. A multi-stage, cluster-sample, household survey was conducted using a World Health Organization methodology. "Coverage" was defined as swallowing the drug during the last MDA. Standardized questionnaires were administered to collect demographic, programmatic, and behavioral data from consenting participants. In data analysis, logistic regression modelling was used to identify correlates of MDA coverage. The survey had 1,688 respondents and a response rate of 98.1%. In Moroto, surveyed MDA coverage was 81.2% (CI: 79.3-83.0) versus a reported coverage of 92.8%. Beyond validating MDA coverage, specific findings are actionable. Sub-county and survey cluster-level MDA coverage ranged from 40.6%-96.5% and 0-100%, respectively. Knowledge of trachoma was positively associated with coverage (OR=2.0, CI:1.2-3.5). Farmers had lower coverage (OR=0.6, CI: 0.4-0.8); students had higher coverage (OR=1.8, CI: 1.2-2.8). Minority ethnic group membership, dominated by the Tepeth, was negatively associated with MDA coverage (OR=0.11, CI: 0.08-0.14) in a logistic regression model adjusting for age, sex, and education. Among non-covered respondents, 119 (38.1%, n=312) were unaware of the MDA; 37 (11.9%) reported a drug stock out. About 80% of respondents live in households practicing open defecation. MOHs can use a CES to investigate areas with persistent transmission. In Moroto, the MOH achieved MDA coverage only slightly below target. And specific geographic areas and populations require increased resources. Pre-MDA social mobilization and advocacy for water, sanitation, and hygiene interventions are examples of district-wide priorities.

0037

MALI ACHIEVES A CRITICAL MILESTONE TOWARD ELIMINATION OF LYMPHATIC FILARIASIS: ALL PREVIOUSLY ENDEMIC DISTRICTS HAVE MET CRITERIA TO STOP MASS DRUG ADMINISTRATION (MDA)

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In Mali, all 75 health districts (HD) were endemic for lymphatic filariasis (LF). Initial mapping with ICT cards in 2004 revealed an average national prevalence of 7.1% (ranging from 1% in the north to 18.6% in the south). The Government of Mali has committed to eliminating LF as a

public health problem by 2024. In 2005, the implementation of mass drug administration (MDA) for LF with albendazole and ivermectin began in areas co-endemic with onchocerciasis in southern Mali and scaled up to reach 100% geographical coverage in 2009. Due to political unrest and insecurity, 50 out of the 75 endemic HDs missed 1 to 3 annual rounds of MDA between 2011 and 2014. However, all HDs implemented at least 5 effective rounds of MDA before undergoing pre-transmission assessment surveys (pre-TAS), following the World Health Organization (WHO) guidelines. TAS1 in insecure HDs was also delayed by two to three years after successful pre-TAS. The Survey Sample Builder (SSB) was used to determine sample sizes and number of clusters for TAS1. By 2020, TAS1 were conducted in all 75 HDs (48 Evaluation Units - EUs) across Mali. Of 75 HDs, two conducted TAS 1 in 2012, 15 HDs in 2015, 33 HDs in 2016, 11 HDs in 2019 and 15 HDs in 2020. A community-based cluster sampling (in 40 EUs) and school-based cluster sampling (in 8 EUs) were used depending on school enrollment rates. All 48 EUs surveyed passed TAS1 with the number of positive cases being below the critical cut-off values determined by the SSB (18 or 20 positive cases) with a range of positive between 0 and 11 per EU. Currently, 100% (75/75) of the originally LF endemic districts have reached the criteria to stop MDA despite the delay caused by political unrest and insecurity, of which 62 are in post-MDA surveillance and 13 are in post elimination surveillance (passed TAS3). Current and future challenges for the LF program include establishing post elimination surveillance strategy for HDs passing TAS3 and those joining them in future years. Despite insecurity in the north causing delays in reaching LF elimination by 2020, Mali is on track to validate LF elimination as a public health problem by 2024.

0038

IVERMECTIN, DIETHYLCARBAMAZINE AND ALBENDAZOLE MASS DRUG ADMINISTRATION: IMPACT ON PREVALENCE OF SCABIES, IMPETIGO AND SOIL-TRANSMITTED HELMINTH (STH) INFECTIONS IN THREE MUNICIPALITIES OF TIMOR-LESTE

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Scabies and soil-transmitted helminth (STH) infections are neglected tropical diseases (NTDs) endemic in many developing countries, including Timor-Leste. In 2019, the Ministry of Health implemented an ivermectin, diethylcarbamazine, and albendazole (IDA) mass drug administration (MDA) programme for lymphatic filariasis control, which was also likely to have benefits for scabies and STH control. We aimed to evaluate the impact of IDA MDA on the prevalence of scabies, impetigo, and STH in Timor-Leste. Cross-sectional surveys were conducted in 6 schools across 3 municipalities prior to MDA and 18-months following MDA. At baseline, 1043 children underwent a clinical skin exam to assess scabies and stool samples were collected from 541 children for STH diagnosis. At follow-up (FU), 1196 children completed a skin exam and 621 provided a stool sample. Quantitative PCR was used to detect and quantify infection intensity of 6 STH species. Generalised linear models were built to estimate the relative and absolute differences in scabies, impetigo, and STH prevalence between timepoints while accounting for school-level clustering. The baseline prevalence of scabies was 37.7% (95% CI 29.2 - 46.1), which decreased to 13.3% (95% CI 7.5 - 19.1) at FU, corresponding to a relative reduction of 64.7% (95% CI 52.4 - 76.9, p<0.000) and absolute reduction of 24.4% (95% CI 17.6 - 31.1, p<0.000). Impetigo prevalence decreased from 12.3% (95% CI 8.2 - 16.5) to 2.2% (95% CI 1.0 - 3.4) at FU, corresponding to a relative reduction of 82.2% (95% CI 74.5 - 90.0, p<0.000) and absolute reduction of 10.1% (95% CI 6.6 - 13.7, p<0.000). The overall baseline STH prevalence was 27.5% (95% CI 23.7 - 31.3) with *A. lumbricoides* being the most prevalent species (17.5% [95% CI 14.3 - 20.8]), followed by *N. americanus* (7.5% [95% CI 5.3 - 9.8]). STH diagnosis of follow-up samples is underway. This study demonstrates the impact of 1 dose of ivermectin delivered in the context of IDA MDA on scabies, impetigo, and STH, and can contribute to informing the integrated control of NTDs both locally and globally.

0039

MEASURING THE IMPACT OF INTEGRATED MASS TREATMENT WITH IVERMECTIN AND ALBENDAZOLE FOR ONCHOCERCIASIS IN FOUR COUNTIES IN LIBERIA &ASSESSING THE EFFECTIVENESS OF THE PREVENTIVE CHEMOTHERAPY IN REDUCING TRANSMISSION OF INFECTION 2018 AND 2019

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Onchocerciasis is among the neglected tropical diseases (NTDs) which remains a serious public health problem. Rapid Epidemiological Mapping of Onchocerciasis (REMO) was conducted in 1999 by the African Program for Onchocerciasis Control which estimated that the disease affected all the 15 counties in Liberia with 1,113,213 populations at risk. Unlike the other diseases (Lymphatic Filariasis, Schistosomiasis and STH) where the target is elimination as a public health problem, onchocerciasis has it's a goal of elimination of transmission by 2025. In accordance with the WHO guidelines, during the National Onchocerciasis Elimination Expert Committee (NOEEC) meeting in May 2017 it was decided that Liberia conduct an impact assessment using OV 16 to enable the committee to assess the impact of treatment and review progress towards elimination of onchocerciasis. By May 2018, the Ministry of Health conducted an epidemiological impact assessment that provided evidence on the status of transmission of Onchocerciasis in the Southwest Region and to determine the village level sero-prevalence of onchocerciasis in children using OV16 rapid diagnostic test (RDT), allowing assessment of recent or ongoing transmission of O*nchocerca volvulus* parasite in Liberia. The target was to test three thousand three hundred (3,300) children ages 5-9 years using OV16 rapid diagnostic test and DBS (Dried Blood Spot) in Bomi, Grand Cape Mount, Margibi and Grand Bassa counties. However, during the research, 2,432 children were successfully tested with 104 testing positives giving the prevalence rates of 3.7%, 3.2%,1% and 0.7% across the four counties. Out of the negative samples collected, 10% were randomly selected for confirmatory testing using OV16 ELIZA at the Filariasis Laboratory in Yaounde, Cameroon. Out of the 342 negative samples analysed, only 3.51% were tested positive. The results analysis indicates that there is slightly more Onchocerciasis detectable by ELISA than by RDT. This indicates that MDA has been successful in controlling the transmission of Onchocerciasis in Liberia and limiting exposure of children to the infection.

0040

EFFECT OF COVID-19 RISK MITIGATION MEASURES ON THE COVERAGE OF MASS TREATMENT CAMPAIGNS AGAINST SCHISTOSOMIASIS AND VITAMIN A SUPPLEMENTATION IN THE REGIONS OF KAYES AND SÉGOU IN MALI IN 2020

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The Government of Mali organizes vitamin A supplementation (VAS) bi-annually for children under 5 years and mass drug administration (MDA) against neglected tropical diseases (NTDs), such as schistosomiasis (SCH) for school age children annually. The first COVID-19 case was confirmed in Mali on March 25, 2020. The first round of VAS (VAS1) was held between March 25-28, 2020. The second round of VAS (VAS2) and the SCH MDA began months later after the first COVID-19 case (October - December 2020). The VAS1 campaign was implemented at the start of the COVID-19 pandemic before any large-scale sensitization or national guidelines for the distribution of Vitamin A were developed. The VAS2 and SCH MDA campaigns took place after national programs had developed tools, such as guidelines, contingency plans, and standard operating procedures in the context of COVID-19. These tools were aligned with the WHO recommendations for NTD interventions in the context of COVID-19 and the Global Alliance for Vitamin A. The program coverage of VAS1 in the Kayes region, where one of the first cases of COVID-19 was confirmed, was 77%, while VAS2 achieved 88% coverage. In Ségou, the program coverage was 80% for the VAS1, while that of VAS2 was 86%. The VAS2 coverage was higher than VAS1 in both regions. Case refusals were reported in eight villages during VAS1 in Kayes region. The program coverage for the SCH MDA were 91% and 99% in Kayes and Ségou, respectively, comparable to program coverage in 2019 (94% in the region) before COVID-19. This analysis suggests that the availability and use of appropriate contingency tools and mitigation measures of COVID-19 may be one of many factors that contribute to adequate campaign coverage during the COVID-19 pandemic. The results indicate that preparation and availability of risk mitigation plans and tools should be prioritized during times of threat to a program (e.g. at the start of an epidemic) prior to the resumption of field-based activities.

0041

EVALUATING THE ADOPTION OF COVID-19 PREVENTION MEASURES DURING MASS DRUG ADMINISTRATION IN ANAMBRA STATE, NIGERIA

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The COVID-19 pandemic has had profound impacts on health programs around the world. Anambra State in southern Nigeria instituted measures designed to prevent the spread of the virus during mass drug administration (MDA) for onchocerciasis, lymphatic filariasis, schistosomiasis, and soil-transmitted helminthiases. We explored the adherence to and experience of these activities using a mixed methods approach beginning in December 2020. Training sessions and community-based MDA were observed using structured checklists. Semi-structured interviews and focus groups were conducted with health officials and community members. In 46 trainings of community drug distributors (CDDs), we observed 100% adherence to screening for symptoms and exposure to SARS-CoV-2. Ninety-eight percent of trainings were done outdoors with social distancing, while all required hand washing and small group sizes. Only 11% of observations saw CDDs being tested before participation. All CDDs in 108 MDA observations had access to masks (100%), but only a few wore gloves (10%). All CDDs wore their masks during distribution (100%), while 93% always wore them while moving between households. All washed their hands frequently. Dose poles, used to determine the number of pills to be given, were disinfected in 98% of observations. All CDDs encouraged physical distancing and gave COVID-19 prevention messages. Mitigation measures were less common in the general community, with only 43% wearing masks and 53% maintaining distance from other households in 108 observations. However, 97% of observations saw people washing their hands. The qualitative data spoke to the upheaval caused by the pandemic, but that the program had adapted well to meet the challenge. Financial stressors, additional logistics, and intense health education messaging made this round of MDA more exhausting than in previous years, however the treatment coverage was high. The perception among health staff was that the public responded well to the messaging and respected the mitigation measures. Health staff were impressed with the response but noted that the extra measures were not likely to be sustainable.

0042

OPPORTUNITIES AND BARRIERS TO HEALTH CAMPAIGN INTEGRATION DURING THE COVID-19 ERA ACROSS VITAMIN A, IMMUNISATION, NEGLECTED TROPICAL DISEASES, POLIO AND INSECTICIDE TREATED BEDNETS: A KEY INFORMANT INTERVIEW STUDY OF CAMPAIGN STAKEHOLDERS

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In low and middle-income countries, health campaigns are used to rapidly deploy high-priority health interventions. Many distinct campaigns occur in communities every year. Campaign integration offers opportunities to increase efficiencies across programs and limit the strain on health systems. We aimed to understand the impact of COVID-19 on campaign delivery and the opportunities it brought for integration across five health domains (Vitamin A, Vaccine-preventable diseases, Polio, Malaria, and Neglected Tropical Diseases). We conducted 26 virtual in-depth interviews with purposively sampled informants among donors, implementing partners and Ministry of Health officials in Côte d'Ivoire, Ethiopia, Guyana, Indonesia, and Nigeria. All verbatim transcripts were analyzed using NVivo12. Due to the pandemic, delays to campaigns disrupted routine vaccination, bed net delivery schedules, and mass drug administrations. As campaigns restarted, informants faced challenges of restriction of movement, redirection of resources to COVID-19 activities, school closures affecting school-based campaigns, and increased expenditures on COVID-19 mitigation measures. Informants reported that communities had concerns about visiting health facilities and receiving people from outside their communities. Opportunities that COVID-19 brought for campaign integration included the possibility of aligning activities, funding, and commodities while reducing health worker workload. Combining delivery of some commodities resulted in higher coverage. Other areas of integration were public health surveillance, catch-up immunization, and integration of behavior change messages. Barriers to integration included complex data collection tools, number of indicators, and efficiency of supply chain management. Personal and professional relationships, leadership qualities, and political will facilitated campaign integration. The COVID-19 pandemic was described as an opportunity to integrate or consider integration of campaigns. The gains made towards effective integration and increased efficiencies should be maintained.

0043

SINGLE-CELL RNA-SEQUENCING REVEALS DISTINCT IMMUNE POPULATIONS IN CEREBROSPINAL FLUID OF NEUROCYSTICERCOSIS PATIENTS AT EARLY AND LATE TREATMENT STAGES

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Neurocysticercosis (NCC) is a parasitic infection that causes significant global morbidity. Subarachnoid NCC (SANCC), the most severe form of the disease, is associated with an exuberant inflammatory response that

leads to significant morbidity and mortality. At present, treatment of SANCC consists of anthelmintics and corticosteroids. However, frequent adverse events result from the prolonged use of these corticosteroids. A deeper understanding of the specific cell populations involved in the inflammatory response and how the inflammation evolves over the course of treatment would allow for the identification of more targeted, steroid-sparing anti-inflammatories. We used single-cell RNA-sequencing (scRNA-seq) of cryopreserved cells isolated from the cerebrospinal fluid (CSF) of 3 patients with subarachnoid neurocysticercosis both early and late in treatment to study changes in the immune milieu at these 2 disease stages. Using exome sequencing data from each of the patients and standard transcriptome analyses from the scRNA-seq allowed for the successful deconvolution of patient samples and identification of mononuclear cell populations including B cells, monocytes/macrophages, dendritic cells, T cells and NK cells. In all patients, the later timepoint was associated with an expansion of T cells when compared to early in treatment. Early timepoint samples (when the SANCC was most active) had a larger proportion of naïve CD4+ T cells, cytotoxic CD8+ T cells, and natural regulatory T cells (nTregs [FoxP3+, CD25+]) while later timepoints had a higher frequency of induced regulatory T cells (iTregs [FoxP3- , CTLA4+, LAG3+]), MAF+ lymphocytes, and B cells. From a methodological standpoint, this experiment demonstrated that transcriptomic data at a single-cell level can be extracted from cryopreserved CSF cells. This project has served as an important pilot study, providing us with a deeper understanding of the cellular milieu in the CSF of patients with SANCC, as well as a technological framework to more broadly assess the local inflammatory process that occurs before and after treatment.

0044

LONGITUDINAL METAGENOMIC SEQUENCING ANALYSIS OF THE GUT MICROBIOME OF HELMINTH INFECTED INDIGENOUS MALAYSIANS

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The ecological relationship between intestinal helminths and gut-resident microbes in endemic human populations is complex and poorly understood. Here, we examined 669 metagenomes from a cohort of 426 indigenous Malaysians from 6 villages with different prevalence (14.9%-100.0%) of helminth infections. Samples were collected longitudinally before and after treatment with the anthelminthic albendazole. Standard mapping approaches to RefSeq complete genomes resulted in large numbers of unmapped reads, however mapping onto Human Reference Gut Microbiome (HRGM), which incorporates metagenome-assembled genomes (MAGs) resulted in >90% mapping. Additionally, we also conducted reference independent approaches such as using k-mers to estimate differences in diversity. By using Growth Rate Index (GRiD) analysis, we compared bacterial replication rates between groups and identified specific replicating taxa associated with helminth infection. Overall, we found that microbial community diversity and composition is most strongly associated with individual villages. Effects of albendazole treatment on metagenomes varied considerably depending on the village and can be observed even in helminth negative individuals. These results indicated that effects of helminths on the microbiota is highly dependent on context and direct effects of albendazole on the microbiota can be confounding for the interpretation of deworming studies. In conclusion, our study identifies several key species and pathways associated with helminth infection and provides evidence for some co-abundance relationships, hence yielding a better understanding of helminth-microbe-host interactions.

0045

MODULATION OF SARS-COV-2-IMMUNE REACTIVITY BY HELMINTH ANTIGENS

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We investigated the ability of helminth antigens to modulate T cell-reactivity in 50 COVID-19 patients and 50 healthy unexposed individuals. PBMCs were stimulated with SARS-CoV-2-peptides alone or in combination with Onchocerca volvulus, *Brugia malayi*, or Ascaris lumbricoides antigens. Flow cytometry was used to identify the frequency of SARS-CoV-2-reactive T cells in CD4 and CD8 T cell populations according to the expression of CD154, CD69, and CD137. SARS-CoV-2-reactive CD4+ T cells were identified in 35.71 % of unexposed healthy individuals and 85.71 % of COVID-19 patients. Reactive CD8+ T cells were found in 29.17 % of healthy individuals, while 71.4 % of COVID-19 patients presented reactive CD8 T cells. The frequency of SARS-CoV-2 reactive CD4+ T cells was significantly reduced, both in healthy individuals and in COVID-19 patients, when stimulation with viral peptides was done in the presence of helminth antigens. In contrast, activation and proliferation of SARS-CoV-2 reactive CD8+ T cells were not affected but slightly increased in the presence of the helminth antigens. These findings suggest that helminth antigens shift the immune response to SARS-CoV-2 peptides towards cytotoxic T cell-mediated viral clearance while inhibiting helper T cell responses. To define the mechanisms underlying this differential modulation of CD4+ and CD8+ T cells by helminth antigens, the expression of different cytokines was quantified in the culture supernatants using a Luminex-based multiplex immunoassay. IL-10 concentrations were significantly increased in the presence of the helminth antigens, whereas IFNγ and TNFα concentrations were decreased. Overall, our data support the hypothesis that in the presence of helminth antigens, immune responses to SARS-CoV-2 might be attenuated in regards to helper T cells and pro-inflammatory cytokines, while SARS-CoV-2 reactive cytotoxic T cells are maintained. This may ultimately shift the immune response to SARS-CoV-2 so that overreaction is avoided. These findings may at least partially explain why African countries endemic for helminth parasites are moderately affected by the COVID-19 pandemic.

0046

REPROGRAMING MACROPHAGES TO DECREASE INFLAMMATORY RESPONSE IN CUTANEOUS LEISHMANIASIS

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Cutaneous leishmaniasis (CL) lesions, due to *L. braziliensis* infection, present intense inflammatory infiltrate with high numbers of mononuclear phagocytes. Furthermore, few parasites are observed. High levels of TNF and IL-1β observed at lesion site of these individuals, is associated with tissue damage and lesion development. Eicosapentaenoic (EPA) and docosahexaenoic (DHA) polyunsaturated fatty acids are known to be natural ligands of peroxosomal proliferator-activated receptor-gamma (PPAR-γ). Activation of PPAR-γ can trigger anti-inflammatory actions including inhibition of NF-κβ. Our aim was to evaluate the role of PPAR-γ ligands in regulating the inflammatory response observed in CL patients. Biopsies and serum were obtained from patients with CL and

healthy subjects (HS). The gene expression of PPAR-γ, NF-κβ, IL-1β, IL-6, TNF, ALOX5 were determined by RNAseq. PBMC was obtained from CL patients and cultured for 72 hours in the presence or absence of soluble *Leishmania* antigen (SLA), GW9662 (irreversible PPAR-γ inhibitor), EPA, DHA and Pioglitazone for 72 hours. Macrophages were infected with *L. braziliensis* and treated with EPA, DHA and Pioglitazone for 2, 48 or 72 hours. Levels of TNF, IL-6, IL-1β, LTB4 and omega 3 were determined in culture supernatants or serum by ELISA. We observed that the genes NF-κβ, IL-1β, IL-6, TNF and ALOX5 were increased in patient's lesions, whereas the PPAR-γ gene was suppressed when compared to healthy skin. In addition, we found that the PPAR-γ gene was negatively correlated with the NF-κβ, IL-1β and IL-6. Higher concentrations of IL-6, IL-1β, and LTB4 were observed in patients' serum, but no difference was found on TNF and omega-3 levels between CL and HS. However, EPA and DHA negatively regulated the production of IL-6, TNF and IL-1β, but increased production of LTB4 in PBMC. Also, EPA and DHA increased LTB4 production by macrophages infected with *Leishmania* decreasing the percentage of infected cells and the number of amastigotes within these cells. Our results show that EPA and DHA decrease *in vitro* inflammatory response and enhance parasite killing in CL, and may serve as adjuvant therapy in CL.

0047

CELLULAR DYNAMICS OF IMMUNE EVASION DURING LEISHMANIA MAJOR INFECTION

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Leishmania major parasites elicit a strong T cell response yet evade complete clearance and persistently infect a small pool of cells. This mechanism of immune evasion remains unclear. Understanding of the persistence mechanisms is lacking, but *Leishmania major* driven induction of the immunosuppressive microenvironment through recruitment of regulatory T cells at the site of infection has been proposed to prevent parasite clearance *in vivo*. In the presented study, we used a novel TCR transgenic mouse model, where $CD4^+$ T cells recognize an immunodominant peptide derived from *Leishmania*- glycosomal phosphoenolpyruvate carboxykinase (PEPCK), to visualize the dynamics of anti-*L. major* $CD4^+$ T cell responses and to characterize mechanisms which restrain their effector function. We show that monocyte-derived macrophage:T cell interaction dynamics were transient at steady-state, but prolonged upon antigen recognition. This activation leads to a production of high levels of IFNγ and can be significantly suppressed by PEPCK-specific Tregs *in vitro*, as compared to polyclonal Treg controls. Co-culture of PEPCK-specific $CD4^+$ T cells, *L. major*-infected monocyte-derived macrophages, and Tregs shows that antigen activation leads to a substantial increase in IL-10 levels, while decreasing IL-12, TNF, and IL-2 production in the culture. Intravital microscopy studies characterizing PEPCK-specific $CD4^+$ T cell migration dynamics and tissue localization within skin lesions directly in live mice show a significant recruitment of adoptively transferred effector T cells to the lesion site *in vivo*, displaying cellular behaviours consistent with antigen recognition. We are currently characterizing whether effector T cell responses are altered in healed lesions, where persistently-infected cells are readily observed. Collectively, our findings show for the first time that *Leishmania*-specific Tregs influence effector $CD4^+$ T cell responses and this could be a mechanism that derives antigen persistence in *L. major* infection.

0048

DIMINISHED Vδ2+ γδ T CELL CYTOKINE PRODUCTION AND DEGRANULATION FOLLOWING *IN VITRO* MALARIA EXPOSURE

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Natural immunity to *Plasmodium falciparum (Pf)* malaria provides some protection against symptomatic disease in older children and adults, but is unable to eliminate parasite replication. Though there are many unanswered questions surrounding this incomplete immunity, a major contributing factor is that innate immune cells that kill the malaria parasite also cause inflammation associated with clinical symptoms. In contrast, repeated malaria exposure leads to attenuation of the pro-inflammatory response from immune cells such as Vδ2+ γδ T cells, which associates with a reduced likelihood of symptoms upon subsequent infection. We are utilizing several innovative approaches to identify mechanisms underlying Vδ2+ T cell dysfunction among a longitudinal cohort in Uganda, as well as to replicate this phenotype *in vitro* using purified Vδ2+ T cells *vs.* Vδ2+ T cells in culture with other immune cells. In these *in vitro* assays, malaria-naïve Vδ2+ cells stimulated with *Pf*-infected red blood cells (iRBCs) or the phosphoantigen HMBPP produce less TNFα and IFNγ and degranulate less in response to secondary stimulation compared to unstimulated cells. In contrast, 6-day stimulation with iRBCs or HMBPP does not impact the ability of the cells to respond to control stimuli, indicating that the reduced response is *Pf*-specific. Rest following stimulation could partially rescue the decreased responsivity to iRBCs. The presence of monocytes did not impact purified Vδ2+ T cell response to iRBCs, indicating that Vδ2+ T cells may be able to recognize *Pf* antigens independent of other cell-cell interactions. In parallel, we are performing paired RNA-Seq and ATAC-Seq experiments among Vδ2+ cells from Ugandan children at multiple timepoints in order to define transcriptional and epigenetic changes underlying altered cell function following repeated malaria. Our *in vitro* system replicating this phenotype will enable a deepened understanding of mechanisms driving inefficient acquisition of antimalarial immunity. Ultimately, this work could inspire novel therapeutic approaches that enhance parasite clearance and/or reduce disease severity.

0049

MALARIA-DRIVEN EXPANSION OF MATURE, SHORT-LIVED FUNCTIONAL CD56NEG NK CELLS CORRELATES WITH CLINICAL IMMUNITY TO MALARIA

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Natural Killer (NK) cells may play an important role in immunity to malaria, but whether repeated malaria modifies the NK cell response remains unclear. To profile the NK cell response, we utilized PBMCs collected from 290 Ugandan children followed in the East African International Centers of Excellence in Malaria Research (ICEMR) cohorts. NK cells were phenotypically defined as CD3-, CD14-, CD19-, CD7+ cells and classified by the expression of CD56 and CD16. We unexpectedly found that children living in highly endemic Tororo had significantly higher

percentages of CD56neg NK cells (31.2% vs 10.5%, P<0.001), and lower percentages of CD56dim NK cells (40.5% vs 65.7%, P <0.001) than children living in Jinja, a lower transmission setting. Among Tororo children, percentages of CD56neg NK cells positively correlated with household-level entomologic inoculation rate (R=0.64, P<0.001). CD56neg NK cells were CD57+, CD85j+ NKG2A+ and FcεRIγ-, a phenotype of mature NK cells. Following stimulation with *Plasmodium*-falciparum infected red blood cells (irbc), NK cells did not produce IFNγ, but in response to antibody opsonized-irbc, 27.9% CD56neg NK cells - compared to 10.1% CD56dim NK cells (P=0.0084) - degranulate, suggesting that CD56neg NK cells have heightened capacity to perform antibody-dependent cellular cytotoxicity. Children with higher levels of CD56neg NK cells had lower parasite densities (Rho=-0.36, P<0.0001), and were less likely to show symptoms when infected, in the year following the immunologic assessment, independent of age. We assessed the stability of NK cell phenotypes over time by performing longitudinal assessments pre- and post- indoor residual spraying of insecticides (IRS). Six months following IRS in Tororo, the percentage of CD56neg NK cells significantly decreased (53.07% to 42.47%, P<0.01) with a concomitant increase of CD56dim cells. CD56neg NK cells also exhibited a reduced ability to degranulate in response to opsonized irbc post-IRS. Together, these data suggest that repeated malaria leads to expansion of mature, but short-lived, functional CD56neg NK cells that correlate with clinical immunity to malaria.

0050

LESIONS OF FEMALE GENITAL SCHISTOSOMIASIS ARE ASSOCIATED WITH THE PRESENCE OF LIVE WORMS AS INDICATED BY CIRCULATING ANODIC ANTIGEN TESTING

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Schistosoma haematobium ova in genital tissue may cause four different lesions: grainy sandy patches, homogenous yellow patches, rubbery papules and abnormal blood vessels. In areas where reinfection with schistosomiasis is rampant, it is not known if the lesions of Female Genital Schistosomaisis (FGS) are a consequence of live worms, or caused by dead ova. Live schistosome worms regurgitate Circulating Anodic Antigen (CAA). We sought to explore if the typical FGS lesions are associated with the presence of live worms. The study was conducted in randomly selected rural high schools on the East Coast of South Africa. Female learners aged 16 - 23 years who were willing to undergo gynecological examination were included in the study. We excluded virgins, pregnant women, and those who had undergone anti-schistosomal mass-treatment the last year. CAA was positive in 82/246 (33%) of the participants, while 39/243 (17%) had schistosome eggs in urine as measured by microscopy. Sandy patches were found in 123 (50%) of the study population. Current risk water contact was found in 118 (48%). Grainy sandy patches were found in 63 (26%) participants; 37/63 (59%) were CAA positive (Age-Adjusted Odds Ratio (AOR) 4.2, 95% Confidence Interval (CI) 2.3-7.8, p < 0.001). Abnormal blood vessels were associated with CAA (AOR 3.0, 95%CI 1.5-4.5, p=0.001) whereas homogenous yellow patches were not associated with CAA (p = 0.57). No rubbery papules were found. Thick and lumpy vaginal discharge and red urine were the only subjective symptoms associated with CAA. No association was found between CAA and warts, polyps or ulcers (p > 0.4 for all). The study indicates that homogenous yellow patches may indicate chronic infection due to dead ova whilst grainy sandy patches and abnormal blood vessels may be associated with more recent infection caused by the deposition of eggs from living worms. Further studies are needed to explore the implications for HIV transmission, reproductive health, patient information, prevention and treatment.

0051

VILLAGE, HOUSEHOLD AND INDIVIDUAL LEVEL PREDICTORS OF BOVINE SCHISTOSOMIASIS INFECTION IN RURAL VILLAGES OF SICHUAN, CHINA

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In China, several domestic animal species are known to be capable of carrying and transmitting *Schistosoma japonicum*. Of the domestic animals commonly found in rural farming villages, bovines have been repeatedly highlighted as a major reservoir of human schistosomiasis, with some estimates suggesting that they may be responsible for as much as 75% of human transmission. However, little is known about the individual and environmental conditions that drive bovine schistosomiasis infection. Using household surveys and infection data collected as a part of a longitudinal study conducted in 39 rural villages in Sichuan, China from 2007 to 2016, we aimed to identify the strongest individual, household and village-level predictors of bovine *S. japonicum* infection. Candidate predictors for this assessment included: 1) biological characteristics of bovines such as sex or age, 2) potential human sources of environmental schistosomes, such as human infection prevalence at the village-level, 3) socio-economic indicators such as the prevalence of improved sanitation, 4) presence of other animal reservoirs including cats, dogs and pigs and 5) agricultural factors including the area of rice planted by a given household. A Random Forests machine learning approach was used to determine which of our candidate predictors serve as the best predictors of bovine schistosomiasis infection in each survey year. Of the five categories of candidate predictors, human sources of schistosomes and agricultural factors were repeatedly found to be among the top predictors of bovine *S. japonicum* infection, highlighting the potential utility of presumptively treating bovines in households where one or more people have tested positive, and those who engage in high-risk agricultural practices such as planting rice. Additionally, household-level predictors tended to be better predictors of bovine infection than village-level predictors, suggesting that targeted, multipronged approaches can be used to address intra-village sources of transmission heterogeneities.

0052

THE SENSITIVITY OF SCHISTOSOMA MANSONI CERCARIAE TO CHLORINE

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Schistosomiasis is a water-based neglected tropical disease acquired through contact with cercaria-infested water. Communities living in endemic parts of the world often rely on unsafe cercaria-contaminated freshwater bodies for their daily water contact activities (e.g. laundry, bathing), resulting in recurring schistosomiasis infection. Water treatment can provide safe water on a household or community scale. However, there are no water treatment guidelines that provide information on how to treat water containing cercariae. We tested the effectiveness of chlorine against *Schistosoma mansoni* cercariae under controlled conditions (pH 6.5, 7.0 or 7.5, and temperature of 20°C or 27°C). Chlorine was dosed at 1, 2 or 3 mg/l and experiments were run up to contact times of 45 minutes. 100 cercariae were used per experiment, thereby achieving up to 2-log inactivations of cercariae. Experiments were conducted with both fresh (<1 hour old) and aged (6-8h old) cercariae, and replicated under lab conditions and field settings at Lake Victoria, Tanzania. A CT value (product of the residual chlorine concentration and chlorine contact time) of 26±4 mg·min/l is required to achieve a 2-log (or 99%) inactivation

of *S. mansoni* cercariae under the most conservative condition tested (pH 7.5, 20°C). Field and lab-cultivated cercariae show similar chlorine sensitivities, and aged cercariae are significantly more sensitive than fresh cercariae. Overall, a CT value of 30 mg·min/l is recommended to disinfect cercaria-infested water, though additional safety factors may be required, depending on water quality and operating conditions. This CT value can be achieved with a chlorine residual of 1 mg/l after a contact time of 30 minutes, for example. The resulting chlorination recommendation can be used to treat water and provide schistosomiasis-endemic communities with safe water facilities (e.g. laundry or bathing stations).

0053

DEVELOPMENT OF A MULTIPLEX BEAD ASSAY FOR DETECTION OF ANTIBODIES AGAINST SCHISTOSOMA MANSONI INFECTION

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Schistosoma mansoni is endemic across sub-Saharan Africa and in several South American and Caribbean countries. The CDC uses purified native *S. mansoni* antigens for serologic diagnoses of suspected cases among returning travelers/immigrants. The native antigens-based assay is challenging to produce for scale-up to evaluate larger populations. We, therefore, attempted to identify recombinant antigens appropriate for use in a multiplex-based assay (MBA) to detect antibodies indicative of *S. mansoni* infection. We collected the soluble fraction of *S. mansoni* adult worms, separated the proteins using size exclusion fast-performing liquid chromatography followed by 1-D gel electrophoresis, and screened immunoblots with sera from *S. mansoni* infected individuals. Fractions still reactive after carbohydrate disruption were subjected to tandem mass spectrometry (MS). From 3731 MS protein sequences, 99 proteins were selected based on size, presence of transmembrane domain, and signal peptide sequences. Selected proteins were expressed, purified, and tested for reactivity against positive sera on immunoblot. Thirty-eight of the expressed proteins were selectively recognized by IgG1 or IgG4 antibodies in sera from infected persons. We selected the top 22 proteins based on the strength of the signal-to-noise (S/N) ratio on immunoblot for the MBA evaluation. Twelve proteins showed good signal-to-noise (S/N) ratio: Sm-25, Sm-13, Sh-13, Sm-tubulin I, Sm-Krueppel-associated box (KRAB) domain-containing protein, Sm-peroxiredoxin (Prx 2), Sm calumenin B, Sm Cathepsin B, Sm-CD63-like, and Sm-adaptin ear-binding coat-associated protein (NECAP)-like, Sm-translational controlled tumor protein (TCTP). Although the sequences are all from *S. mansoni,* seven of the proteins had higher S/N ratios with sera from persons with *S. japonicum* infections. None of the antigens were recognized by sera of *S. haematobium*-positive individuals. In conclusion, we have identified antigens for *S. mansoni* serological diagnosis, and these antigens will be tested on an MBA to determine the best combination for diagnostic testing and serosurveillance.

0054

RAPID PARASITE DNA AMPLIFICATION USING A DIPSTICK WITH LAMP ASSAY

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Schistosomiasis is a neglected tropical disease in many developing countries in tropical Africa, the Middle East, Asia, and Latin America. It affects the poorest communities and is a significant cause of morbidity and can be fatal. School-aged children are mostly at risk of infection. There have been increased efforts aimed at controlling and eliminating schistosomiasis in recent decades; however, the global disease burden remains high, in part due to inadequate diagnostics. Molecular diagnostics are powerful tools for disease detection that are usually confined to the laboratory setting due to burdensome and expensive methods required to extract nucleic acids from biological samples. The DNA dipstick is a simple and rapid method for DNA extraction requiring minimal equipment and when combined with LAMP, may be an ideal tool for point-of-care diagnostics. This study aimed to determine the effectiveness of the dipstick in detecting *Schistosoma japonicum* in parasite and clinical samples. *S. japonicum* from different life cycles including cercariae, eggs and adult worms were isolated and purified from infected snails and livers of experimentally infected mice and homogenized in lysis buffer. The DNA was extracted by inserting dipsticks into the lysed samples, washed to eliminate contaminants, and dipped into the amplification mix, all in less than 30 secs. The mix was incubated at 65°C for 60 mins. Similarly, cell-free DNA was also extracted from mice and naturally infected human urine samples using dipsticks. The DNA dipstick successfully identified *S. japonicum* from infected snails, cracked eggs, worms and human urine spiked with cracked eggs or genomic DNA from *S. japonicum* as proof of concept. This method was able to detect as little as 0.01 fg/µl of target DNA in urine. Positive results were also obtained from experimentally infected mice and naturally infected human urine samples. The DNA dipstick combined with LAMP is a promising cost effective and simple method for detecting schistosomiasis infection in endemic regions.

0055

VALIDATION OF THE ISOTHERMAL RECOMBINASE POLYMERASE AMPLIFICATION (RPA) ASSAY FOR THE POINT-OF-CARE MOLECULAR DIAGNOSIS OF FEMALE GENITAL SCHISTOSOMIASIS USING CERVICOVAGINAL LAVAGE AND VAGINAL SELF-SWAB SAMPLES

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Female Genital Schistosomiasis (FGS) is a gynaecological disabling condition caused by the parasite *Schistosoma haematobium* affecting over 56 million women across Sub-Saharan that gets confounded by sexually transmitted infections, as they share symptomatology. Africa. reproductive health and increases the risk of HIV-1 infection. Improved diagnostics, close to the point-of-care (POC) are urgently needed to increase surveillance. Molecular diagnostics offer the best way to diagnose active FGS by detection of *Schistosoma* DNA within cervical and vaginal samples. Our study validated and assessed the performance of a field-applicable isothermal Recombinase Polymerase Amplification (RPA) assay for *S. haematobium* on standard cervicovaginal lavage (CVL) and home-based self-collected vaginal swabs (VS) samples compared to the laboratory-based gold standard real-time polymerase chain reaction (qPCR). Samples were obtained from the Bilharzia and HIV (BILHIV) project in Zambia. DNA was extracted from the CVL samples using both a crude (CCVL) and traditional pure (PCVL) extraction method. VS samples were extracted using the pure method (PVS). Out of a total of 527 samples, 223 CCVL, 133 PCVL and 190 PVS samples were were tested using the RPA assay that has been developed for urogenital schistosomiasis diagnosis. All data were evaluated using refence qPCR data available for each sample. For CVL samples, The RPA sensitivity for PCVL were 76.9% and 85.7%, for CCVL and 95% and 98.1% specificity respectively. For the self-collected PVS samples sensitivity and specificity was 94.1% and 97.7% respectively. PCR is currently unsuited for use at the POC. In contrast, the RPA assay is portable, rapid (~20 minutes) and can be performed on crude sample preparations relieving laboratory resource needs. In our study the RPA assay had optimal performance, particularly associated with self-collected

swab samples. Self-collected samples paired with the POC use of the RPA assay has great potential to support FGS diagnosis and surveillance in endemic settings.

0056

IDENTIFICATION OF NOVEL THERAPEUTICS AGAINST HUMAN SCHISTOSOMIASIS

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Human schistosomiasis is a neglected tropical disease caused by parasitic worms. It affects over 250 million people globally. Most human infections are caused by *Schistosoma mansoni, S. haematobium,* and *S. japonicum.* Currently there is only one method of treatment for human schistosomiasis, the drug praziquantel. Constant selection pressure has caused a serious concern because of rise in resistance to praziquantel leading to the urgent need for additional pharmaceuticals, with a distinctly different mechanism of action, to be used in combination therapy with praziquantel. Previous treatment of *Schistosoma mansoni* included the use of oxamniquine (OXA), a prodrug that is enzymatically activated by a sulfotransferase, an enzyme produced by *S. mansoni*. Although sulfotransferases are produced by *S. haematobium* and *S. japonicum,* OXA is not effective against these two species. OXA kills about 90% of *S. mansoni* in 14 days *in vitro.* Structural data have allowed for directed drug development in reengineering oxamniquine to be effective against *S. haematobium* and *S. japonicum*. Guided by data from Xray crystallographic studies and *Schistosoma* worm killing assays more than 300 OXA derivatives were designed synthesized and tested *in vitro* against the adult parasites. All of the derivatives synthesized are racemic mixtures. Currently, we were able to identify four of them as powerful derivatives that kill 100 % of all three *Schistosoma* species *in vitro*. Two of these derivatives were able to kill 100% of the three species in less than 7 days. Both derivatives were able to kill 100% of the three species with half the dose required for OXA. Although adult male worms produced higher amount of SULT than female worms, paired female and single-sex female worms were highly susceptible to CIDD-0150610 and CIDD-0150303 like paired males and single-sex males. In addition, our *in vivo* results show a significant reduction in worm burden when infected animals were treated with CIDD-0150303. Therefore, we conclude that CIDD-0150303 is a potential novel drug that can be used in combination with praziquantel to treat schistosomiasis.

0057

FEATURE IMPORTANCE: OPENING A SOIL-TRANSMITTED HELMINTH MACHINE LEARNING MODEL WITH SHAP

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In the field of landscape epidemiology, the contribution of machine learning (ML) to modeling of epidemiological risk scenarios presents itself as a good alternative. This study aims to break with the black box paradigm that underlies the application of automatic learning techniques by using SHAP to determine the contribution of each variable in ML models applied to geospatial health. For this purpose, the prevalence of hookworm from an area of Ethiopia was used since the country bears the third-highest burden of hookworm in sub-Saharan Africa. The machine learning model XGBoost software was used to fit and analyze the data while the Python SHAP library was used to understand the importance of the variables as predictors in the trained model. The description of the contribution of these variables on a particular prediction was obtained, using different types of plot methods. The results show that the ML models are superior to the classical statistical models; not only demonstrating similar results but also explaining, by using the SHAP package, the influence and interactions between the variables in the generated models. This analysis provides information to help understand the epidemiological problem presented and provides a tool for similar studies.

0058

SAMPLE COMPUTATIONAL STRUCTURES TOWARDS AN EPIDEMIC SIMULATION TOOL COMPRISING COMMUNICATING PROGRAMS ON MOBILE PHONES AND SERVERS

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Here we explore possible computational structures for an epidemic simulation tool comprising communicating programs on smart phones and a server or servers. Through calculations distributed among devices, such a tool or computational structure could in ~real-time access and directly incorporate the location or distance information of individual smart devices into an individual-level epidemiological model in order to assemble and collect simulated epidemic data; multiple epidemic simulations could be run simultaneously with various simulated control measures. We build programs towards the tool in phases. As a first phase, we built a rough sample individual-level model similar in structure but not rigor to what is found in the research literature. As a second phase, we simulated an epidemic simulation tool being used in a simulated world, with humans carrying smart devices which along with a server contain a distributed program for an epidemic simulation tool that uses either location-based or signal/distance-based interaction inference or simulation. As a third phase, we built a sample demo (demonstration) of the computational structure for the epidemic simulation tool. The demo comprises communicating processes running ~simultaneously on one computer for demonstrating program structures with multiple processes, one process for each smart device and one for a server, smart device detection of synthetic location information in time (fast-forwarded for the demo) for place-based interaction inference, and for each smart device a set of sim-humans (one for each epidemic simulation). Sample epidemic simulation outputs from these phases show what information each smart device can opt to see about its own sim-humans and what simulation data is assembled and gathered by the server. Through the descriptions and initial phases here, we contribute discussion towards some of the structural possibilities for such an epidemic simulation tool.

0059

LANGUAGE, POPULAR CULTURE AND MYTHOLOGY UPHELD FEMALE GENITAL CUTTING AND CHILD MARRIAGE IN SUDAN

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Harmful practices such as Female Genital Cutting (FGC) and Child Marriage (CM) are often upheld by core social norms such as differential treatment of girls and boys, of men and women and of children of different socio-economic groups. According to anthropological and historical research, FGC pre-dates Islam and Christianity tracing the practice to Egypt in the fifth-century BC and arguing that the practice is spread across wide cultures and in different parts of the world, suggesting that the practice may have independently existed among different groups and for various reasons. In Sudan FGC is known historically to have existed more than 30 centuries relating to cross border interaction in various directions. The persistent high prevalence rate has led the author to closely observe the practice. Through qualitative research and the use of focused group discussion among various sectors of the Sudanese society, the author attempts to unveil some of the unspoken of reasons

of the practice. The behavioral rules applying to practices such as FGC and CM identify the social constructs of the beliefs of members of a community embedded into scripts and are part of a thick network of beliefs and practices. It is assumed here that the empirical expectations for practicing stem from the connectivity of language and its connotations in the local value system coupled by myths that leads a sufficiently large group to uphold the practices. The normative expectations are beliefs that a sufficiently large subset of people is expected to conform such that the norm is almost universally endorsed. This generates a widespread conformity and the result is following the norm by the majority by preference. The FGC terminologies and the proverbs and stories of mythology are analyzed in this study and presented within the frame of normative expectations that have held FGC and CM in the Sudan for such a long time. It was found that the language by which FGC is labelled and the popular culture of proverbs, stories of mythology and statements transmitted through generations define the scripts that are adhered to and strongly uphold the practices.

0060

CLINEPIDB.ORG: LOWERING THE BARRIER FOR EXPLORATORY DATA ANALYSIS OF GLOBAL HEALTH STUDIES

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Open access to data from epidemiological studies has tremendous potential to preserve data over time, increase secondary data use, and accelerate discovery and translational impact, but there are significant technical, practical, and confidentiality barriers. The clinical epidemiological database, ClinEpiDB.org, was developed three years ago to facilitate access to de-identified data from large, high-quality global health studies. As of April 2021, ClinEpiDB hosts data from over 1.1 million participants representing 29 global epidemiological studies in three major domains - maternal, newborn & child health; malaria; and neglected tropical diseases. These include case-control studies, longitudinal cohort studies, cross-sectional surveys, randomized controlled trials and surveillance datasets. ClinEpiDB enables investigators to not only meet, but surpass, the requirements of journals and funders to make data publicly available by integrating study data with standardized ontologies to make data more easily reusable. Linked study pages provide context and study-related documentation such as consent forms, case report forms and codebooks. The ClinEpiDB platform lowers barriers to data use with an intuitive point-and-click interface that allows users to understand complex epidemiological studies, visualize aggregate data, and explore associations between variables. Entire datasets or customized subsets may be downloaded for further analysis. We have implemented a tiered data access system with a simple interface for users to submit an access request and for data providers to manage requests. ClinEpiDB will expand in 2021 to encompass a new exploratory data analysis workspace, enhanced visualization tools, significant international user outreach and education, and integration of new datasets.

0061

OPERATIONALIZING ROUTINE HMIS DATA FOR A DISEASE BULLETIN IN GUINEA

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The National Malaria Control Program (NMCP) in Guinea produces a monthly report, the Bulletin Mensuel de Paludisme, on the status of Malaria in the country. This report includes indicators describing the current status of malaria at a national and district level such as the number of confirmed cases, the number of tests conducted, and the number of treated cases. The NMCP distributes the bulletin as a Microsoft Word document by e-mail to a list of more than 400 people including MOH officials, implementation partners, and other stakeholders. However, compiling the report is time consuming and involves manual data cleaning. Thus, the NMCP wanted a new system to reduce the time to produce monthly reports.The PMI funded StopPalu+ project implemented by RTI International that delivers ongoing technical assistance to the NMCP provided a solution to this problem. By leveraging the data within Système National d'Information Sanitaire, and its DHIS2 software platform, the project team developed an open source DHIS2 module1 to fully automate the creation of the report. The module is called the Bulletin Mensuel de Paludisme Exportateur and runs in the national HMIS software in DHIS2 and leverages its data security framework. The process is more sustainable than what was in place before as it allows all users of the national HMIS, not just those with programming skills, to generate the monthly malaria report. If maintenance is required, the source code is publicly available and can be modified by a DHIS2 application developer. The structure of this app was designed to be a flexible framework that can be adapted to generate any type of disease bulletin in other health areas. Its small innovations like this one that can help the NCMP in Guinea eliminate data silos and provide timely and accurate data more efficiently.

0062

SPATIOTEMPORAL VARIATION IN CHILDHOOD RISK OF ENTERIC SHIGELLA INFECTION: A GLOBAL PREDICTIVE MODEL AND RISK MAPPING TOOL

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Diarrheal disease is a major cause of childhood illness caused by numerous, diverse species of infectious microorganisms, which are sensitive to environmental conditions. The bacterium *Shigella* is responsible for 64,000 annual deaths in young children and thrives in warm, moist conditions. This study aimed to model the spatiotemporal variation in pediatric *Shigella* infection risk using covariates with quasi-global coverage and use these to map the predicted prevalence of shigellosis across Low- and Middle-Income Countries (LMICs). Data were combined from numerous studies that used PCR to diagnose *Shigella* in stool samples collected from children aged ≤59 months in LMICs and a standard list of covariates was generated that included: household- and subject-level covariates ascertained at enrollment; environmental and demographic spatial covariates extracted from global rasters; time-varying hydrometeorological variables extracted from historical daily Earth Observation- and model-based re-analysis-derived estimates. Variable selection was carried out by forward stepwise addition of covariates and a predictive projection method was implemented. Spatiotemporal predictions were made separately for 0-11-, 12-23-, and 24-59-month age groups. The final database included results from over 64,000 stool samples collected from 21,000 subjects in 19 LMICs. Daily temperature averaged over a 7-day lagged time

window was the most important variable, followed by the interaction of precipitation with soil moisture. Accessibility to the nearest city was the most important non-hydrometeorological covariate. The model predicted wide belts of elevated *Shigella* risk in tropical Sub-Saharan Africa, India, and Brazil, as well as smaller pockets of high prevalence in New Guinea, Ethiopia, the Sahel, coastal Central America, and Colombia, among others. Once finalized, these predictions will be made available to the public via an online portal for use in decision-making, such as identifying populations living in hotspots of *Shigella* transmission risk that can be prioritized as vaccines become available.

0063

EVALUATION OF A POINT-OF-CARE SCREENING DEVICE "GAZELLE" FOR CHILDREN WITH SICKLE CELL DISEASE IN GHANA

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Sickle cell disease (SCD) is the commonest genetic disorder in Ghana, affecting up to 16,000 newborns (approximately 2%) per year, which can lead to severe complications causing significant morbidity and mortality. Timely diagnosis is key to prevent or manage these complications. Current standard practices for diagnosing SCD are Haemoglobin electrophoresis high performance liquid chromatography (HPLC) and Isoelectric focusing. However, these methods rely on expensive laboratory systems which are time consuming or unavailable in resource-limited settings. Here, we describe the field performance of microchip based cellulose acetate electrophoresis "Gazelle™". The current study was conducted at Korle Bu Teaching Hospital, Accra, Ghana. Gazelle is a low-cost, point-of-care translation of the electrophoresis method that has been well established and widely applied in SCD diagnosis. Gazelle is a fast (<10 minutes), easy-to-use point of care test which can be performed by minimally trained personnel using only a finger-prick volume of blood. The primary study objective is to evaluate the utility of the Gazelle for the diagnosis of children with SCD. Blood samples were collected from 1250 subjects who were enrolled in the study, out of which 541 were newborn samples and 709 samples were from the vaccination clinic (age < 5 years). Each sample was tested with Gazelle, and the results compared to HPLC. Interim analysis of Gazelle yielded a high (99%) diagnostic accuracy for all Hb variants compared to HPLC. Gazelle demonstrated a high sensitivity of 100% for HbSS (sickle cell disease, SCD) and 97.9% for HbAS (sickle cell trait), and specificities of 99.3% for disease vs. HbAA, 100% for disease vs. trait, and 97.3% for trait. Gazelle demonstrated high sensitivity and high specificity for identifying SCD and sickle cell trait (HbAS). Gazelle™ offers an innovative and inexpensive solution, leveraging a novel engineering approach, to point of care (POC) diagnosis of SCD. Gazelle can be a potential clinical tool and hence will increase the rates of screening, diagnosis and timely treatment of SCD impacting the lives of underserved population.

0064

PUTTING POPULATIONS ON THE MAP: LANDSCAPING GEOSPATIAL SOLUTIONS FOR PRIMARY HEALTH CARE

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Geography is a critical dimension for monitoring the health of populations, ensuring access to care, and formulating public health policies. However, population and other geospatial data are often out of date or not available at the resolution required for operational decision-making and strategic planning. To address this challenge, the Geo-Referenced Infrastructure and Demographic Data for Development (GRID3) consortium generate geospatial data for development including population, settlements, infrastructure, and boundaries together with government agencies. While these products have been used extensively to plan vertical delivery campaigns, uptake into routine general health services has been limited. From 2020 to 2021, landscape assessments were conducted in nine countries: Burkina Faso, DRC, Ethiopia, Ghana, Kenya, Mozambique, Namibia, Sierra Leone, and Zambia. Assessments evaluated the availability and use of population datasets including GRID3 products, the stakeholders involved, and barriers to adopting novel geospatial products in primary health care planning and operations. General results will be presented detailing opportunities for GRID3 data to be integrated into existing processes and operations to improve primary health care outcomes, such as ensuring equitable access through expanded community-level healthcare delivery, improving targeting and forecasting of commodities, or designing and implementing universal health coverage reforms. Use-cases prioritized based on feasibility and impact will be tested by MoH and local partners with support from CHAI by conducting analysis using GRID3 and other geospatial data to inform activities related to the use-case. Through a consultative, inclusive process, this project aims to improve uptake of cutting-edge geospatial data use to improve primary health outcomes such as access to care and coverage of public health interventions.

0065

PROJECTING TEMPORAL AND SEASONAL PATTERNS OF DENGUE CASES USING CLIMATE ADJUSTED MODELS IN THREE BRAZILIAN METROPOLITAN AREAS

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Global dengue incidence may be increasing due to climate factors and globalization. Brazil is estimated to have the highest dengue caseload globally. We assessed the temporal and seasonal patterns of dengue in three Brazilian metropolitan areas (Rio de Janeiro, Fortaleza, and Belo Horizonte) from 2009 to 2019. We analyzed monthly dengue surveillance data from the national Notifiable Diseases Information System (SINAN) using Seasonal Auto-Regressive Integrated Moving Average (SARIMA) models with precipitation (total), temperature (min, mean, or max), and humidity (mean) as covariates. To determine a best-fit model, we generated a matrix of all combinations of 0 to 4 months of lag for the climate covariates (for 575 possible models per city), and selected models that minimized the root-mean-square error . We used SARIMA models to forecast monthly dengue cases during the year 2020 in three ways: using unadjusted models, models adjusted with actual 2020 climate data, and models adjusted with simple climate data projections (5-year monthly averages). Reported annual dengue incidence was highly variable between cities and year (from 6.9/100,000 in Rio in 2014 to 4,401/100,000 in Belo Horizonte in 2016). Per the model structure SARIMA(p,d,q)(P,D,Q)[m], best fit models were: Rio de Janeiro (1,0,1)(1,1,1)[12]; Fortaleza (2,0,0)(0,0,1)[12]; and Belo Horizonte (2,0,2)(1,0,0)[12]. All adjusted models included the three climate covariates but with different lag combinations. Ljung-Box tests indicated that all models achieved stationarity. Actual monthly 2020 dengue cases in the three cities were within 50% prediction intervals for 36.1% (13/36) of months in the unadjusted models, 63.9% (23/36) in the real climate data models, and 61.1% (22/36) in the climate projection models. These results suggest that climate adjusted SARIMA models can forecast dengue cases with moderate accuracy even in areas with highly variable seasonal and temporal dengue patterns given sufficient baseline data. Simple climate projections may be sufficient for use in future forecasts, which can help to inform outbreak prevention and treatment efforts.

0066

INTRODUCING A MULTIFACETED PLATFORM TO ENGAGE COMMUNITIES IN FIGHTING AGAINST VECTOR CONTROL AND CLIMATE CHANGE

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The Global Vector Control Response (2017-2030) outlines a new strategic plan to strengthen vector control programs worldwide by improving capacity building and surveillance, better coordination and integrated action across government sectors and diseases. However, the 2020 World Health Organisation malaria report indicates progress towards malaria ellimination has stalled. One of the pillars proposed within this strategic plan is engagement and mobilisation of communities through increased basic and applied research and innovation. Innovative vector control strategies continue to emerge while climate change is taking a turn for the worse. Lack of community participation continue to undermine the progress of many emerging innovative interventions and climate change policies around the world. Through a program known as "introducing Science, Technology, Engineering and Math for Vector Control and Climate Change" (iSTEMVCCC), we have established a network of vector control and climate change experts across the world to design a platform through which communities can be educated, participate and provide feedback to enhance the performance of vector control programs and mitigate climate change. The main aim of iSTEMVCCC's is to bridge the gap between stakeholders, vector control/climate change experts and the communities by educating the community on 1) existing vector control/ climate change problems, 2) interventions available to solve the problems, 3) how these interventions work and 4) how the communities can enhance the effectiveness of these interventions. The role of iSTEMVCCC is to partner with stakeholders and vector control programs in various countries to design, implement and evaluate progress of vector control by developing capacity building through structured community engagement programs to ensure a streamlined introduction of new interventions and ensure interventions remain as effective as intended. We will discuss the development and implementation of iSTEMVCCC and ways through which the platform can be adopted worldwide for effective community enegagement in vector control and climate change programs.

0067

PERCEPTIONS AND BARRIERS RELATED TO ROUTINE IMMUNIZATION AND COVID-19 VACCINE HESITANCY AND ROLE OF MHEALTH & ELECTRONIC MEDIA IN PAKISTAN DURING THE PANDEMIC: PRELIMINARY DATA FROM AN EXPLORATORY STUDY

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Social media is influential in dissemination of mis/information regarding the pandemic and vaccines. Routine immunization has been affected globally by the COVID-19 pandemic. Attitudes towards adult COVID-19 vaccine are unknown in Pakistan. Our study purpose is to use m-Health and social media interventions in improving childhood immunization coverage during the COVID-19 pandemic, exploring perceptions and barriers among caregivers of infants <1 year of age, and healthcare providers (HCP) towards CRI during the pandemic, and exploring the need for COVID-19 vaccination. This exploratory qualitative study (depending on saturation) utilizes virtual IDIs (60) and FGDs (18) on phone and Zoom calls in urban, peri-urban, rural sites. Preliminary data indicate that irrespective of the COVID-19 pandemic, caregivers favor CRI completion for their infants. Hesitancy towards adult COVID-19 vaccine is more prominent in rural and peri-urban than urban sites. There is high vaccine acceptance among healthcare providers at all sites. HCP are aware of COVID-19 preventive measures, but lack of SOP implementation is observed in hospital and non-hospital settings, while most caregivers in rural and peri urban sites are uncertain about the pandemic's existence. However, caregivers coming to urban hospital for CRI are aware of the pandemic and COVID-19 vaccines. Caregivers and families are skeptical about federal/provincial lockdowns & the resulting financial/psychological impact. Urban caregivers have access to smart phones and social media apps, while rural/peri-urban participants access information about the pandemic and vaccines from local TV/radio news channels & non-smart phones. HCP regularly use social media apps WhatsApp, Facebook on smartphones. Majority caregivers and HCP feel caller tone messaging (updates about the pandemic, vaccines, SOPs) is useful for creating awareness among general population. This work in progress is among the first studies in the region to examine perceptions and barriers related to the role of mHealth in improving childhood routine immunization and COVID-19 vaccine acceptance and uptake.

0068

SUPPORTIVE SUPERVISION VISITS TO IDENTIFY GAPS IN DATA MANAGEMENT AND USE AT THE PROVINCIAL LEVEL IN THE DEMOCRATIC REPUBLIC OF CONGO

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Generating quality data remains a challenge for the malaria surveillance system in the Democratic Republic of Congo. The U.S. President's Malaria Initiative (PMI) Measure Malaria project supports the National Malaria Control Program (NMCP) to improve the quality of routine malaria data and promote data use, including supportive supervision visits to health facilities (HFs). The NMCP team led supportive supervision visits in 58 HFs and 30 health zones (HZs) in the 9 PMI-supported provinces from Oct.-Dec. 2020. Teams reviewed data reporting and management tools, data analysis and use practices and discussed planning and monitoring of malaria activities. Findings revealed that all HFs had data collection tools but only 11% of those tools met the national standards. At the HZs, all providers coordinating malaria activities were trained in District Health Information Software 2 (DHIS2) and data management but only 50% had computers to access DHIS2; 22% had data collection tools and none could identify their need for data reporting tools. Regarding data analysis and use, 78% of HFs held data analysis meetings but only 33% had dashboards and graphs displayed. For HZs, all held data analysis meetings but only 56% had dashboards and graphs displayed; none sent feedback to HFs. In terms of monitoring malaria control activities, 89% of HFs had a micro plan, all received supportive supervision visits from HZs, but none received training in surveillance, monitoring, and evaluation (SME). For HZs, none had a supervision visit schedule and none had received any formal supervision or feedback from the Provincial Health Division on SME. Most of the HFs and HZs did not have optimal data management capacity, and the key reasons for the gaps were a lack of standardized manuals for completing data collection tools, lack of feedback to the HFs, and limited capacity in the use of data at HFs. In response to these findings, corrective actions have been taken at the HFs and HZs. Regular supportive supervision visits help identify and address SME challenges at HFs, and they should be streamlined and supported to optimize data use to inform malaria service delivery

0069

INITIAL ASSESSMENT OF THE QUALITY OF MALARIA SURVEILLANCE DATA IN SELECTED HEALTH FACILITIES SUPPORTED BY THE U.S. PRESIDENT'S MALARIA INITIATIVE IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Quality data is required for measuring progress of malaria control interventions. Since 2014, to improve data quality and use in the Democratic Republic of the Congo (DRC), the country has reformed the national Health Information System (HIS) by revising the normative framework and integrating District Health Information Software 2 (DHIS2) as the platform for managing routine health data. The National Malaria Control Program (NMCP) with support from the U.S. President's Malaria Initiative (PMI) Measure Malaria project, assessed malaria surveillance data quality in the second and third quarters of 2020, covering 99 health facilities (HFs) in 33 of the 178 heath zones (HZs) in the 9 PMI-supported provinces, based on geographic accessibility and reporting performance. Data quality and the data management system were assessed through monthly activity reports, data collection and reporting tools, and operational action plans. Of the 99 HFs visited, 90% had source documents and 68% had standard data collection and reporting tools. Regarding outpatient register data completeness, 62% of HFs had at least one missing value related to either general information or malaria diagnosis and treatment. Regarding data accuracy, 85.9 % of HFs had inconsistent indicator data and differences were observed between data from the monthly summary form and DHIS2. In terms of the data management system, there was lack of training for data managers, a backlog for data quality verification, lack of standard definitions for data elements, and inadequate data collection and reporting tools. Training for providers was not adequately supported by HZ senior teams and feedback was almost nonexistent. Continued challenges in data quality are linked to the under-supported data management system. There is a need to improve the availability of data management tools, develop an instruction manual for data management and analysis including a data dictionary, and train providers on data management, analysis, and validation. The NMCP and HIS department would benefit from prioritizing regular data quality monitoring in HZs and should consider electronic reporting from HFs.

0070

USE OF BULK SMS TO SUPPORT HEALTH WORKER KNOWLEDGE RETENTION ON ANTENATAL CARE AND USE OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY DURING COVID-19 IN BOSSO LOCAL GOVERNMENT AREA OF NIGER STATE, NIGERIA

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Considering COVID-19 travel restrictions, bulk SMS were used to support knowledge retention of health workers following an in-person training held before the pandemic. In December 2019, 72 facility health workers and 260 community health workers (CHWs) in Bosso local government area of Niger State, Nigeria participated in a 12-day training about benefits of early antenatal care (ANC) attendance, CHW referrals to ANC, and use of intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine to prevent malaria. In-person supervision visits were conducted 3 months following training, although three months later in-person supervision was no longer possible due to COVID-19 related travel restrictions. Post-training support transitioned to use of bulk SMS which were sent twice a week to each cadre for two 3-month rounds of messaging. Knowledge tests comprised of 10 multiple choice questions linked to key ANC and IPTp guidelines were administered at 5 time points: 1) baseline; 2) post-training; 3) at in-person supervision visit 3 months after training; 4) after first round of bulk SMS (6 months post-training); and 5) after second round of bulk SMS (9 months post-training). Average test scores for each cadre were calculated at each time point and T-tests were used to assess differences in scores. The results show that facility health workers scored an average of 53% on the pre-test followed by scores of 76%, 74%, 86%, and ending at 80% 9 months following training. CHWs started with an average score of 49% which increased to 67% post-training; subsequent average scores were 83%, 74%, and 94%. Results were compelling with facility health worker knowledge improving from 76% immediately post-training to 80% 9 months later (p-value<0.05) and for CHWs the improvement was from 67% to 94% (p-value<0.05). These findings suggest that use of SMS can support knowledge retention of key ANC and IPTp guidelines following an in-person training. Program managers, trainers and supervisors may consider using this approach to support health workers where resources and/or movement are restricted.

0071

ASSESSING NURSING EDUCATIONAL NEEDS AND BARRIERS TO CARE THROUGH SEMI-STRUCTURED INTERVIEWS IN A LOW RESOURCE SETTING: A QUALITATIVE STUDY OF PEDIATRIC NURSES IN PORT AU PRINCE, HAITI

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After Port Au Prince's 2010 earthquake, Hospital Bernard Mevs (HBM) developed a collaborative with international medical volunteers. While initially providing clinical care, the volunteer role evolved to include medical education. With limited literature addressing volunteer coordination efforts to support low-resource setting nursing educational needs, there has been little coordination amongst volunteers or input from local providers about ways volunteers can best serve the clinical, educational, or material needs of the hospital. Objectives for this qualitative study included classifying barriers to clinical care at HBM, identifying strengths of the hospital, and eliciting commonly encountered diagnoses, perceived educational needs and preferred modes of learning. In October 2020, HBM pediatric nurses from the neonatal and pediatric intensive care units and general pediatric ward participated in semi-structured small group interviews that were transcribed, underwent content analysis and were coded independently by two investigators. Forty percent of the pediatric nursing staff was interviewed. The most common barrier to care identified was lack of clinical supplies. The most common strengths were team dynamics and training. Interviewees highlighted education provided by both HBM and international volunteers as a strength of the hospital. Requested future training topics had a neurologic and resuscitation focus, and preferred education modalities were didactics and hands-on workshops. Based on our findings, plans at HBM include development of an educational curriculum prioritizing preferred learning modalities with identified relevant and requested content. Coordination amongst volunteers and sharing of educational content may enable volunteers to meet the educational needs of the HBM team in a robust and systematic way. By providing education to Haitian pediatric providers, we hope to support autonomy and capacity building in an efficient and coordinated fashion. This model may inform other programs with a volunteer presence in resource limited countries to promote autonomy and self-directed learning.

0072

INFECTION PREVENTION AND CONTROL KNOWLEDGE AND PRACTICES OF FRONTLINE HEALTH CARE WORKERS DURING THE COVID-19 PANDEMIC IN NIGERIA

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Given the importance of infection prevention and control (IPC) measures for reducing the transmission of COVID-19, a cross-sectional, mixed-methods study was conducted to assess IPC knowledge and practices of frontline health workers – both facility- and community-based health workers (CHWs) – in three local government areas (LGA) in Nigeria: Akure South, Bosso and Ohaukwu. In November 2020, a structured survey was administered to 622 health workers – 294 facility-based (47%) and 328 CHWs (53%) – at 174 primary health care centers. In each LGA, key informant interviews (KII) were conducted with health department management teams; 5 members from LGA and 3 from State health teams. Of all health workers surveyed, 58% had been trained in IPC during COVID and 5 of 11 questions were correctly answered by >94% of providers; however, 3 questions were correctly answered by 65% or fewer. More health workers reported that they practiced recommended IPC behaviors during than before the COVID 19. Significant differences were seen for reported washing hands before glove use (70.5% vs 95.2% during COVID), washing hands after glove use (70.5% vs 95.2%), and using a surgical mask in the workplace (61.7% vs 97.6%). There was no change in reported washing and disinfecting of hands after contact with each patient (69.0% vs 68.6%) or use of an N95 mask in the workplace (8.2% vs 9.1%). Incorrect use of hand sanitizer when hands are visibly soiled however increased (35.8% vs 95.7%). The KIIs confirmed these findings. As one CHW in Ohaukwu said, "We increased our use of face masks, hand sanitizer and gloves," while a facility-based health worker from Bosso said, ". . . now we take more precaution than then . . . unlike before we palpate with our hands but now we use gloves." KII showed that fear of contracting the disease was the reason for change in behaviors. Despite reports of improved IPC measures, use of N-95 masks and hand sanitizer practices remain sub-optimal. There is need for continued support for correct hand hygiene, and to reinforce the relative importance of different IPC practices to ensure adherence to COVID-19 preventive measures.

0073

TRENDS AND SOCIOECONOMIC-RELATED INEQUALITIES IN CHILD GROWTH FAILURE FROM 2000 TO 2016 IN ETHIOPIA: A DECOMPOSITION ANALYSIS

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Despite continued investment by the government of Ethiopia and partners, levels of child growth failure (CGF) are still high. This study examined trends, socioeconomic-related inequalities in CGF and determinants that potentially explain inequalities from 2000 to 2016 in Ethiopia. A total of 43029 child-mother pairs were sampled between 2000 and 2016. Children aged 0 to 59 months and mothers aged 15 to 49 years were included. We categorized CGF indicators (stunting, underweight and wasting) based on height/weight-for-age z-scores. We plotted margins of predicted probabilities to estimate trends of CGF. Concentration curve and indices were used to estimate socioeconomic-related CGF inequalities. Following this, we decomposed the concentration index to identify predictors of CGF inequalities. Margin plot showed evidence of decline in CGF between 2000 and 2016. This reduction varied over time and reached a low between 2005 and 2011. This may be attributable to large-scale nutrition programs over this time. Despite this reduction, we found strong evidence that socioeconomic-related CGF inequalities are increasing and highly concentrated at the lower tail of economic distribution. Between 2000 and 2016, the absolute values of concentration index have increased from -0.072 to -0.139 for stunting, -0.088 to -0.131 for underweight and -0.015 to -0.050 for wasting. These disparities could be explained by key socioeconomic predictors. We found stunting inequalities were mainly explained by geographic region (49.37%), wealth quintile (46.17%) and number of antenatal care visits (31.40%). While underweight was explained by geographic regions (82.21%), wealth quintile (29.18%), access to handwashing (18.59%) and improved water facilities (-17.55%). Wealth quintile (52.91%) and normal body mass index (-66.06%) have explained wasting inequalities. The increased disparities may help us to understand why CGF levels continue to be higher in Ethiopia compared with the World Health Organization cut-offs for public health concern. Multisectoral interventions tailored towards addressing these CGF disparities are required.

0074

FACTORS RELATED TO CHANGES IN HEALTH FACILITY ATTENDANCE AMONG PREGNANT WOMEN DURING THE COVID-19 PANDEMIC IN THREE LOCAL GOVERNMENT AREAS OF NIGERIA

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COVID-19 disrupted public health interventions and weakened global and national health systems. We used a mixed-methods study to explore factors related to changes in health facility attendance during the COVID-19 pandemic in three local government areas (LGAs) in Nigeria: Ohaukwu, Akure South and Bosso. Three hundred fifteen pregnant women seen for antenatal care (ANC) in November 2020 participated in a survey about their attendance before and during the pandemic; 198 women participated in focus group discussions (FGDs). One quarter of women surveyed reported that they reduced the frequency of their visits or did not visit during the pandemic. The biggest reported changes in visits were for immunization (47% visited before the pandemic versus 30% during the pandemic, p<0.001) and other outpatient services (66% to 57%, p=0.027), with small but statistically significant declines in ANC (99% to 94%, p=0.002) and family planning (11% to 5%, p=0.002). Both LGA and religion were significantly correlated with reduced/no visits during the pandemic; other socio-demographic characteristics were not. Whereas 33% of Christian women reported reduced/no care seeking, only 7% of Muslim women did (p<0.001). Women in Ohaukwu were most likely to report reduced/no visits (39%), followed by those in Akure South (26%), and Bosso (7%) (p=0.012). During FGDs transport issues, proximity to health facilities, and fear of contracting COVID-19 or being labeled as COVID-positive were the most common reasons mentioned for not seeking care during the pandemic. Differences by LGA are likely related to differences in both levels of transmission and the State-level response to the pandemic. Ebonyi state, where Ohaukwu is located, had the longest lockdown and most restricted movement; better understanding of differences in the pandemic and state response could inform future actions. The FGDs findings highlight the need for health systems to consider how to facilitate service utilization during a pandemic, such as providing safe transport or increasing outreach, and to minimize stigma for those seeking care.

0075

COMMUNITY ENGAGEMENT TO MANAGE RUMORS IN HEALTH RESEARCH A CASE FROM ETHIOPIA

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The majority of health research projects are conducted for the benefit of public health, but people in many parts of the world often fear or do not trust researchers. WHO (2008) has recognized that communities need to have a voice in health research projects to reduce rumors and suspicions. Still, in many cases, there is more focus on the clinical aspects than on community values, priorities, & interests. In Ethiopia, the Child Health and Mortality Prevention Surveillance (CHAMPS) Network is using community engagement as a key tool in the research. Community engagement includes working with local groups ('*Iddirs'* and '*Afoshas'*), women's associations, health institutions, religious leaders, chief of villages, & health extension programs to encourage notifications when children under five pass away. In addition, radio programs, theatre performances with actors from the communities & regular meetings with Community Advisory Boards are used both for regular feedback and to disseminate information & research findings to the communities. These activities have helped build trust, form relationships & facilitate mutual sharing of knowledge about child & maternal health. Through the involvement of community members in the research, the team has been able to manage rumors about organ theft and vampires & other suspicions about why CHAMPS is focusing on dead children. These engagements contributed to the participation in the research & helped reduce the challenges of conducting the clinical activities. Demonstrating the importance of community engagement in Ethiopia to manage rumors and suspicion, we suggest that health research planning, implementation & outcomes should be developed through close collaboration with local communities.

0076

WESTERN AUSTRALIAN AND PAPUA NEW GUINEAN EUCALYPTUS SPECIES KINOS: VALIDATION FOR THE TRADITIONAL MEDICINAL USES OF KINOS

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Tropical diseases are persistent in tropical developing and underprivileged communities in developed counties where there is lack of water and non-adherence to simple regulations for sanitation and hygiene. Disadvantaged populations use alternative traditional medicines when approved, conventional drugs are inaccessible and costly. Kinos are astringent dried exudates from certain Eucalyptus species and they are used to treat gastrointestinal infections, wounds, fever, and sores by First Australians. In contrast, there is no documentation of traditional medicinal uses of kinos from Papua New Guinean eucalyptus species. This study aimed at validating some of the claims of the First Australian communities for the treatment of various infections with kinos. The kinos from Western Australian *Eucalyptus calophylla* and Papua New Guinean *E. confertiflora* were tested for their immuno-modulatory effects on LPS-stimulated RAW267.4 macrophages *in vitro*. Assays included Enzyme-linked immunosorbent assays for TNFα, interleukin (IL) 6 and 10, and the Griess reaction for nitric oxide. Phagocytic activity of macrophages to Gram-negative and Gram-positive bacteria was evaluated with flow cytometry. Phytochemical screening of the kinos extracts was also performed in this study. Cytokine profiles and phagocytic activity were investigated by treating the cells with different kinos concentrations (62.5-250 µg/mL) at specific time points (6 and 12 hr for IL6, IL10 and TNFα; 24 hr for nitric oxide). Interleukin 6, IL10 and nitric oxide levels were significantly reduced in a dose-dependent manner (from $p<0.05$ to $p<0.0001$) while TNFα significantly increased (from $p<0.05$ to $p<0.0001$). The phagocytic activity of kinos-exposed macrophages increased for both Gram-positive and Gram-negative bacteria. Mostly phenolic compounds and tannins were detected in the kinos. The results show that the kinos of the Eucalyptus species possess immuno-modulatory activity, impacting cytokine profiles and phagocytic activity.

0077

UNDERSTANDING ANTIMICROBIAL RESISTANCE THROUGH THE LENS OF ANTIBIOTIC VULNERABILITIES IN PRIMARY HEALTH CARE IN RURAL MALAWI

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The diminishing effectiveness of antimicrobials raises serious concerns for human health. While policy makers grapple to reduce the overuse of antimicrobial medicines to stem the rise of antimicrobial resistance, insufficient attention has been paid to how this applies to low-income rural contexts. We provide an in-depth understanding of antimicrobial prescribing at primary health care level in rural Chikwawa District, Malawi. Fieldwork took place over 18 months (2018-2020). We surveyed all 22 health facilities in the district, observed 1348 health worker-patient consultations, and carried out 45 in-depth interviews with staff and patients. Care was centred around provision of an antimicrobial. Amid chronic lack of essential medicines and other resources, clinic interactions were tightly scripted, providing patients little time to question or negotiate their treatment. We develop the concept of antibiotic vulnerabilities to reveal the multiple ways in which provision of antimicrobials in rural Malawi impacts people living in extreme scarcity. Antibiotics are central and essential to primary care. As targets for optimal antimicrobial prescribing take a more central role in global policy, we must track the ramifications of this for the delivery of care to ensure that efforts to stem resistance do not undermine the goal of improved health for all.

0078

ENGAGING ISLAMIC RELIGIOUS SCHOLARS AND LEADERS TO INCREASE ACCEPTANCE OF MINIMALLY INVASIVE TISSUE SAMPLING PROCEDURE IN A MORTALITY SURVEILLANCE PROGRAM IN RURAL BANGLADESH

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Minimally invasive tissue sampling (MITS) post-mortem is an alternative to full autopsies to determine etiology of deaths. After obtaining family consent, Child Health and Mortality Prevention Surveillance conduct MITS on stillborn babies and deceased children under-5 years to identify causes of death. Previous studies in Muslim countries revealed Islamic religious challenges to MITS acceptance, which influence and determine broader social acceptance. We aimed to establish endorsement of the MITS procedure among Islamic religious leaders and expand social acceptance in Baliakandi sub-district, a predominantly Muslim community in rural Bangladesh. From May 2017 to December 2020, we arranged two group discussions with four Muftis (qualified legal Islamic religious scholars) and 21 senior Imams. We then conducted 84 meetings with Imams at local Mosques with community residents. We circulated 246 copies of a Fatwa (interpretation on a point of Islamic Shariah) on MITS procedure among Muftis, Imams, presidents, and secretaries of Mosque committees. Muftis agreed to the MITS procedure and senior Imams concurred with their views but noted the importance of continuously engaging with

local level religious leaders and community residents to enhance MITS acceptance. Two Muftis and 10 Imams actively supported explaining the MITS procedure to families when the team approached them for consent, communicating cause of death and providing report to families and contributed with their suggestions (i.e.: importance of resolving 'seepage' from deceased body after MITS is performed) to enhance religious acceptance of MITS. Of the 185 families approached for MITS, 37% consented to the procedure. Among refusals, only ~9% reported religious beliefs as the reason for non-acceptance, which declined over the time, from 50% (3/6) in 2017 to 4.5% in 2020 (1/22). Islamic religious scholars and religious leaders are key stakeholders to endorse and extend religious acceptance of MITS at the community level. Public health programs that include evaluations of deaths or needle biopsy must engage religious scholars and religious leaders throughout the study.

0079

COGNITIVE OUTCOMES AT 18 MONTHS: FINDINGS FROM THE EARLY LIFE INTERVENTIONS FOR CHILDHOOD GROWTH AND DEVELOPMENT IN TANZANIA (ELICIT) TRIAL

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Seasonal subsistence communities are predisposed to seasonal patterns of hunger, micronutrient deficiencies, and enteric pathogens, all of which may be risk factors for long-term child growth and development. Cognitive testing is the most accurate assessment of developmental progress; however, stunting (length-for-age Z-score more than 2 standard deviations below the mean) is frequently used as a surrogate because direct cognitive assessments are harder to measure. Here we assess cognitive outcomes in children enrolled in the ELICIT randomized, placebo-controlled trial in Haydom, Tanzania. The Malawi Developmental Assessment Tool (MDAT), which includes gross motor, fine motor, language, and social assessments, was a pre-specified secondary outcome. There was no effect of the study interventions of nicotinamide (change in development-for-age-Z-score (DAZ) -0.08; 95% CI: -0.16, 0) or antimicrobials (change in DAZ 0.04; 95% CI: -0.06, 0.13) on the overall MDAT score. Additional analyses of MDAT subscores and within subgroups defined by gender, socioeconomic status, birth weight, and birth season showed no effect of the interventions. We then used linear regression to identify risk factors for developmental outcomes. We identified birth in the pre-harvest season (-0.16 difference in DAZ vs. birth in the post-harvest season, 95% CI: -0.26, -0.06) and higher socioeconomic status (SES) (0.26 difference in DAZ score compared to lowest SES quartile, 95% CI: 0.12, 0.40) as factors associated with cognitive outcomes. Interestingly, we found that birth during the pre-harvest season was not associated with attained length at 18 months (estimate -0.02, 95%CI: -0.14, 0.10). This study cohort represents a high-risk population for whom interventions to improve child growth and development are urgently needed. Further analysis of this cohort is needed to determine the best surrogates for cognitive outcomes and the best timing for such interventions to improve cognitive outcomes.

0080

ARE NATIONAL MALARIA CONTROL PROGRAMS (NMCPS) EMBEDDED TECHNICAL ADVISORS MAKING AN IMPACT? LESSONS LEARNED FROM NMCPS IN WEST, CENTRAL, AND EAST AFRICA

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National Malaria Control Programs (NMCPs) must have robust institutional capacity and an empowered health workforce to achieve malaria control and elimination. Since 2016, the USAID Human Resources for Health in 2030 Program (HRH2030) has embedded long-term technical advisors (LTTA) into 10 NMCPs (Burundi, Cameroon, Central African Republic, Chad, Cote d'Ivoire, The Gambia, Guinea, Niger, Sierra Leone, and Togo) to build leadership, management, and governance (LMG) capacity to make the best use of Global Fund grants and U.S. President's Malaria Initiative resources. HRH2030 implemented two evaluation methods to assess effectiveness of the LTTA model: The Capability Maturity Model (CMM) (adapted from Carnegie Mellon University) assesses institutional capacity to implement NMCP functions. The LTTA collaborates with NMCP colleagues to rate organizational performance along a five-scale scoring system ranging from «initial» to «optimizing» and identify actions for maturation. The Confidence Assessment (adapted from the LMG Project approach) is a retrospective self-assessment that elicits NMCP staff feedback on how technical assistance influenced their individual confidence to perform key tasks. These models are complemented by output indicators tracking LTTA activities and Global Fund performance indicators. CMM results in Burundi, Cameroon, The Gambia, and Sierra Leone showed significant organizational capacity improvements from an average of 3.2 to 4.0 out of 5. Confidence assessments in Cameroon, Niger, and Sierra Leone showed an average increase in NMCP staff confidence of 29% (from 4.4 to 5.6 out of 7); most NCMP staff indicated the LTTA had some (37.1%) or significant influence (46.4%) in their confidence. The Niger NMCP's Global Fund grant performance rating increased from B2 in 2015 to A1 in 2018. Other country data collection is ongoing. These approaches illuminate the multi-faceted impact of LTTAs. For stakeholders looking to adapt these approaches, HRH2030 recommends establishing NMCP baseline capacity and using evaluation tools as collaborative opportunities to engage and empower in-country stakeholders.

0081

POPULATION CHANGE IN RURAL EASTERN ETHIOPIA BETWEEN 2007 TO 2019: FINDINGS FROM AN OPEN DYNAMIC COHORT DEMOGRAPHIC SURVEILLANCE

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In the face lack of a vital records system, demographic surveillances are an efficient, cost effective and reliable method to collect population-based data in a resource limited setting. This report focuses on characterizing the population of Kersa Health and demographic Surveillance System (Kersa HDSS) in Eastern Ethiopia, under surveillance since 2007, to examine population and demographic changes over the 12-year period. Kersa HDSS was established by enumerating population in catchment of 12 sub-districts. At the baseline the population was 50,692 and at the end of 2019 it was 136,650. A person-time analysis was used to determine population increase, population growth rate, birth and death rates, sex ratio, and dependency ratio. The surveillance had produced 1,026,580 person-year over 12-year period. The overall population sex ratio was balanced at 102.7, while the overall sex ratio at birth was 110. The young dependency ratio had reduced from 88.8 % in 2008 to 73.3% in 2019 and the old dependency ratio had slightly increased from 3.9 to 5.6% for the same period. The crude birth and death rates were 31.9 and 11.2 in 2008 and became 24.3 and 5.9 in 2019. The average crude population

increase was 2.4 per year. The life expectancy had increased by 11.8 from 56.1 in 2008 to 67.8 in 2019. There is a significant change in life expectancy as a result of significant reduction in mortality. More young population is entering to the productive segment of the population. There is an opportunity to harness these work force for the national development.

0082

TOWARDS A SUSTAINABLE CONFERENCING: COMPARING THE CARBON FOOTPRINT OF IN-PERSON, VIRTUAL AND HYBRID ASTMH EDITIONS

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The carbon footprint of academic conferencing exceeds that of some countries. Carbon emissions contribute to the climate crisis that has severe consequences for human health. In the years prior to the COVID-19 pandemic, thousands of participants from across the globe travelled to the Annual Meeting of the American Society of Tropical Medicine and Hygiene (ASTMH). In 2020, a virtual conference was organized that will be followed by a hybrid meeting in 2021. For the 68th edition of ASTMH in National Harbor, Maryland (2019), we determined the most likely route of travel based on country or state of residence. We used this to determine total distance travelled and the CO_2 equivalent (CO_2e) emissions, considering differences in emissions per km between long- and short-haul flights, and accounting for indirect emissions from radiatively active substances and aerosol formation. The 4834 participants together travelled an estimated 27.7miles/44.6 kilometres, or the distance of 58 return trips to the moon. The estimated CO_2e emissions were 8646 metric ton. In the coming months, we will calculate the CO_2e emissions from the 69th ASTMH conference in 2020 that was a virtual edition with 4011 participants, incorporating network-, laptop- and server-related emissions at 240 gCO_2e/kWh. The 70th edition of ASTMH will be held in National Harbor, Maryland in 2021 and offers a choice of in-person and virtual attendance. In the month prior to the conference, we will acquire an approximation of the number of virtual and in-person participants and calculate their carbon footprint. In conclusion, in addition to the quality of knowledge dissemination and networking, securing annual revenues to maintain ASTMH as an organization, the carbon footprint of conferencing is an increasingly important factor that needs to be considered when organizing conferences. Data from the 68th, 69th and 70th editions of ASTMH offer an opportunity to directly quantify these emissions to support discussions on how to best organize this conference in this era of climate change.

0083

FACILITATORS AND BARRIERS TO ADOPTING SUSTAINABLE PRACTICES IN GLOBAL HEALTH INSTITUTIONS

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The climate crisis threatens human health and wellbeing and disproportionally affects populations in low and middle-income countries. Given that the purpose of global health institutions is to improve the health of populations, they have a critical role to play in mitigating the health effects of climate change as well as rapidly reducing greenhouse gas emissions from the global health enterprise. This study examines how global health institutions are reducing the greenhouse gas emissions from their own operations and analyzes the facilitators and barriers to achieving decarbonization goals. We reviewed the sustainability goals and implementation plans of 10 global health universities from the 'TropEd' network, and the top 10 international non-governmental organizations (NGO) ranked by 'NGO Advisor' working in a health-related field. We systematically collected information from institutional websites and annual reports. Through online interviews, key informants validated the information and shared their opinions regarding what factors are helping their institutions decarbonize and what factors are hindering progress. Initial findings show that 9/10 universities sampled have a sustainability policy, 6/10 have specific decarbonization goals, and 8/10 have implementation plans. However, the systematic online search shows that only 3/10 non-governmental organizations sampled have a sustainability policy, 1/10 have specific decarbonization goals, and 1/10 have implementation plans. Findings from the interviews with the key informants of the 10 global health universities are summarized. Institutions across all sectors need to rapidly decarbonize to mitigate the worst effects of climate change, but results suggest institutional responses are lagging amongst global health NGOs. This study identifies lessons and best practices that global health institutions are utilizing to successfully decarbonize their operations as well as highlighting critical gaps that still need to be tackled.

0084

LESSONS LEARNED FROM THE EXPERIENCE OF MOBILIZING COMMUNITY VOLUNTEERS FOR REAL-TIME IDENTIFICATIONS OF STILLBIRTHS AND < 5 CHILD DEATHS IN A CHILD HEALTH PROGRAM IN RURAL BANGLADESH

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The Child Health and Mortality Prevention Surveillance (CHAMPS) uses minimally invasive tissue sampling (MITS), a post-mortem procedure, at 3 sentinel facilities and in the community in a rural sub-district *Baliakandi*, Bangladesh to identify the causes of stillbirths and <5 child deaths. The major challenge of conducting MITS among children who die in the community is receiving notification of death before burial so that the team can approach the guardians about participation in the MITS procedure. To receive rapid notifications, we recruited 1,190 community volunteers from 261 villages in 2018 and requested them to notify stillbirths and <5 deaths through a CHAMPS hotline number. Later, we contacted 296 volunteers from March-November 2019 over the phone, to explore the barriers why they missed notifying 156 deaths in their villages that were later notified by the Demographic Surveillance System of the CHAMPS. The major reasons stated by the volunteers were: The residence of the deceased child was not nearby their house, the hotline number was not saved in their mobile phone, they heard about the death once the body had already been buried, and the CHAMPS hotline number was unreachable due to poor network connection. In response, we took certain actions to overcome the barriers. We identified 63 places in the catchment area where no volunteer was available and recruited new volunteers. We included an additional hotline mobile number. We arranged refresher training from December 2019 to February 2020 with 995 volunteers where we ensured all volunteers saved hotline numbers in their mobile phones on the spot and also clarified that they should report any deaths even if the body was already buried. We have done a comparative analysis between two equal durations before and after implementing the necessary actions to assess the changes in receiving notifications. We found that after implementing the necessary actions, overall the number of notifications rose 30%; those within 5 hours rose 23%. Identifying the bottleneck of an ongoing community-based death notification system and implementing need-based actions increased the number of notifications.

0085

THE IMPACT OF CLIMATE CHANGE ON NEGLECTED TROPICAL DISEASES OF THE SKIN

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The climate crisis is a serious threat to global human health, disproportionally affecting low-resource settings and vulnerable populations. Importantly, climate change increases the incidence of neglected tropical diseases (NTDs) of the skin, which WHO lists as Buruli ulcer, cutaneous leishmaniasis, leprosy, mycetoma, yaws, onchocerciasis, lymphatic filariasis, scabies and fungal infections. Global warming drives changes in precipitation and humidity and increases extreme weather events. Alterations in these climatic variables increase the risk of bacterial and fungal infections and expand the range and odds of survival of insects, leading to a rising incidence in vector-borne skin NTDs and the emergence of these diseases in new geographic regions. Increasingly, millions are fleeing climate change-related alterations in their natural environment as impacts from climate change such as extreme weather events, sea-level rise, and drought exacerbate food insecurity and civil unrest, fueling mass migration. Consequently, rising rates of skin NTDs among climate refugees are observed due to poor nutrition and hygiene, overcrowding, and confinement in refugee settlements, enhancing the transmission of highly communicable diseases such as scabies and yaws. Extreme weather events also curtail surveillance and treatment efforts aimed at reducing skin NTDs. During wildfires, flooding and storms, rapid evacuation may result in lost or forgotten medications and access to health care services becomes severely limited. Additionally, community surveillance programs and mass drug administration services may be suspended, and damage to telecommunications infrastructure diminishes accessibility to remote health care services delivered on digital platforms including telehealth. Skin NTDs are associated with substantial morbidity and disfigurement, resulting in stigmatization that reinforces socioeconomic barriers such as discrimination and poverty. The impact of climate change on the incidence of skin NTDs must be emphasized. Moreover, lack of climate mitigation risks serious setbacks to controlling skin NTDs globally.

0086

DENGUE TRANSMISSION RISK IN A CHANGING CLIMATE: BANGLADESH COULD EXPERIENCE A LONGER DENGUE FEVER SEASON IN THE FUTURE

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Our changing climate is already affecting the transmission of vector borne diseases such as dengue fever. This issue presents a significant public health concern for some nations, such as Bangladesh, which already experience regular seasonal outbreaks of dengue fever under present day conditions. To provide guidance for proactive public health planning to potentially mitigate future infections, we explore the impact of climate change on dengue infections by calculating the change in vectorial capacity of *Aedes aegypti* mosquito at a seasonal level for all regions in Bangladesh for two atmospheric greenhouse gas concentrations for the period 2050-2099. For each of the four climate models used, and for both scenarios, our analysis reveals that the annual vectorial capacity remains at a level that would enable potential dengue epidemic transmission in all regions during the time period examined. We found a slight decline in vectorial capacity in half of the regions examined during the last two decades of 21st Century for the lower-concentration scenario, with a pronounced decline in vectorial capacity in all geographic regions beginning in 2060 for the higher-concentration scenario. The likely reason is that in many regions greenhouse warming is leading to temperatures beyond the optimum for mosquito breeding. However, seasonal differences in vectorial capacity dissipate as the climate warms, to the point that there is almost no observable seasonality for the higher-concentration scenario during the last two decades of the century. This suggests the potential for the dengue season to extend all year, with outbreaks occurring at any time. These findings suggest that disease surveillance and control activities would need to be geographically and temporally adapted to mitigate dengue epidemic risk.

0087

PRIORITIES FOR VECTOR-BORNE DISEASE RESEARCH UNDER A PLANETARY HEALTH FRAMEWORK: A SCOPING REVIEW AND BIBLIOMETRIC ANALYSIS OF CLIMATE CHANGE AND AEDES-VECTORED DISEASES IN AUSTRALIA AND INDO-PACIFIC REGION

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In recent years, three key arboviruses transmitted by Aedes mosquitoes, Zika, Chikungunya and Dengue have emerged and re-emerged in both hemispheres of the world to cause major human epidemics. The distribution if these viruses are driven by changes in climate and it is estimated that by 2050, almost half the world's population will live in areas where Aedes mosquitoes have established. The two main vectors transmission, *Aedes aegypti* and Aedes albopictus have already caused outbreaks and epidemics of dengue, chikungunya and Zika in the Pacific region and in the Torres Strait in Australia. Understanding the distribution and spread of these diseases under future climate scenarios therefore remains a priority. We conducted a scoping review of peer-reviewed literature from five scientific databases with the aim of providing a synthesis of the current state of literature on the emergence and re-emergence of the mosquito-borne arboviruses to identify key drivers of transmission, changing risk under climate change and other drivers, current strategies for risk management and research gaps and priorities. We analyse these studies through the lens of an integrated risk framework based on the theoretical concepts of planetary health and the IPCC risk assessment framework to identify key drivers of hazard, exposure and vulnerability. We found that compared to other regions, there are limited studies (n=43) that address climate change and Aedes vectored arboviruses. Further we find that 32% of these studies are review studies, providing a broad overview of the research landscape. While the remaining studies acknowledged the influence of climate on spread of disease, only 6 studies assessed how climate factors and/or climate change will influence disease transmission and all of these studies were on Dengue virus. This study highlights the need for more understanding of factors driving risks of emergence and re-emergence of Aedes-vectored arboviruses in the region.

0088

CLIMATE CHANGE AND MALARIA RISK IN EAST AFRICA - UNPACKING IMPACTING FACTORS AND INTERVENTION OPTIONS USING BAYESIAN NETWORK MODELLING: A PLANETARY HEALTH APPROACH TO A WICKED PROBLEM

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Malaria is a disease with a complex transmission cycle, influenced by biophysical and socio-ecological drivers. While malaria is the most studied mosquito-borne disease in the context of climate change, there are limited studies that model the many inter-related pathways of causality and hierarchical relationships between these drivers of transmission. Thus, model projections still contain a high degree of uncertainty. We address this uncertainty using a participatory systems approach and Bayesian Network modelling. We develop the model using key drivers of

malaria transmission. We parametrize the BN model using quantitative and qualitative data from global climate change models, Demographic and Health Surveys, Malaria Indicator Survey, Expert knowledge, key informant interviews and focus group discussions to provide a robust assessment of malaria risk. We estimate the posterior probability of risk of malaria infection given conditional prior probabilities under different climate change scenarios to suggest potential adaptation options for risk management. Results of our sensitivity analysis indicate that under baseline conditions, health system services, agricultural land use and sand quarrying are significant drivers of optimum malaria transmission at the community level. Under RCP4.5 projected to 2080, air temperature and to a lesser extent, sand quarrying, health system services and agricultural land use are the significant drivers of malaria risk. Under RCP8.5 projected to 2080, air temperature is the significant driver of optimum malaria transmission. Our results suggest that in the short-term, interventions to manage climate change and malaria transmission risk at the community level should focus on health systems strengthening and sustainable land use practice while in the long term, focus should shift to managing the risks associated with higher temperatures. Our results highlight which adaptation interventions are likely have the most influence on risk reduction under changing climate and have important implications for climate change and health adaptation policy and practice.

0089

CLIMATOLOGICAL VARIABLES AND THE INCIDENCE OF LEPTOSPIROSIS IN CARTAGENA DE INDIAS, COLOMBIA: AN ECOLOGICAL STUDY FROM 2008 TO 2017

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Leptospirosis is an acute febrile illness caused by bacteria of the genus Leptospira. The prevalence of human leptospirosis largely varies across geographic locations and is frequently associated with occupational factors and exposure to animals and rodents. Seasonality and climatic factors also describe as consistent factors related to the increase of leptospirosis cases in humans. Here, we described the relationship between the incidence of Leptospirosis cases and multiple climatological variables in Cartagena de Indias - Colombia from 2008 to 2017. Cases were collected from the National Public Health Surveillance System of Colombia (SIVIGILA), and meteorological data regarding precipitation, days with precipitation, relative humidity, and average temperature were obtained from the Oceanographic and Hydrographic Research Center (CIOH) located in Cartagena. Additionally, the Oceanic Niño Index (ONI) was used to monitor the "El Niño" and "La Niña" phenomenon across the evaluated period. We identified a total of 360 laboratory-confirmed cases through the study period and an increased incidence of leptospirosis during wet periods with heavy rainfall and rainy days. Almost half of the cases (156/360, 43.4%) occurred during 2010 and 2011. Remarkably, these years had the highest accumulated precipitation. The highest incidence periods consistently occurred during August to December, months that constitute the rainy season in Cartagena. Additionally, "El Niño" and "La Niña" seem to affect the occurrence of leptospirosis cases. Our findings suggest that wet periods and rains are correlated factors to leptospirosis outbreaks in Cartagena. For this reason, these variables could potentially be used as early indicators to establish prevention and control policies in affected areas with a high risk of contagion

0090

THE ROLE OF THE UGANDA PUBLIC HEALTH FELLOWSHIP PROGRAM IN UGANDA'S COVID-19 RESPONSE

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Local, trained field epidemiologists are key to health security. However, many countries still face shortages in this workforce. Uganda has been working since 2015 to build its field epidemiology capacity through the Public Health Fellowship Program (PHFP). We describe the PHFP and its role addressing COVID-19 in Uganda. PHFP is a two-year, full-time, post-Master's degree field epidemiology training program integrated into the Uganda Ministry of Health (MoH). It is implemented by the US CDC, Makerere University School of Public Health (MakSPH), and MoH. Fellows work in MoH priority host sites under close supervision from host site mentors, PHFP, CDC, and MakSPH staff, and others. They lead outbreak investigations and respond to public health emergencies, conduct planned epidemiologic studies, analyze surveillance data, undergo leadership training, conduct quality improvement projects, and write and present their work. Since 2015, 52 fellows have graduated; 27 are enrolled. Most graduates work for MoH or partners. PHFP fellows conducted 44 COVID-19 response activities from February 2020-March 2021, including 24-hour airport screening, contact tracing, operational readiness assessments at health facilities, investigating infection clusters, evaluating adherence to prevention measures, assessing the impact of COVID-19 among refugees and truckers, and many others. Graduates working for MoH and partners oversaw major activities, including designing and overseeing contact tracing databases and activities, tracking healthcare worker infections, conducting COVID-19 death investigations, designing and executing district trainings, and mentoring investigators. Such activities were sometimes, but not always, responses to direct requests from the MoH or MakSPH. In conclusion, Uganda's response to COVID-19 was strongly supported by PHFP fellows and graduates, who filled key roles and provided critical skillsets otherwise not available in the workforce. Similar programs should be considered to address needs in other countries and increase capacity to respond to public health emergencies.

0091

EFFECT OF ALTITUDE ON COVID-19 MORTALITY IN ECUADOR: AN ECOLOGICAL STUDY

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The SARS-CoV-2/ COVID-19 pandemic has claimed nearly 900,000 lives worldwide and infected more than 27 million people. Researchers worldwide are studying ways to decrease SARS-CoV-2 transmission and COVID-19 related deaths. Several studies found altitude having a negative association with COVID-19 incidence and mortality. Using publicly available province-level Ecuadorian data we performed an ecological study to explore a relationship between altitude and COVID-19. ANOVA, correlation statistics, and a multivariate linear model explored the relationship between different Ecuadorian altitudes against incidence, mortality, and case-fatality rates. Population statistics attributed to COVID-19 were included in the linear model to control for confounding factors. Statistically significant differences were observed in the Amazónica, Sierra, and Costa regions for incidence, mortality, and case fatality rates, suggesting an association between altitude and SARS-CoV-2 transmission and COVID-19 disease severity (p-value ≤ 0.05). In the univariate analysis, altitude had a negative association to mortality rate with a 1-unit change in altitude resulting in the decrease of 0.006 units in mortality rate (p-value = 0.03). The multiple linear model adjusted for population statistics showed a statistically significant negative association of altitude with mortality

rate (p-value = 0.01) with a 1-unit change in altitude resulting in the decrease in mortality rate by 0.015 units. This study suggests altitude may influence mortality associated with COVID-19. More research is needed to understand why altitude may have a protective effect against COVID-19 mortality and how this may be applicable in a clinical setting.

0092

SETTING UP A HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM IN A SEMI-INFORMAL SETTLEMENT: INITIAL EXPERIENCES AND FINDINGS FROM MANYATTA CHAMPS SITE IN KISUMU, KENYA 2016-2018

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Lack of effective and comprehensive national civil registration and vital statistics systems in low-middle income countries (LMICs) entail the need for tracking population health using Health and Demographic Surveillance Systems (HDSS). As part of Child Health and Mortality and Prevention Surveillance (CHAMPS), we established an HDSS in Manyatta urban informal settlement in Kisumu county, Kenya to provide a platform for conducting high-priority research and as a primary alternative source of data to extrapolate estimates of national cause-specific mortalities among children < 5 years.We hired and trained a local community-based research team; planned and held consultative meetings with national and community based administrative, political and health leaders; conducted community and household consenting. We mapped boundaries and locations of households and villages from November 2016 to February 2017. We performed an initial population-based census enumeration in 2017 and subsequently repeated household follow up census visits in 2018. Overall, 121 villages, 31,000 households and resident population of 77,800 people (including 11,866 (15.3%) children < 5years) were mapped and enumerated in 2017. In 2018, the number of mapped households was 3.0% lower than baseline (30,074) and the resident population dropped by ~1% (77,339 people who included 10,016 (12.9%) children < 5years, 4, 900 (6.3%) in-migrants and 1,575 (2%) new births (1,575)). Out-migration and deaths accounted for 5,960 (7.7%) and 204 (0.3) of reduction in the baseline population and 0.1% of initially enumerated households refused further participation. The HDSS launch coincided with the general election held in Kenya in August 2017 and subsequent disputed election results. However, hiring members of our study team from the study region enabled basic surveillance despite displacement of populations due to post-election violence. Although experience in setting up a new HDSS site might vary globally, our Kenyan experience demonstrates feasibility of a strategy that could potentially be replicated in other similar settings.

0093

PERCEPTIONS AND IMPACT OF COVID-19 ON RESIDENTS IN RURAL COMMUNITY IN BANGLADESH: A QUALITATIVE RAPID ASSESSMENT

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Bangladesh has taken multiple steps, to control COVID-19 spread, including restricting human movement. We conducted 16 key informant interviews and 2 focus group discussions (FGD) in rural Baliakandi, Bangladesh to explore perceptions of COVID-19, including transmission and prevention; and the impact of pandemic response on food consumption, healthcare, and livelihoods. Participants included residents previously diagnosed with COVID-19, healthcare providers, drug sellers, and local volunteers with a child health project. Respondents perceived that international travelers introduced 'Corona' to the country and it then spread from cities to rural areas. Local spread continued through social interactions in public places including mosques, local markets and public transport. To prevent spread, respondents washed hands, wore masks, drank hot water or herbal tea, disinfected household surfaces, refrained from shaking hands and avoided crowds. Residents erected a fence to isolate households of those testing positive but also supplied food and necessities to the household residents. During lockdown, residents primarily sought healthcare, including antenatal and pediatric care, from village doctors or drug shops because of fear of infection from health facilities. Respondents believed that childbirth by unskilled birth attendants increased while movement was restricted. One woman who recovered from COVID-19 and experienced a miscarriage reported that she had not been able to attend antenatal visits and blamed the lockdown for her miscarriage. Healthcare providers reported a low PPE (coverall) supply, noting that PPE reuse increased the risk of infection. Food insecurity and financial hardship were widely noted. Members of low-to-middle income groups lost their jobs as businesses were shut. Due to this hardship, residents cut out high-protein foods and stopped non-essential expenditures. Some took out micro-credit loans or sold their land. The findings demonstrate the importance of ensuring access to essential food and healthcare, if movement restrictions are used, to avoid any adverse maternal and child health outcomes.

0094

MALARIA RESPONSE SYSTEM - A FOUNDATION FOR TARGETED COVID-19 INTERVENTIONS AMONG VULNERABLE POPULATIONS IN SURINAME

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To assess the contribution of the Suriname Malaria Program to the national COVID-19 response toward mobile migrant populations. Following the first case of COVID-19 in Suriname in March 2020 the Malaria Program, with support from the Ministry of Health, established capacity for COVID-19 response with a focus on its own priority risk population of mobile migrants. COVID-19 diagnosis was implemented according to national guidelines and protocol, using the structures and expertise that were in place for malaria elimination. Outreach was done using multi-lingual communications, via formal and social media and via the Malaria community health workers. Low threshold access to COVID-19 diagnosis was established by the multilingual health personnel in the Malaria Program clinic. A total of 1446 people were tested for COVID-19 at the Malaria Program clinic between June and December 2020. This constituted 4.5% of the national number of people tested during this period. The overall trend of number of cases over time was in line with the national trend. Non-Surinamese subjects tested (29% of total) were primarily of Brazilian Cuban, Chinese, Venezuelan, and Dominican nationality. A total of 317 people tested COVID-19 positive. The majority of these (91%) were aged between 15 and 59 years old. The overall positivity rate was 21.9% (varying from 5.45.% to 64.79.% per month). High overall positivity rates were found among people of Brazilian (54.03%) and Venezuelan (47.2%) nationalities. Mortality was zero. The health structures and expertise that were in place for Malaria Elimination in Suriname aided a quick and effective COVID-19 response of the Ministry of Health toward (mobile) migrant populations, who were considered a key vulnerable risk group. The high positivity rate among migrants of Brazilian and Venezuelan origin confirmed the need for inclusion of these populations in the national response as well as the need for a targeted approach. This is an example of how (emerging) priority health services can be provided to marginalized populations by integrating them in the capacities already established for infectious diseases, such as malaria.

0095

USING MHEALTH SMS SURVEYS FOR ACTIVE SURVEILLANCE OF FEBRILE ILLNESS IN KENYA

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Febrile illness is a common symptom of disease and motivates a substantial portion of healthcare seeking behavior. Current research underestimates the burden of febrile disease, especially in low- to mid-resource settings, such as sub-Saharan Africa, where some febrile illness goes undetected by clinical presentation and passive surveillance. Surveillance data requires advancements to document baseline occurrence and identify etiologies of fever in these settings, such as the contribution of arboviral disease outbreaks. mHealth surveys are an accessible method to report febrile symptoms. Starting in October 2020, we distributed semiweekly, incentivized mobile SMS surveys to a randomized sub-cohort of adult household representatives (n=750) from a larger cohort of participants (n=1353 households) across two clinical study sites in Kisumu and Mombasa, Kenya to surveil febrile disease. Our survey captured the presence and amount of fever in a household, identity of the febrile individual(s), and healthcare-seeking behaviors in five questions. Mean response rates are 55% per survey, and 13% of households report fever on average with minor mean difference across sites (2.2%, p<.01) but comparable temporal trends. The most cited reasons for not seeking care were insufficient money and utilizing home remedies. Using logistic regression, we found respondents who complete over 65% of surveys have more education (OR for college =2.52 [1.58,4.06]) but did not differ on other demographic variables. Survey referrals accounted for 30.9% of clinic sick visits but lacked differences in percentage febrile at presentation compared to walk-in clinic visits. And cases referred via survey follow-up had associations with diagnoses of tonsilitis/pharyngitis (OR=2.71 [1.66,4.45]), upper respiratory tract infections (OR= 0.66 [.46,.95]), and possibly urinary tract infections (4.49 [1.40, 17.01]) but not malaria (OR= 0.82 [.55, 1.21]). Though challenges with validity of self-reported fever, non-response, and technological hurdles remain, these results suggest mHealth surveys may improve follow-up in clinical research efforts.

0096

GEOGRAPHIC REPRESENTATION OF ACUTE FEBRILE ILLNESS SURVEILLANCE: AN UPDATED GLOBAL SCOPING REVIEW

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Fever is a common symptom that brings many patients to health facilities worldwide. However, etiology can vary significantly by region, exposure, and patient. Moreover, acute febrile illness (AFI) surveillance lacks a clear case definition and a standardized approach, leading to limitations in comparing and interpreting results across settings to understand etiologies. We updated a previously published scoping review (from January 2005-December 2017) to characterize recently published AFI research and identify knowledge gaps to inform future studies and programs. In the update, publications from December 2017-December 2020 were obtained through searches of databases with search terms including "undifferentiated fever," "acute febrile illness," and "non-specific fever." After de-duplication, 258 titles and abstracts were screened; 155 (60%) were excluded as they did not identify AFI etiology or were case reports, clinical trials, outbreak investigations, or lab comparisons. An additional 33 articles were excluded after full text review and the remaining 70 publications were combined with the previous review (190 publications). Data extraction was performed on 260 publications using a standardized tool to obtain characteristics of study designs and results. Of the six World Health Organization Regions, the South-East Asian Region (SEAR) is most commonly represented in AFI publications (104) with the fewest studies in the European (EUR, 5) and Eastern Mediterranean (19) Regions. Country coverage within all regions was below 50%, with EUR and the African Region (AFR) having the lowest percentage of countries represented in studies (6% and 29%). AFR had the greatest diversity of pathogens investigated (82 pathogens), followed by the Western Pacific Region (60) and SEAR (54). We found significant increases in publications in SEAR and the Region of the Americas, compared with other regions (p<0.05). While the annual number of publications increased from 2005-2020, future AFI studies should aim to expand regional and global understanding of the causes and burden of AFI by focusing on underrepresented geographic settings.

0097

ACUTE FEBRILE ILLNESS SURVEILLANCE IN TWO URBAN HEALTH FACILITIES IN LIBERIA TO IDENTIFY ENDEMIC AND NEW PATHOGENS

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Little is known about the causes of acute febrile illness (AFI) in Liberia. AFI surveillance can identify known and newly emerging diseases of potential public health importance to inform public health responses, improve clinical care, and build laboratory capacity. We enrolled patients who had measured temperatures of ≥37.5°C or self-reported fever in the past seven days in two urban health facilities in Monrovia, Liberia. We collected demographic, clinical, and exposure data. We collected blood samples and conducted an analysis using real-time polymerase chain reactions in the TaqMan Array Card (TAC) format for more than 20 viral, bacterial, and parasitic pathogens. Testing was performed at Liberia's National Public Health Reference Laboratory. We enrolled 1,506 patients with AFI recruited between 12/2018 and 3/2020. Using TAC, we were able to detect an etiology in 47% of the patients that presented with a fever or a history of fever. The median age was 18 years (IQR 7-27) and 463 (31%) were hospitalized. The most commonly detected etiologies included *Plasmodium spp.* [670 (44%)], dengue[16 (1%)], *Streptococcus pneumoniae* [13 (0.8%)], *Rickettsia spp.* [10 (0.7%)], hepatitis E [2 (0.1%)], *Salmonella enterica* [2 (0.1%)], *Neisseria meningitis* [2 (0.1%)], Lassa Fever [1 (0.07%)], and *Leptospira spp.* [1 (0.07%)]. In conclusion, AFI surveillance and the use of TAC identified nine pathogens, including three (dengue, *Leptospira spp*., and *Rickettsia spp.*) not previously detected in Liberia. Knowledge of circulating high consequence pathogens such as Lassa fever will assist policymakers and public health systems with improved prevention and response. Our findings highlight the need for expanded surveillance systems and laboratory testing capacity for infectious diseases that are endemic or newly emerging in Liberia. Enhanced AFI surveillance will assist with efforts to improve public health systems, clinical care, and laboratory capacity.

0098

EVALUATION OF OCCUPATIONAL CADRE AND EBOLA VIRUS DISEASE RISK PROFILES DURING PREVENTIVE VACCINATION CAMPAIGN - SOUTH SUDAN, 2019

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Healthcare workers are at increased risk for Ebola Virus Disease (EVD) infection due to occupational and community exposures. In response to the 2018-2020 EVD outbreak in the Democratic Republic of Congo (DRC), South Sudan implemented EVD vaccination targeting frontline and health care workers in high-risk states bordering DRC. We described demographics (age), occupational cadre, and EVD risk profiles of vaccinated individuals in South Sudan. Paper campaign vaccination records (January-July 2019) were transcribed into an electronic database to obtain age and occupation. An EVD risk variable was based on the likelihood of occupational exposure with a known or unknown symptomatic EVD infected person and or body fluids. The variable levels were high, medium, or low. High risk occupations included activities with direct patient contact or direct contact with bodily fluids (e.g., clinicians, cleaners, and laboratorians). Jobs with limited exposure to body fluids were classified as medium risk (e.g., vaccinators and security). The remaining occupations (e.g. social mobilizers, community health workers) were labeled as low risk. Frontline workers included occupations not associated with a designated health facility (e.g., burial team, rapid response team). A total of 2,931 individuals were vaccinated. Of those, all had information on age and 97% (2844/2931) had occupations with identifiable job descriptions. The mean age of vaccinated individuals was 41.1 (SD ±12.6) years. Nursing was the most commonly vaccinated occupation [20% (601/2844)], followed by cleaners [14% (404/2844)] and clinical officers [8% (227/2844)]. High risk occupations made up 61% (1749/2844) of vaccinations, followed by low risk [26% (740/2844)] and medium risk [12% (355/2844)]. Frontline workers made up 4.7% (135/2844). The majority of vaccinations in South Sudan included high risk occupational groups, serving to protect vital occupations and prevent further transmission in the event of an EVD outbreak. In the future, preparedness efforts in countries at risk for EVD outbreaks should prioritize persons in high-risk occupations for vaccination.

0099

COMMUNITY-BASED SURVEILLANCE OF RESPIRATORY SYNCYTIAL VIRUS IN INFANT MORTALITY: PROTOCOL FOR MINIMALLY INVASIVE TISSUE SAMPLING STUDY IN PAKISTAN

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The burden of hospitalization and mortality of young infants due to respiratory syncytial virus (RSV) is estimated to be high, compounded by challenges in obtaining post-mortem specimens in religiously conservative community settings. To understand the underlying pathology of RSV and to improve classification of upper respiratory tract infections in deceased infants, minimally invasive tissue sampling (MITS) was incorporated into an ongoing parent study of community-based nasopharyngeal specimen collection from recently deceased infants. The primary study objective is to assess and analyze the burden and determinants of respiratory syncytial virus mortality by obtaining nasopharyngeal swabs and lung/thorax MITS specimens in deceased infants (<6 months of age) in peri-urban HDSS catchment areas of Karachi, Pakistan. Outcome measures include RT-PCR findings of nasopharyngeal swab specimens and lung tissue, and a histopathology report of lung tissue specimens, to improve classification of upper respiratory tract infections. Specimens were collected in a designated purpose-built vehicle parked outside community households. During the study formative phase, strategies to implement surveillance were formulated, such as using religious rulings and key community partners to help gain parental consent, and a purpose-built van was used in community-based specimen collection for infant mortality surveillance in Pakistan for the first time. Innovations included bringing the laboratory to the household and developing an in-house data program (e-CRFs, monitoring system through live dashboard and social media platform) for documenting death alerts and specimen collection. These measures allowed us to collect 13 lung/thorax tissue and 14 nasopharyngeal specimens between November 2020 and March 2021. There are many operational, cultural challenges in obtaining minimally invasive tissue specimens from deceased infants in the community. A designated vehicle can be utilized, along with community mobilizers, health workers and leaders advocating for the study procedure to obtain parental consent for lung/thorax MITS.

0100

ADDRESSING COVID-19 RUMORS AND BEHAVIORS USING THEORY IN GUYANA

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The USAID-funded Breakthrough ACTION project, in collaboration with the Guyana Ministry of Health, developed a theory-based rumour tracking system to inform the existing national COVID-19 communication campaign. Breakthrough ACTION used the Extended Parallel Processing Model (EPPM) to identify rumors that reflected perceived vulnerability to COVID-19 ("Is the rumour associated with a belief that people are vulnerable to COVID-19?") and self-efficacy to engage in recommended COVID-19 prevention behaviors ("Is the rumour associated with the belief that people feel enabled/able to take action to mitigate COVID-19?"). Rumors were categorized as high versus low perceived vulnerability and self-efficacy, respectively. Contextually relevant social and behavior change messages, called "MythBusters", were designed based on the EPPM rumor classification model. Most rumors emanated from Region 4 (29%) and Region 8 (25%). Two-thirds of the rumors (67%) were recurring. Rumours were typically related to COVID-19 treatment (32%) and transmission (31%). Most rumors (38%) reflected high perceived vulnerability and low self-efficacy, 29% reflected low perceived vulnerability and low self-efficacy, 24% reflected high perceived vulnerability and high self-efficacy, while 9% reflected low perceived vulnerability and high self-efficacy. A total of 38 MythBusters were developed to address these rumors. These were integrated into the national COVID-19 communication campaign, disseminated via radio, television, and Facebook, and are estimated to have reached the majority of the Guyanese population. The EPPM was found to be a particularly useful tool in giving direction on countering myths with appropriate messaging to affect relevant behaviours. The COVID-19 MythBusters provided the Guyanese public with valid and verifiable information in addition to promoting preventive and protective behaviours.

0101

IMPLEMENTATION OF MULTIPLEX BEAD ASSAY AT THE NATIONAL REFERENCE LABORATORY, NIGERIA CENTRE FOR DISEASE CONTROL, GADUWA, ABUJA-NIGERIA: A AESOURCE LIMITED SETTING

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Serologic assays can monitor population exposure to pathogens and population immunity estimates for vaccine-preventable diseases (VPDs). Multiplex bead assays (MBA) test for multiple analytes simultaneously, which has cost and time advantages compared to single-analyte immunoassays. We used dried blood spot (DBS) specimens collected from the Nigeria HIV/AIDS Indicator and Impact Survey (NAIIS), a nationally-representative sample of >200,000 people of all ages, to assess for exposure to 19 infectious diseases VPDs, and malaria antigens. We established an MBA Laboratory equipped with MAGPIX™ instruments at the National Reference Laboratory (NRL), Nigeria Centre for Disease Control (NCDC), in Gaduwa, Nigeria. A year of planning included development and approval of a study protocol, identification of support for funding from multiple sources, and establishing a laboratory workflow for high-volume antibody and antigen testing. DBS from children 0-14 years of age and a subset of women of reproductive age (WRA) were prioritized. Staff from US Centers for Disease Control and Prevention conducted site visits to NRL to assess infrastructure requirements and coordinate support. MBA laboratory leads were first trained on MBA laboratory management and then staff were trained on testing DBS using MBA. Infrastructure support, including equipment and supplies, was provided. A plan for ongoing technical assistance and logistical support, including monthly in-person site visits and routine shipments, was started in 2019. The plan was heavily modified in 2020 due to COVID mitigation efforts in Nigeria and the United States. Weekly videoconferences replaced site visits when travel was restricted. Within five months of starting testing, the NRL MBA Laboratory completed antibody and antigen assays for approximately 42,000 NAIIS specimens (~10,000 DBS from WRA and ~32,000 DBS from children 0-14 years). Data from these assays supported Nigerian requests for funding for the childhood immunization program and the next round of malaria program support from Global Fund. The remaining specimens from adults are currently undergoing analysis.

0102

FORMATIVE PHASE OF COMMUNITY-BASED SURVEILLANCE OF INFANT RESPIRATORY SYNCYTIAL VIRUS - MINIMALLY INVASIVE TISSUE SAMPLING STUDY IN PAKISTAN

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We conducted a community-based RSV mortality surveillance and minimally invasive tissue sampling study in deceased infants in low-income areas of Karachi, Pakistan. Out study objective is to explore the acceptability of minimally invasive tissue sampling (MITS) of lungs/thorax and nasopharyngeal swab collection from deceased infants under 6 months of age and stillbirths, in 2 peri-urban areas of Karachi. We conducted 21 in-depth interviews and 2 focus group discussions with key stakeholders, including parents of deceased infants, community leaders and elders, religious leaders, graveyard undertakers, bathers, burial good vendors, and physicians to ascertain perceptions and barriers regarding MITS. The stakeholders commented on the feasibility and appearance of a potential MITS collection van, that the van should be like a fully equipped ambulance. Most agreed that the sampling van could be parked outside households for sample collection. Formative phase findings indicated community fears about the MITS procedure involving removal of body organs from infants. Religious leaders recommended obtaining and using MITS religious rulings (fatwas) & seeking permission from different ethnicities/communities. Respondents also identified possible challenges in taking the infant body from household to van, since it is not religiously permissible to cause pain or harm to the deceased body, but parents would require counseling concerning the benefits of MITS to save upcoming generations. Respondents' beliefs, religious and community buy-in are the key to success of MITS surveillance phase. The deceased infant's parents appreciated grief support home visits, counseling and cause of death consultation visit by a doctor. This study showed bereaved parents want to know more about their child's cause of death. This study suggests community stakeholders' involvement along with healthcare professionals to explain MITS procedure and purpose of collecting MITS samples in a van in the community. Potential barriers and facilitators were identified at the community level, for local tailoring of exhortations for future MITS surveillance studies.

0103

IMPROVING VITAMIN A COVERAGE THROUGH INTEGRATION WITH SEASONAL MALARIA CHEMOPREVENTION: AN IMPLEMENTATION RESEARCH IN RURAL AND URBAN SETTINGS IN NIGERIA

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Vitamin A deficiency is associated with a 3 to 12-fold increase in mortality from acute gastroenteritis, pneumonia, and measles. Though vitamin A supplementation (VAS) can reduce all-cause under-five mortality by 24 percent, its coverage remains low in Nigeria (49%). Seasonal malaria chemoprevention (SMC), targeting children under-five in the Sahel region, including over 12 million children in Nigeria, provides a ready platform for VAS delivery to improve coverage and access. A preliminary feasibility study integrating SMC and VAS in one local government area (LGA) in northwest Nigeria in 2019 provided evidence for improved VAS coverage (from 2% to 59%, p-value =0.001). While it also demonstrated a good fit with the SMC infrastructure and high acceptability among providers and beneficiaries, there were other questions unanswered. A follow up study aims to provide more answers and strengthen the evidence to support policy adoption regarding full integration of VAS with SMC campaigns at scale, targeting twice as many children as the initial pilot. The study objectives are therefore, to design and implement, in collaboration with key stakeholders, an integrated SMC plus VAS campaign in diverse settings – urban and rural; develop and implement a research uptake plan; assess the feasibility (including effectiveness, equity, efficiency, safety and cost), and acceptability of integration among caregivers, field implementers, and policy makers. The primary research question is: what is the effect of full integration of SMC and VAS at scale on vitamin A coverage, SMC coverage, safety, equity, efficiency and cost? This implementation research will use a convergent mixed methods approach to arrive at an overall descriptive evaluation of feasibility and acceptability. The study is planned to take place between May and October 2021 in two LGAs, Bauchi State, Northeast Nigeria. The data sources will include cross-sectional pre- and post-intervention surveys with a sample size of 540 children aged 6-59

months; focus group discussions among health workers, caregivers and community distributors, and key informant interviews among key stakeholders.

0104

UNDERSTANDING INDIVIDUAL AND COMMUNITY CARE-SEEKING BEHAVIORS DURING COVID-19 PANDEMIC IN NIGERIA

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Understanding individual and community care seeking behaviours is important for maximizing receipt of critical health services. Factors that influence sub-optimal use of health services, especially during public health emergencies like COVID-19, should be determined to tailor behavioural interventions to promote continued prompt care seeking. A cross-sectional household (HH) survey was conducted in January–February 2021 among women of reproductive age in Benue and Plateau states, Nigeria, to assess predictors of intentions to seek care for fever during the COVID-19 pandemic. A compact segment sampling technique was used to select 500 HH from catchment areas of 50 health facilities (HF); one woman was interviewed per HH. Factors assessed were knowledge on fever care, risk perception, self-efficacy to seek care, attitude towards care-seeking, perception of outpatient department (OPD) providers, and community norms on care seeking. Individual factors and sample means of settlement clusters for community factors, including proportion of HH who normally seek fever care (0: < 25%, 1: ≥25% <50%, 2:≥50% <75%, 3: ≥75%), decision-making autonomy for fever care (0 = index respondents, 1= others), and community perceptions of HF safety during COVID-19 (0 = unsafe, 1 = safe) were included in a logistic regression model. We interviewed 500 women; 92.8% were married. Mean age was 31 years. Individuals' knowledge of fever care (OR = 1.31, p = 0.05), risk perception of fever illness (OR = 1.40, p <0.01), self-efficacy to seek care (OR = 1.58, p <0.01) and positive perceptions of OPD providers (OR = 1.28, p = 0.001) were positively related to intention to seek fever care. Those that perceived care seeking for fever as a community norm were more likely to seek fever care (OR = 3.20, p = 0.05). Fever care seeking behaviour was associated with individual- and community-level factors. Effective social and behavioural change programs in the era of COVID-19 may benefit from a holistic approach that addresses both individual and community level factors. In this study population, such an approach would engage women caregivers as well as their catchment HF and providers.

0105

NEGOTIATING MYANMAR'S LAW AND (DIS)ORDER AMIDST ANTIMICROBIAL RESISTANCE POLICY IMPLEMENTATION

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The WHO has declared antibiotic resistance (often referred to as antimicrobial resistance, AMR) an emergency and co-authored a Global Action Plan (GAP) to address it. Over a hundred countries have already followed the WHO's prescription and adopted their own national action plans. Myanmar, prior to the 2021 coup, was one of them and had actively promoted their NAP as the solution to the issue of AMR in Myanmar. Additionally, global health policymakers have identified Myanmar, like other countries in Asia, as a source of high drug resistance and informal pharmaceutical markets, in need of tighter state regulation. On paper, the Myanmar government appeared to be following the WHO's AMR GAP. However, in our article we show that in practice the realities are very different, this difference is further pronounced after the February 2021 military coup. We foreground historical and contemporary aspects of Myanmar and draw on extensive in-depth ethnographic research to explore how global plans for AMR, such as restricting access to antibiotics, when it merges with national action on drug regulation can end up dramatically deviating from the intentions of the WHO GAP and its values. Our paper argues that those working to promote the regulation of medicines must attend more carefully and explicitly to different modes of political governance, state sovereignty, histories of health systems and rule of law rather than uncritically and apolitically pushing state-centric programmes. Otherwise, they risk contributing to, if not intensifying, already existing health inequities and social injustices, whilst also failing to generate their intended outcomes, such as meaningful changes to antibiotic sales and reductions in resistance.

0106

PROTECTING HEALTHCARE PERSONNEL FROM COVID-19 INFECTION: ARE WE DOING ENOUGH? NIGERIA EXPERIENCE

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Health workers (HWs)' risk for contracting COVID-19 increases in settings with higher community transmission of COVID-19 and strained health systems. It is vital for health systems to protect HWs as they carry out their duties. We conducted an evaluation to assess measures for COVID-19 prevention among health facilities (HFs) and HWs in Benue (30 HF) and Plateau (20 HF) states in Nigeria. Various HF types (public, private, primary health centres, hospitals) supported by PEPFAR or PMI were purposively sampled. HF assessments of COVID-19 prevention measures were conducted in January–February 2021 using a checklist adapted from CDC infection prevention and control (IPC) tools. The HF in charge and 1-2 additional HWs providing clinical care were randomly selected, interviewed, and observed on COVID-19 prevention measures and compliance using pretested tablet-based questionnaires. Data were analysed using Stata 16. Only 27 of 50 HFs (54%) had an IPC committee, and 10 (20%) had written IPC policies. While 34 (68%) HFs had functional hand hygiene stations, only 7 (14%) had uninterrupted supplies of personal protective equipment (PPE). Of 199 HWs interviewed, 81 (41%) had been trained on IPC in the context of COVID-19; 112 (56%) reported continuous access to medical masks during patient consultations, and 163 (82%) had access to functional hand hygiene stations. Half (n=100) of the HWs interviewed perceived their HF had adequate PPE to keep them and their patients safe from COVID-19 exposure. Survey teams observed HWs during patient consultations and reported that all HWs practiced hand hygiene during patient consultations in only 28 (56%) HFs. Similarly, all HWs were observed wearing face masks in only 6 (12%) HFs. Overall, gaps existed in IPC administrative structures, HW trainings, access to COVID-19

IPC materials, and compliance with COVID-19 prevention measures among HFs and HWs in these two states of Nigeria. Additional support for establishing IPC committees, training HWs on IPC measures, and provision of continuous PPE and IPC materials is warranted to improve IPC practices in health care settings.

0107

FACTORS INFLUENCING INTENTIONS TO SEEK CARE FROM COMMUNITY HEALTH WORKERS FOR CHILDHOOD ILLNESS IN THE COVID-19 CONTEXT IN UGANDA—DECEMBER 2020

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Management of childhood illness by community health workers (CHW) is a critical component of healthcare in Uganda; unfortunately, care-seeking from CHWs declined during the COVID-19 pandemic. We conducted a cross-sectional household survey in Moyo and Adjumani Districts in December 2020 to assess caregivers' intentions to seek care for sick children, attitudes, and perceptions of CHWs, and community norms during the COVID-19 pandemic. We used composite scores to evaluate responses, logistic regression to identify factors associated with intentions to seek care, and Pearson's chi-squared test to compare proportions. Of 306 women (18-49 years) interviewed, intention to seek prompt care from a CHW was higher for fever (94%) than cough (67%; *p*-value<0.01). Most (93%) respondents perceived that CHWs provide high quality services; 89% were confident in CHW's COVID-19 preventive measures. The perception that they and their family were at risk for COVID-19 was higher among respondents intending to seek care for a child's cough than for fever (86% vs. 78%, *p*-value=0.03). Respondents intending to seek care recognized that cough (42%) and fever (47%) could have potentially severe consequences. Of those intending to seek fever and cough care, 87% and 80%, respectively, believed the majority of their community would also promptly seek care for similar symptoms (perceived norms). The multivariable analysis included COVID-19 risk perception, perceived symptom severity and community norms; intention to seek prompt cough care was significantly associated with COVID-19 risk perception (adjusted odds ratio (aOR)=2.8, 95%CI: 1.3-5.3) and perceived norms (aOR=3.0, 95%CI: 1.4-4.7). Intention to seek prompt fever care was not significantly associated with any explored variable. Despite overall positive regard for CHWs, including their COVID-19 prevention measures, there is a behavioral gap in prompt care-seeking from CHWs, particularly for cough. Social and behavior change interventions highlighting risks of COVID-19 and promoting community norms of prompt care-seeking could help to close this gap and improve the health and safety of communities.

0108

COVID-19 SEROLOGY CONTROL PANEL FOR SEROSURVEILLANCE AND CALIBRATION OF IMMUNOLOGICAL TEST PLATFORMS

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Currently, there are >60 serology diagnostic tests on the FDA EUA list and 70 serology test kits in the CE-marked European market. A review of 200 serosurveillance papers showed there are many more laboratory-developed tests being used in clinics and research laboratories. With so many tests in use and lack of standardized material to evaluate their performance, it is imperative that we prioritize quality calibration of test performance, especially in low resource settings. In summer of 2020, the University of Colorado developed the COVID-19 Serology Control Panel (CSCP), a kit of five well-characterized dried tube specimens with strong/weak/negative SARS-COV-2 reactivity for laboratories to use: (1) to evaluate SARS-SoV-2 serology platforms, (2) as a training resource, and (3) as an inter/intra-laboratory comparator. The CSCP materials were sourced from pooled donor convalescent plasma and characterized by multiple test platforms (ELISA, multiplex bead array, focus reduction neutralization, and pseudovirus neutralization) against target antigens (S, N, RBD). The CSCP kit contains 5 coded samples: strong/ reactive, weak reactive and non-reactive. All dried CSCP samples retained reactivity for up to 6 months after continuous storage at -20°C, 4°C, and 25°C. The strong reactive CSCP sample had equivalent reactivity to the WHO International Standard (NIBSC 20/136).. Samples were stable at ambient temperature for international shipments to laboratories in Australia, Africa, and Asia. Preliminary CSCP results from 15 laboratories showed 88%, 70% and 90% inter-laboratory concordance respectively for the strong/weak/negative samples. Widespread use of the CSCP to compare COVID-19 antibody tests will help laboratories reinforce confidence in their results and deter other laboratories from poorly performing tests. Additionally, the CSCP will help clinical laboratories inform their decision when choosing COVID-19 antibody tests. The CSCP could serve as the connecting calibrator samples in vaccine efficacy and serosurveillance studies.

0109

THE EFFECT OF NOVEL SCABICIDES ON SCABIES-ASSOCIATED BACTERIAL PATHOGENS

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Scabies has a prevalence of approximately 300 million cases, and is one the most common infectious dermatological diseases. As of 2017 it is recognised as a neglected tropical disease by the World Health Organization. Prevalence is high in tropical regions where there is an established link between scabies and severe secondary bacterial infections. Research has demonstrated that scabies mites promote opportunistic bacterial infections, especially with *Staphylococcus aureus* and *Streptococcus pyogenes*. As scabies is a neglected disease the molecular biology of these mites and their associated pathogens is poorly understood. We aim to provide the fundamental knowledge required to deliver better treatment outcomes to patients, and to understand the role scabies mites play in severe secondary bacterial infections. This research investigated the antimicrobial effects of emerging scabicides on the most relevant scabies pathogens. Using the broth micro dilution method, the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration of these drugs was determined for 3 strains each of *S. aureus, S. pyogenes and A. baumannii.* The strains investigated were selected to provide an overview of clinical, laboratory and pathogenic strains. The research found that Manuka Oil and its Beta-Triketones exhibit antimicrobial activity on *S. aureus, S. pyogenes* and *A. baumannii.* The MIC for these pathogenic bacteria are lower than the effective concentration for scabies mites and eggs. The emerging head lice treatment Abametapir also exhibited antimicrobial activity against gram positive bacteria but had limited effectiveness on gram negative bacteria. Pre-clinical trials are underway to determine the effectiveness of Abametapir and Manuka Oil on scabies mites, and on the associated microbial communities to determine the effectiveness of these drugs at controlling scabies-associated secondary bacterial infections. This research will help to determine new effective treatment protocols for the control of scabies and its associated diseases.

0110

SURVEILLANCE GAP ANALYSIS AND ECOLOGICAL NICHE MODELING OF MEDICALLY IMPORTANT TICK DISTRIBUTIONS IN EAST AFRICA

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Tick-borne disease outbreaks impacting human and animal health are a growing concern in East Africa. Effective disease mitigation depends on accurate vector distribution data, elucidation of variables governing habitat suitability, and development of robust ecological niche models (ENMs) to accurately predict disease risk and inform health policy decision-makers. A comprehensive distribution compendium detailing tick distributions in five East African countries (Chad, Djibouti, Ethiopia, Kenya, Uganda) was generated using surveillance data produced by our project, VectorMap records, and a systematic online literature search of over 300 published papers. Collection records represented 721,628 counts of 105 tick species distributed in 1358 unique occurrences in 30 of the 52 collective states of these countries. *Amblyomma variegatum* and *Rhipicephalus appendiculatus* accounted for 11% of total observations and 39% of total abundance, respectively. Tick occurrence and abundance rates were highest in Ethiopia (45%) and Kenya (52%), respectively, and lowest in Chad (0.2% and 0.1%). Species diversity was highest in Kenya (based on incidence) and Djibouti (based on abundance) and lowest in Chad, likely reflecting limited historical faunal surveys. Surveillance gap analysis identified key sites for future surveillance to increase sample size, minimize sampling bias, and provide a geospatially equitable distribution of sampling effort for future modeling analyses. Significant topographic and bioclimatic covariates governing habitat suitability were identified for each targeted medically important species. Statistically robust ENMs were developed using species-specific covariates in the MaxEnt maximum entropy model to generate habitat suitability maps. Model predictions reflected the geospatial distribution of the collection records. Regions of low predicted suitability may be due to unfavorable environmental conditions and/or inadequate sampling effort. Sampling bias can be alleviated iteratively by sampling targeted sites, augmenting collection records for modeling, and conducting follow-up gap analyses.

0111

SEROPREVALENCE OF SPOTTED FEVER GROUP AND TYPHUS GROUP RICKETTSIAE IN THREE INDIGENOUS COMMUNITIES OF THE PERUVIAN AMAZON

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Spotted fever group (SFG) and typhus group (TG) rickettsiae are widely distributed vector-borne bacteria. Infection results in an acute undifferentiated febrile illness. In Peru, rickettsioses are seldom recognized - likely due to their undifferentiated signs and symptoms and lack of testing capability. We conducted a study to determine the seroprevalence of SFG and TG antibodies in an indigenous population in the southeastern Peruvian rainforest. An evaluation of a Water-Sanitation-Hygiene Education program was performed in three communities (Yomibato, Tayacome and Huacaria) of the southeastern Peruvian rainforest in October 2019. Asymptomatic children and adults were asked to answer a health questionnaire, provide a stool sample to evaluate for helminth infections, and a blood sample to evaluate for anemia. One drop of this blood was blotted on Whatman paper, left to dry at room temperature, and packed individually. The dry blood blots were identified only by the subjects 'code and sent to the University of Texas Medical Branch for serologic analysis for rickettsiae. The subjects belonged to the Matzigenka ethnic group living in isolation inside the Manu Natural Reserve. Sera was eluted by cutting a 4 mm punch from each blood blot and placing in 250 µlof phosphate buffered saline at 4°C overnight. Samples were tested for SFG and TG antibodies using the indirect immunofluorescence assay (IFA). Samples were considered reactive if titers were ≥ 1:128. Sera from 352 participants were tested by IFA. The mean age was 15.9, females represented 52.1%, and 59.8% were from the community of Yomibato. SFG antibodies were detected in 4/352 (1.1%). From reactive samples, 2 were from Yomibato and 2 from Tayacome communities. None of the specimens demonstrated reactive TG antibodies. In conclusion, in this indigenous young asymptomatic population from the Peruvian Amazon, we found a low but demonstrable seroprevalence to SFG rickettsiae. We found no evidence of seropositivity to TG organisms. Additional studies to determine circulating rickettsial agents and vectors in this region should be pursued.

0112

RNA INTERFERENCE IN SARCOPTES SCABIEI EGGS: A USEFUL TOOL TO FIND NOVEL OVICIDAL DRUGS

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Scabies is a parasitic skin disease caused by the burrowing mite *Sarcoptes scabiei*. It is a worldwide problem with high morbidity and the secondary complications caused by pathogenic bacteria can be life threatening. Current treatments for scabies are suboptimal, mainly due to their lack of ovicidal activity. A female mite lays 2-3 eggs per day and this is the main amplification step in the scabies mite life cycle. RNA interference (RNAi) is a powerful tool to investigate genes encoding proteins that can potentially be targeted in therapeutic intervention. RNAi has not been widely established for parasitic mite eggs. Here we establish a method to introduce double-stranded RNA (dsRNA) into scabies mite eggs and induce gene silencing. Eggs were treated with sodium hypochlorite to increase the eggshell permeability. Double stranded RNA (dsRNA) intake was determined using fluorescent tagged dsRNA. *S. scabiei* Deadpan, a single copy gene with predicted functions in embryo development, was used as the target gene. Eggs were incubated with 2.5 µg/µl of dsRNA encoding a fragment of the target gene or the *E. coli* LacZ gene (negative control) at room temperature for 24h. Gene expression was quantified by quantitative PCR and the data were normalised with expression data of the *S. scabiei* EF1alpha gene. The results were analysed by student t test. RNAi-treated eggs were also incubated at 37^0C for 3 days to observe hatchability. Eggs immersed in *S. scabiei* Deadpan dsRNA showed significant reduction ($p<0.05$) in gene expression (21913± 7536 copies/µl) compared to the negative control (105620± 25190 copies/µl). In addition, 46.67 ± 7.265% hatchability reduction was observed when incubated with Deadpan dsRNA compared to the negative control. Our findings suggest that the RNAi can be achieved in the scabies mite eggs *in-vitro*. The *S. scabiei* Deadpan gene is potentially contributing to egg hatching and may bea therapeutic target.

0113

ANALYSIS OF IGG RESPONSES TO THE 34K2 SALIVARY PROTEINS FROM AEDES ALBOPICTUS AND AE. AEGYPTI TO ASSESS HUMAN EXPOSURE TO ARBOVIRAL VECTORS

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The rapid world-wide spread of *Aedes albopictus*, a vector of arboviral diseases like chikungunya, dengue or Zika, points up the need for better

vector surveillance and control. Human antibody response to mosquito salivary antigens is emerging as a relevant additional tool to directly assess vector-human contact, monitor efficacy of control interventions and evaluate the risk of arboviral transmission. Recently, we showed that IgG responses to the *Ae. albopictus* 34k2 salivary protein (al34k2) appear suitable to evaluate seasonal and spatial variations of human exposure to *Ae. albopictus* in conditions of natural exposure in a non-endemic area of Italy. The aim of this study was the validation of the al34k2 antigen in epidemiological settings with ongoing arboviral transmission maintained by *Ae. albopictus*. ELISA were used to measure human IgG responses to the al34k2 in adults from an area of Reunion Island where *Ae. aegypti* is absent and *Ae. albopictus* represents the unique vector of chikungunya. In addition, to check the specificity and/or cross-reactivity of this biomarker, we also analyzed the IgG responses i) of the same individuals from Reunion Island to the orthologous 34k2 salivary protein from *Ae. aegypti* (ae34k2) and ii) of Bolivian subjects, only exposed to *Ae. aegypti*, to both al34k2 and ae34k2. A group of French individuals, not exposed to either *Ae. albopictus* and *Ae. aegypti*, was used as control group. Individuals from Reunion showed significantly higher IgG responses to al34k2 than to ae34k2 validating this antigen as a good and specific marker of human exposure to *Ae. albopictus* in an endemic area. In contrast, IgG responses to the ae34k2 showed in both areas a low specificity and yielded a relatively high background, even in unexposed controls. These results provided a clear evidence that IgG responses to al34k2 may represent a suitable marker of human exposure to *Ae. albopictus*. On the contrary, the *Ae. aegypti* orthologue ae34k2 does not appear suitable as marker of human exposure to *Ae. aegypti* due to the unspecific IgG response and a high background.

0114

PRELIMINARY BLOOD MEAL ANALYSIS OF CULICOIDES SPECIES IN LEISHMANIA (MUNDINIA) ENRIETTII COMPLEX-ENDEMIC REGION IN GHANA

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Cutaneous leishmaniasis (CL), since 1992, has been endemic in the south-eastern part of Ghana. During the periods 2002 and 2003, a total of 8876 human leishmaniasis were recorded in this endemic focus. The *Leishmania* parasites have long been known to be transmitted by female phlebotomine sand fly vectors. However, in recent laboratory models of infection studies, the new species of *Leishmania* belonging to the *Leishmania* (*Mundinia*) *enriettii* complex found in Ghana does not survive in sand flies but rather, multiply and develop successfully in *Culicoides* species. *Culicoides* species are distributed worldwide and are capable of transmitting several infectious agents including parasites. In this study we analyze the hosts feeding preferences of *Culicoides* found in the leishmaniasis endemic foci in Ghana to expedite the study of a member of *Leishmania* (*Mundinia*) *enriettii* complex transmission routes and vector implication. The biting midges were collected in August to December 2019 at two weeks intervals using CDC light traps and sweep nets. A total of 185 midges were collected, pooled together in groups of ten and subjected to DNA extraction. Molecular analyses were conducted using the universal mammalian primers (Mammal-F, Mammal-R) in the meantime. PCR revealed that 34.7% of flies collected fed on mammalian hosts. The primers amplified the 772bp band size which is the identical to the size of the mammalian amplified product. This result indicates that the *Culicoides* analyzed so far prefer mammalian host for blood meal. The preliminary results obtained indicate the host preferences of some *Culicoides* species feed on mammals. We conclude that *Culicoides* species collected in the CL endemic communities can potentially serve as a vector for the novel *Leishmania* species found in Ghana. Keywords: Ghana, *Leishmania* (*Mundinia*) *enriettii* complex, *Culicoides* species

0115

INCRIMINATING CULICOIDES BITING MIDGES AS VECTORS OF HUMAN CUTANEOUS LEISHMANIASIS IN GHANA AND VALIDATION OF STANDARD LIGHT TRAPS AGAINST EXPLICIT HUMAN BITING RATES

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Human cutaneous leishmaniasis (CL) is a neglected tropical disease endemic to some communities in Ghana and is caused by a newly identified species of *Leishmania* belonging to the *L. enriettii* complex. The vector(s) responsible for transmitting human CL in Ghana remain unknown as very few sandflies have been collected in the various endemic foci in Ghana. Previous laboratory investigations have shown that the new species of *Leishmania* colonized and replicated successfully within the gut of *Culicoides* biting midges. In this study, we validated CO_2-baited CDC light traps against human landing catches (HLC) for *Culicoides* and sandflies caught in the endemic Ho district of Ghana and characterised the *Culicoides* fauna to incriminate species of *Culicoides* as vector candidates responsible for CL in Ghana. *Culicoides* biting midges and sandflies were collected from May 2020 to August 2020 using CO_2-baited CDC light traps and filtered mechanical aspirators for HLCs. Light traps and human volunteers were rotated around four collection locations using a Latin square design. A morphological key was developed for the identification of *Culicoides* midges collected. Statistical analyses were carried out using R to determine whether CO_2- baited light traps could be accurately used as a proxy for human biting rates. Significantly, more *Culicoides* were collected by HLCs than by the traps ($p<0.001$) showing that CO2-baited CDC traps do not accurately reflect biting rates of Culicoides on humans. *Culicoides grahami* (98.5%) were the predominant species identified from both CDC traps and HLCs. Other species were identified in very small numbers (1.5%) and included *C. imicola*, *C. inornatipennis*, and *C. leucostictus* . Therefore, *C. grahami* is proposed as the putative vector of *Leishmania* in the Ho District of Ghana.

0116

UTILITY OF MALDI-TOF MS IN SPECIES IDENTIFICATION AND BLOOD MEAL ANALYSIS

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Protein profiling using MALDI-TOF MS is a promising technology in the field of medical entomology. Studies have shown its ability to rapidly identify mosquito species and their associated blood meal sources. The technology overcomes challenges posed by the current methods used for entomological surveillance. The purpose of this study was to test the discriminative power of MALDI-TOF MS to distinguish between members of *An. gambiae* complex collected in coastal Kenya and their trophic preferences. Template mosquitoes obtained from the field and insectary were characterized using gold standard approaches. Proteins were extracted from legs and abdomens of individual mosquitoes for spectra acquisition and ClinProTools software was used for preprocessing and database creation. A total of 167 *An. gambiae* produced quality spectra

that were subsequently used for database creation and validation (query). These comprised of *An. merus* (143), *An. arabiensis* (n=34) and a pool of 45 *An. gambaie* s.s. (Kilifi strain) mosquitoes from the insectary were also processed. MALDI-TOF MS provided accurate identification of members of *An. gambiae* complex collected in the field including *An. arabiensis* (31/31) and *An. merus* (139/139) and insectary (*An. gambaie* s.s) (40/40). Analysis of abdominal proteins of blood fed mosquitos provided accurate identification of host sources. Majority of the samples had fed on Goat (n=63), Bovine and Human (n=6) and the rest (n=3) had fed goat-bovine. This study provides further evidence on the utility of MALDI-TOF MS approach for entomological surveillance by accurately identifying *An. gambiae* sibling species and their associated blood meals.

0117

SPOTLIGHT REPORTS: TICKS AND TICK-BORNE DISEASE THREATS IN ETHIOPIA

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Tick-borne diseases are a rising health concern across Africa. Understanding the geographic distribution of ticks at the country level can inform Ethiopian and international stakeholders about potential pathogens in circulation. Over 6,000 peer-reviewed articles from 1901-2020 were systematically screened for data meeting our inclusion/exclusion criteria. A total of 116 articles met final inclusion criteria with data extraction and geo-referencing, before being combined into a single database. Tick species identified came from seven different genera including five hard ticks (Amblyomma, Haemaphysalis, Hyalomma, Ixodes, and Rhipicephalus) and two soft tick genera (Argas and Ornithodoros). Fifteen species of Rhipicephalus ticks were reported, followed by 11 different species of Hyalomma ticks. The most commonly survey tick species was Amblyomma variegatum (Fabricius, 1794) with approximately 64% of articles detecting this species; with Rhipicephalus decoloratus Koch, 1844 and Rhipicephalus evertsi s.l. in 61% and 57% of studies, respectively. A total of 28 pathogens were reported in Ethiopian ticks, including medically important genera: Anaplasma, Borrelia, Coxiella, Ehrlichia, Haemoplasma, Rickettsia, Theileria, and Trypanosoma, along with four viruses (Congo, Dugbe, Jos and Thogoto). Amblyomma and Rhipicephalus ticks were the most commonly reported genera with identifiable pathogens. The data collected from this systematic review provides valuable information on historic high-risk areas for transmission of tick-borne disease, as correlated with tick vector distributions, and can be used to enhance ongoing surveillance and targeted sampling efforts.

0118

STUDY OF RED MEAT ALLERGY USING A MOUSE MODEL WITH BITES OF THE LONE STAR TICK, AMBLYOMMA AMERICANUM

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Recent studies have provided strong evidence supporting the Lone star tick bites as a cause of red meat allergy (RMA) in humans. RMA is caused by an increase of Immunoglobulin E (IgE) antibody production against galactose-alpha-1,3-galactose (aGal), which is a common glycan in mammals with the exceptions of old word monkey and human. The main factor causing the RMA, the lone star tick (*Amblyomma americanum*), is broadly distributed throughout the East and Midwest of the United States, which is also a vector of a wide range of human and animal pathogens. We previously found that the salivary glands of male and female ticks contained high levels of aGal epitopes after feeding on bovine blood. In our current work, we aimed to test whether the ticks mediate the transmission of the aGal sensitizer, acquired from non-human blood to humans through salivary secretions. In the course of tick feeding in the field, intrastadial host switches in completely fed ticks could occur as an episode of transmission of the aGal sensitizer. To test this hypothesis, we used an alpha-galactosyltransferase knockout mutant mouse (aGT-KO) model system, where untreated ticks (unfed) and pretreated ticks (partially fed with bovine blood) were placed. Using the enzyme-linked immunosorbent assay (ELISA), we measured the IgE and IgG levels, the total and specific antibody against aGal, after tick feedings with the pretreated ticks. Our results showed that aGT-KO mice significantly responded to tick feedings and injections of aGal (Galα1-3Galβ1-4GlcNAc) conjugated to human serum albumin or mouse serum albumin (aGal-HAS or aGal-MSA) by increased total IgE and aGal-specific IgE compared to those in C57BL/6 control mice. All the treatments by tick feedings with pretreated and untreated ticks functioned as sensitizer for increased specific IgE against aGal with large individual variations. This study confirmed that the aGT-KO mouse can be used as the model for RMA study while more study is required for understanding the individual variations in the immune responses to aGal.

0119

SPOTLIGHT REPORT: TICKS AND TICK-BORNE DISEASE THREATS IN CHAD: A CASE FOR INCREASED SURVEILLANCE

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Ticks and their associated pathogens pose a significant risk to animal and human health in the Sahel, where pastoralist lifestyles increase the interface and possibility of zoonotic spillover. To augment our surveillance activities in Chad, to develop effective risk assessments for tick-borne diseases of humans and livestock, additional high-quality surveillance data is needed to better document the distribution and pathogen-association of ticks in Chad. A systematic literature review was conducted to compile tick surveillance data from peer-reviewed literature. In this systematic review, terms were searched within titles and abstracts in PubMed, CABI, Web of Science, Scopus, with date limits of 1901–2020 in languages limited to English and French. Reported surveillance findings were standardized using

the VectorMap data schema and collection localities were georeferenced. This search generated 114 unique titles for literature in Chad, of which 35 articles met inclusion criteria for a full review and 2 articles produced extractable data meeting *a priori* inclusion criteria. These articles produced surveillance records for two unique taxa: *Hyalomma impeltatum* Schulze and Schlottke, 1930; *Rhipicephalus decoloratus* Koch, 1844 as well as an unconfirmed collection of *Hyalomma* spp. Limited pathogen detection data indicates TBDs may be found in Chad, however more surveillance is needed to confirm prevalence. While rigorous in approach, our literature search produced a limited output of useable data again highlighting the need for additional surveillance. Chad remains largely un-surveyed, increasing the importance of our current active biosurveillance efforts in the country.

0120

MOSQUITOES FROM CO-ENDEMIC AREAS OF MALARIA AND DENGUE IN CENTRAL EASTERN AND SOUTHWESTERN VIETNAM: BIODIVERSITY AND BARCODE

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A mosquito survey with focus on those recognized as vectors of malaria and dengue was carried out to assess the diversity of mosquitoes (Diptera: Culicidae) in Khanh Thanh and Cau Ba communes, Khanh Hoa Province (central-eastern), and Bu Gia Map and Dac O communes, Binh Phuoc Province (south-western) in October 2018 and March 2019. In order to confirm species level diversity, an integrated identification approach based on morphological diagnostic characters of adult mosquitoes using taxonomic keys, in conjunction with DNA barcoding sequences was conducted. A total of 4,702 specimens were collected, including 23 taxa. Among them, many specimens of the main dengue vectors, *Aedes* (*Stegomyia*) *aegypti* (Linnaeus) and *Ae.* (*Stg.*) *albopictus* (Skuse), and many secondary vectors were found in greater abundance. Although, fewer of the main malaria vectors, *An*. (*Cel*.) *dirus* Peyton and Harrison, and *An*. (*Cel*.) *minimus* Theobald were found. Sequences of the mitochondrial DNA (mtDNA), cytochrome c oxidase subunit I (*COI*) gene are presented for 42 individuals. *Anopheles dissidens* Taai & Harbach and *An. wejchoochotei* Taai & Harbach comprise new country records. Sequences of *COI* were useful to distinguish species complex/groups. The high density of secondary malaria vectors was not adequate to estimate the risk of malaria transmission. However, in both studied provinces malaria is still a problem, and dengue a major threat. Additional ecological and vector status studies are required to understand the importance of sympatric secondary malaria and primary dengue vector species, which may impact the implementation of strategies to prevent human infections of both diseases in Vietnam. This study provides diversity and molecular taxonomic information and emphasizes the significance on the use of molecular data as an integrated systematic approach in mosquito taxonomy.

0121

METAGENOMIC PROFILE OF THE MICROBIAL COMMUNITIES ASSOCIATED WITH ANOPHELES DARLINGI FROM NORTHWESTERN COLOMBIA

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Anopheles mosquitoes harbor microbial communities that carry out an essential role in their biology given the functional association with their host. *Anopheles darlingi* is the main malaria vector in the Neotropics, and one of the primary malaria vectors in Colombia. In this work, the metagenomic profile of the *Anopheles darlingi* microbiota was characterized in mosquitoes from an important malaria-endemic region of Colombia using RNA-seq methodology. Field collected *An. darlingi* were grouped into two pools of 15 specimens each. RNA was extracted, and rRNA depleted cDNA libraries were prepared. Sequencing was performed on Illumina NovaSeq at 60M PE reads. Sequencing quality was determined with FastQC, and host reads were excluded from analysis by mapping against the *An. darlingi* genome (A_darlingi_v1). The metagenomic classification was performed through *K-mer* matches (K = 31) against the One-Codex Database under the *Lowest Common Ancestor* concept (LCA). A total of 137,977,398 reads were obtained; 1,562,976 of these were classified as of microbial origin. Fungi comprised the highest number of reads (55.7%), followed by Bacteria (39.5%), Protista (4.8%), and Archaea (0.02%). The phyla with the highest number of reads were, in Bacteria: *Proteobacteria, Actinobacteria, Bacteroidetes*, and *Firmicutes*; Fungi: *Basidiomycota* and *Ascomycota*, and Protists: *Bacillariophyta* and *Apicomplexa*. Only 230 reads corresponded to Archaea with the phyla *Euryarchaeota* and *Crenarchaeota*. Furthermore, the sequencing depth and workflow implemented allowed identifying more than 800 microbial genera in the two *An. darlingi* pools. This work shows the first microbial profile for *An. darlingi* and confirms previous observations about the wide microbial diversity in mosquitoes. The results showed that metagenomic classification by RNA-seq allows a reasonable resolution of this mosquito microbiota composition. Determining the microbial-communities composition of disease vectors will allow future work to investigate the effect of these microorganisms on mosquito biology and the detection of vector control candidates.

0122

A HABITAT SUITABILITY MODEL FOR AMBLYOMM AVARIEGATUM (FABRICIUS, 1794) IN KENYA

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The tick *Amblyomma variegatum* (Fabricius, 1794) is an important vector of both human and animal diseases across Africa. In Kenya alone, numerous etiological agents of parasitic, bacterial, and viral diseases have been detected in this species. Thus, understanding the ecological parameters governing the distribution of this species is imperative to predict and mitigate disease outbreaks. This study investigated the potential distribution of A. variegatum in Kenya by creating an ecological niche model, using presence-only collection data compiled during a systematic literature review of data records (1901-2020), and accessing publicly available data resources such as VectorMap. A covariate significance analysis was performed to assess the relative significance of 25 environmental and climactic variables associated with predicting habitat suitability. Six significant covariates were determined through the covariate significance analysis: flow accumulation, soil demography, precipitation seasonality, isothermality, precipitation of wettest quarter, and

annual precipitation. These variables were used to model A. variegatum distribution in Kenya using the maximum entropy (MaxEnt) approach. Our results indicate distinct variation in habitat suitability and highlight the need for additional A. variegatum surveillance in some regions of Kenya.

0123

DISSECTING THE ROLE OF ORNITHINE DECARBOXYLASE IN SUGAR- AND BLOOD-FED AEDES AEGYPTI FEMALES

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We previously reported that ornithine decarboxylase (ODC) mRNA levels increased in the fat body of RNAi-driven xanthine dehydrogenase1-deficient *Aedes aegypti*, suggesting that polyamines play a key role in mitigating ammonia and free radical toxicity. To identify the potential role of polyamines in the regulation of nitrogen (N) disposal we performed several molecular and biochemical analysis on ODC (EC 4.1.1.17), an enzyme that catalyzes the decarboxylation of ornithine into putrescine, the first and rate-limiting step in the polyamine synthesis. First, we monitored the ODC gene expression in tissues dissected from sugar- and blood-fed mosquitoes by qPCR. We found that ODC transcript levels in fat body increased at 6 h post blood meal (PBM) and reached a peak of expression at 36 h PBM compared to control, whereas the relative abundance of ODC mRNA in midgut and Malpighian tubules increased between 12 h and 36 h PBM. These peaks of ODC expression coincide with the timing of intense blood meal digestion (12-24 h) and N waste synthesis and excretion (12-48 h). In thorax, the ODC mRNA abundance increased at 12 h and 24 h PBM and reached a peak of expression at 36 h PBM, whereas in ovary, ODC transcript levels increased at 72 h PBM and reached the highest abundance at 96 h PBM. Next, we monitored the ODC protein expression in mosquito tissues by western blotting using a custom-made *A. aegypti* ODC antibody. We observed a distinct ODC protein expression in different tissues dissected from sugar- and blood-fed mosquitoes during the first gonotrophic cycle. We are currently analyzing the phenotypes of ODC knockdown by RNAi. Mosquitoes injected with dsRNA-ODC started to exhibit a significant mortality at 48 h PBM compared to control group. A dramatic decrease in survival at 10 days post-injection was observed, with approximately 66% survival rate compared to the control group. At 22 days after dsRNA injection, 100% of dsRNA-ODC mosquitoes died. In addition, we have observed that ODC deficiency causes a reduction of N waste excretion and a decrease of female fecundity. Overall, our results indicate that ODC plays a critical role in blood-fed *A. aegypti* metabolism.

0124

FLYNAP (TRIETHYLAMINE) IS A SUITABLE ANESTHETIC FOR BIOLOGICAL ASSAYS IN MOSQUITOES

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Mosquitoes are often anesthetized when studying certain physiological and behavioral life-history traits. Cold and CO2 are commonly used sedatives for several insect species; however, these require samples to be continuously exposed to low temperatures or CO2, which can adversely affect their fitness. Some alternatives include chemical compounds such as chloroform and triethylamine (commercially known as FlyNap*). Chloroform is frequently used for entomological procedures and requires safety measures because of its toxicity, whereas FlyNap is a non-toxic component that has long been used in *Drosophila* spp. studies. However, the effectiveness and potential fitness cost of using these compounds for mosquito experimentation have been poorly evaluated. We analyzed the effectiveness of using a small volume (20µl) of chloroform or of FlyNap on a cotton ball placed over the mesh of a cup containing 30 mosquitoes and introduced into a glass container with a lid. Male and female *Anopheles gambiae* were independently exposed to one of the two chemicals at different exposure times (30 sec, 1 min, 5 min), and their recovery and survival rates were recorded over three days. Results from three biological replicates indicated that FlyNap-treated mosquitoes remained anesthetized for significantly ($P<0.05$) longer (2-5 hrs.) than chloroform-treated individuals (15 min-1 hr.) regardless of exposure time or sex. Also, survival was not affected by either sedative when exposed for 30 sec, however, increased mortality was observed when exposing mosquitoes to FlyNap for 1 or 5 minutes compared to shorter exposure times or any of the exposures with chloroform. Our results indicate that FlyNap is an alternative for mosquito anesthesia, with a significantly longer recovery time and no impact on survival when using 20µl for up to 30 sec compared to chloroform. The effectiveness of FlyNap and chloroform on other vector species (*Aedes aegypti, Culex quinquefasciatus*) as well as on forced mating and fecundity, fertility, and pupation rates in *Anopheles dirus* are currently being assessed. *Use of this product does not constitute an endorsement.

0125

THE IMPACT OF PARTIAL BLOOD MEALS ON MOSQUITO MIDGUT INTEGRITY AND ARBOVIRUS DISSEMINATION

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Studies of vector competence rarely consider the impacts that successive blood meals have on arboviral transmission by mosquitoes. *Aedes aegypti* mosquitoes readily feed more than once and often take partial blood meals. The effect this behavior has on viral transmission needs to be better understood and incorporated into models of mosquito-borne disease epidemics. Previously it was shown that *Ae. aegypti* infected with dengue virus (DENV) via a primary blood meal had earlier viral dissemination when given a second non-infectious blood meal three days later. Evidence suggests that gut distention during blood feeding leads to damage of the midgut basal lamina and faster viral escape. While mosquitoes are usually allowed to feed to repletion in the laboratory, mosquitoes in the wild are often interrupted and only acquire partial blood meals. Therefore, we examined the effects that partial blood feeding has on midgut basal lamina damage and DENV dissemination. To assess midgut basal lamina integrity, we performed a collagen hybridizing assay on cohorts of *Ae. aegypti* given either a full, partial or no blood meal. *Ae. aegypti* provided a partial blood meal had an intermediate degree of damage compared to fully engorged or naive cohorts. We also assessed whether the size of a second blood meal impacted damage and if damage accumulated across blood meals. Mosquitoes given a partial blood meal three days after an initial full feed had less midgut damage than mosquitoes given a full second blood meal, but more damage than the cohorts provided no additional blood meals. Thus, midgut damage appears proportional to distention and feeding volume and is not cumulative. Consistent with this, individuals provided a partial second blood meal had an intermediate early dissemination phenotype for DENV. This indicates that damage from a partial feed is sufficient to cause accelerated dissemination, further demonstrating the significance of sequential blood meals on arbovirus epidemiology. This work has strong implications for our understanding of disease transmission in the field and will be useful in creating more accurate models of viral spread and maintenance.

0126

A NEW MOSQUITO MIDGUT PROTEIN AGAP008138 FACILITATES PLASMODIUM FALCIPARUM TRANSMISSION IN ANOPHELES GAMBIAE MOSQUITOES

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Malaria is one of the most dangerous tropical infectious diseases: up to 229 million new clinical infections occur every year, with an annual death toll of about 500,000. The malaria mosquito vector, *Anopheles gambiae*, is largely responsible for *Plasmodium falciparum* transmission and spreading of malaria. Some mosquito midgut proteins are critical for malaria invasion, as the *P. falciparum* ookinetes travel through midgut endothelial cells and develops into oocysts between the endothelia and basal membrane. Hence, the discovery of a new key protein facilitating the invasion of ookinetes into the mosquito midgut will aid to uncover a molecular infection mechanism. To date, parasite transmission and the many specific pathways the parasite utilizes to gain access into the midgut, remain unclear and paradoxical. We hypothesize that parasitic malaria transmission to humans relies on the interaction with specific mosquito midgut proteins. Earlier groundwork by our lab revealed several protein candidates, including AGAP008138, that have shown to interact with parasites. In order to investigate the transmission potential of AGAP008138 and carry out further analysis, mosquitoes were infected with *P. falciparum* parasites and anti-AGAP008138 IgG at various concentrations. The oocysts and mosquitoes were dissected 7 days post-infection. The results demonstrated that IgG antibodies specifically target AGAP008138 and significantly reduce the number of oocysts as compared to the control. This shows that AGAP008138 assists in malaria transmission and could be a potent target for malaria control.

0127

X-RAY TOMOGRAPHY AS A USEFUL TECHNIQUE TO EXPLORE THE TIMELINE OF MOSQUITO PROBOSCIS DEVELOPMENT

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We characterized the morphological features of the head and the buccal apparatus of larvae, pupae and adult mosquitoes of the species *Aedes albopictus, Aedes aegypti* and *Anopheles stephensi* using X-ray Tomography. The datasets were acquired using a synchrotron light source. This approach represents a valid alternative to Transmission Electron Microscopy analysis, due to challenging embedding and sectioning into resins of the cuticular layer covering appendices of mosquito proboscis and pupal case. The adult mosquito proboscis is composed of an external sheet retracted during feeding, the labium, and of a fascicle of stylets which penetrates the host skin. The labrum forms the entry of the food channel, the hypopharynx carries the salivary duct, and two pair of mandibles and toothed maxillae are used to penetrate the host skin. How and when this apparatus forms during development is unknown. We collected a total of 48 datasets, 25 from *Aedes albopictus* (4th instar larva, 24 female pupae at different time points after pupation, 7 male pupae, female and male adults), 11 from *Aedes aegypti* (4th instar larva, 9 female pupae and the female adult), 12 from *Anopheles stephensi* (4th instar larva, 10 female pupae and the female adult). The datasets were analysed in both the absorption based and edge-enhanced radiography using Gridrec and Paganini reconstruction pipelines at the synchrotron facility. 3D reconstruction and segmentations have been carried out using Slicer 3D software. Our analyses provide new insights into the development of mosquito during pupal metamorphosis, particularly our focus was the mouth apparatus. We were able to compare male and female differential development and highlight similarity and differences comparing close-related (*Ae. aegypti* Vs *Ae. albopictus*) or distantly related species (*Aedes* vs *Anopheles*). Our study sets a step-forward in the study of mosquito development and the datasets that we have generated might be used to study other organs.

0128

TOWARDS A MALE LETHAL SEXING STRAIN OF ANOPHELES STEPHENSI MOSQUITOES FOR THE MANUFACTURE OF PFSPZ VACCINES

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Sanaria® PfSPZ Vaccine and PfSPZ-CVac vaccines are composed of aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ), and are manufactured using aseptically grown *Anopheles stephensi* female mosquitoes. Removing male mosquitoes at the embryonic stage using conditional expression of a male specific lethal gene will make the aseptic rearing process of immature mosquito stages up to 100% more efficient at no additional cost. We identified a number of unique regions on the Y-chromosome that are targets for CRSPR/Cas9 homology directed repair (HDR) to integrate lethal gene for male specific expression. As a first step, we generated a transgenic line of *A. stephensi* in which 2 *locus of* X (*lox*) sites and a fluorescent eye color marker DsRed, under the control of eye specific 3xP3 promoter, were integrated into the Y-chromosome. In this line, only male mosquitoes are fluorescent, suggesting Y-chromosome specific integration and male specific expression of DsRed. Work is in progress to integrate the lethal gene, dominant negative of the immune gene Relish 2 (*dnrel2*) which we have shown previously to be lethal to *A. stephensi*. *dnrel2* will be placed under the control of the Tet-on regulatory sequence, TRE (tetracycline responsive element), and integrated using *Cre/lox* recombination to the *lox* site to generate the effector line. In parallel, a driver *A. stephensi* transgenic line was created using *piggyBac* transposon-mediated integration of rtTA (reverse tetracycline-controlled transactivator) under the regulation of the early embryonic bZip1 promoter. Gene expression analysis of this driver line demonstrated that rtTA is expressed only in the early embryonic stages. To test the proof of concept, the driver line will be crossed with the effector line. In the resulting progenies, addition of oxycycline induces bZip1 promoter to express TRE in embryos which will drive the expression of the lethal *dnrel2* gene only in males, resulting in selective male embryo lethality, leaving female embryos alive for vaccine manufacturing.

0129

GENETICALLY ENGINEERED ENDOSYMBIONTS OF MOSQUITOES FOR USE IN PARATRANSGENESIS

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Paratransgenic control of vector-borne viruses and other infectious disease agents can be achieved through exploitation of the bacterial microbiome constitutively present in mosquito populations. Bacteria in the genus *Enterobacter*, among others, have previously been identified as symbionts of the midgut and salivary glands of *Aedes* mosquitoes. Appropriate implementation of *Enterobacter* as a paratransgenic tool requires further analysis of bacterial re-colonization within the mosquito post-genetic manipulation. We hypothesized that if a genetically modified version of *Enterobacter* or *Asaia* is capable of re-colonization of *Ae. aegypti* and/or *An. gambiae* midgut and salivary glands then expression of anti-viral effector molecules can disrupt viral transmission. *Enterobacter* and *Asaia* expressing fluorescent proteins were first screened for multi-organ dissemination within the mosquito. Female mosquitoes 7-10 days old, were exposed to either *Enterobacter* (mCherry) or *Asaia* (GFP) via 1)

blood, 2) protein-rich artificial, or 3) sugar meal. Mosquitoes positive for exposure were then collected at 3, 7, and 14 days. Mosquito midgut, crop, and salivary glands were removed, mounted, and viewed for bacterial colonization via microscopy. Our results demonstrate *Enterobacter* and *Asaia* dissemination is restricted to the midgut and crop when mosquitoes are exposed to infectious sugar meal. Analysis of exposure via blood or alternative protein sources is ongoing. Results of this study will inform future experiments seeking to understand the mosquito microbiome and develop paratransgenic tools to counteract spread of emerging viruses.

0130

WARBURG EFFECT IS REQUIRED FOR IMMUNITY IN MOSQUITOES

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Immune activities require immune cell proliferation. It has been shown in vertebrates that the Warburg effect, i.e., aerobic glycolysis, allows immune cells to turn pyruvate into lactic acid via lactate dehydrogenase (LDH) in the presence of oxygen rather than the traditional path of pyruvate to acetyl-CoA for the tricarboxylic acid cycle. Warburg metabolism provides intermediates and energy required for immune cell proliferation. In this study, we provided phenotypic and genetic evidence that Warburg metabolism is required for immunity in *Anopheles gambiae*, the malaria vector. First, the lactate abundance, measured by a lactate assay, was increased in mosquitoes which survived a hemocoelic infection via a bacterial challenge, as compared to the injury controls. Second, when the glycolic enzyme GAPDH was inhibited pharmacologically by the compound dimethyl fumarate, the antibacterial immunity was compromised as demonstrated by higher mortality upon hemocoelic infection with bacteria. Lastly, the genes *Gapdh* and *Ldh* were knocked down, and mosquitoes exhibited higher mortality upon bacterial challenge. With the pharmacological and genetic evidence, we conclude that the Warburg metabolism is crucial for mosquito immunity.

0131

THE WHOLE-GENOME SEQUENCING AND IMPROVED HYBRID ASSEMBLY OF TWO GEOGRAPHICALLY DISTINCT STRAINS OF THE MALARIA VECTOR ANOPHELES ALBIMANUS

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A principal vector of malaria in the Americas, *Anopheles albimanus*, exhibits diverse geographical distribution and phenotypic variation. Hence, a high-quality reference genome from geographically distant populations is essential is to understand and capture genetic variation in this species. In this study, we sequenced and assembled the whole genome sequences of two reference strains of *An. albimanus*, originating from El Salvador (STECLA) and Colombia (Cartagena, CART), using a combination of long-read PacBio and short-read Illumina-HiSeq technologies. Assemblies were scaffolded against a published reference assembly (AalbS3) using MaSurCA and gap-filled using TGS-GapCloser. Each assembly was then compared with the published reference genomes AalbS2 and AalbS3. Our hybrid assembly approach generated reference-quality genomes for each strain and recovered 96% of the expected genome size (173 Mbp). The genome assemblies of STECLA and CART consisted of 109 and 150 contigs, respectively, organized in 3 chromosomes, with estimated genome sizes of 167.5 Mbp (N_{50}=88 Mbp) and 167.1 Mbp (N_{50}=87 Mbp), respectively. They contained a smaller number of gaps and total missing bases than either of the two published reference genomes for this species. Previously generated RNA-Seq Illumina paired-end reads (18 Gb) were mapped to the assembled genomes, providing an overall higher mapping rate (STECLA: 98%, CART: 83.3%) than the previously published assemblies (AalbS2: 61.5%, AalbS3 : 59.8%), suggesting a considerable improvement in the quality and completeness of the assemblies. In addition, unreported complete mitogenomes of both CART (14 Mbp) and STECLA (15 Mbp) were recovered. Ongoing analysis of transposable elements, gene family characterization and SNP analyses will provide a comprehensive picture of genetic variation between the two strains. Taken together, these preliminary results suggest that the newly assembled genomes of the two geographically distinct *An. albimanus* strains are of high quality and will serve as key resource for future research in genetics and genomics of this important malaria vector.

0132

FINE-SCALE POPULATION GENETIC STRUCTURE OF ANOPHELES VECTORS OF MALARIA IN PAPUA NEW GUINEA: PANMIXIA, HIGH GENE FLOW AND GENETIC BOTTLENECK FOLLOWING AN INSECTICIDAL BEDNET CAMPAIGN

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Villages in coastal and inland regions of northern Papua New Guinea are long term study sites for malaria transmission and control through implementation of long-lasting insecticidal bednets (LLIN). *Anopheles* vectors in villages within these regions may represent isolated populations which respond differentially to such interventions. To address this question, population genetic structure of *Anopheles farauti* sampled from 5 coastal villages in Sumkar district, Madang province from 2010 to 2017 and *Anopheles punctulatus* from 4 hilly inland villages in Drekikier district, East Sepik province from 2008 to 2010 were analyzed using microsatellite allele frequency. Analysis of molecular variance and Bayesian clustering method were used to infer population structure and number of distinct populations. Gene flow among villages was estimated based on effective number of migrants per generation using Wright's formula $N_m = (1\text{-}F_{st})/4F_{st}$, where F_{st} is fixation index. Genetic bottlenecks were assessed with tests of excess heterozygosity. Results show that *An. farauti* in Sumkar comprised a single panmictic population with high gene flow (N_m: 31-125) between villages. Comparison of this population to one from Kivori, Central province revealed population structure and low gene flow ($N_m < 4$) between the two geographically isolated regions (500 km apart). Panmixia and high gene flow was also observed in *An. punctulatus* both before and after LLIN distribution, however, gene flow among villages dropped significantly after LLIN from a mean N_m of 37 ± 5 to 17 ± 2 (*t*-test: t = 3.8, df = 6, P = 0.009). Significant excess heterozygosity was observed for *An. punctulatus*, indicating a genetic bottleneck associated with the LLIN, which was not observed for *An. farauti*. These findings show that the demographic impact of LLIN was stronger on *An. punctulatus* than *An. farauti*. Panmixia and high gene flow may enable emergence of phenotypes such as insecticide resistance to spread rapidly in the region. Discussion of factors affecting heterogeneity in behavior and ecological attributes of malaria vectors can be made with knowledge of their underlying population structure.

0133

GENETIC VARIABILITY WITHIN POPULATIONS OF ANOPHELES COUSTANI FROM MADAGASCAR

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Malaria remains a major public health concern in Madagascar despite multiple malaria control and prevention measures. Aside from the primary malaria vectors (*Anopheles gambiae s.l.* and *Anopheles funestus*), the anthropophilic tendency of secondary or local vectors, primarily zoophilic, suggests their potential contribution to malaria transmission. Secondary vectors, such as *Anopheles coustani*, are thought to be of minor importance and thus understudied in terms of population structure and vectorial competence, despite being found infected with *Plasmodium* in many African countries. The current study is a preliminary molecular analysis to investigate the presence of distinct genetic populations or even sibling species within Malagasy *An. coustani* and potential link with different roles in malaria transmission in Madagascar. Morphologically identified *An. coustani* mosquitoes were collected in two villages (Ambohitromby and Miarinarivo, Maevatanana district) in northwestern Madagascar using human landing catches (HLC) and catches in zebu parks. Additional samples came from a zebu park in the eastern part of the country (Vavatenina, Toamasina). The genetic diversity of twenty-two *An. coustani* was explored using the mitochondrial cytochrome c oxidase subunit I (COI) and the ribosomal internal transcript spacer region 2 (ITS2) markers. Our current analysis based on COI sequences revealed the presence of two phylogenetic groups of *An coustani*, supported by mutation signatures. These two groups included mosquito samples collected in each of the three geographic areas surveyed and were collected using different collection methods. No variation of ITS2 sequences was found in the current study. Our current data suggest the existence of differentiated *An. coustani* populations in Madagascar. To refine these findings and properly determine genetic variations within this species, additional mosquito specimens from other locations of Madagascar, as well as different collection methods, will be analyzed.

0134

AEGYPTI ATLAS: A NEW TRANSCRIPTOMIC RESOURCE REVEALS DETAILED FUNCTIONAL ORGANIZATION OF THE MOSQUITO BODY AND GUT

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Mosquitos are important vectors for human diseases. A clear understanding of mosquito physiology is required to develop informed genetic manipulations and potentiate the development of new strategies of control. Large scale transcriptomics are instrumental both to understanding mosquito physiology and engaging in reverse genetic approaches. I will introduce Aegypti Atlas, a new open-source online database hosting transcriptomes of body parts (head, thoracic carcass, abdominal carcass, gut, Malpighian tubules, ovaries) and gut regions (crop, proventriculus, anterior midgut, posterior midgut, hindgut) of female *Aedes aegypti* mosquitoes, as well as a timeseries of transcriptomes from the blood-fed gut. I will present biological messages from these data encompassing the dynamics of the blood-meal response, the organization of immune defenses, and a cross-species comparison of regional function in the midguts of *Ae. aegypti* and *An. gambiae* mosquitoes.

0135

APPLICATION OF METAGENOMICS AND METABARCODING TO CHARACTERIZE RNA VIRUSES CIRCULATING AMONG BITING MIDGES (CERATOPOGONIDAE) AND RELATED DIPTERA IN KENYA

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Vector-borne diseases account for more than 17% of all infectious diseases worldwide and are responsible for over 700,000 deaths each year. The mitigation and response strategies to fight vector-borne diseases are heavily reliant on surveillance programs. These programs enable early detection and control of vector-borne diseases. Vector surveillance programs rely on insect trapping, taxonomic identification, and subsequent pathogen screening in the trapped vectors. The traditional methods of screening the trapped vectors are prohibitively expensive. In this study, we used metagenomics and metabarcoding as relatively low-cost approaches to identifying RNA viruses and their vector hosts among biting midges from various locations in Kenya. Midges were collected from arid and semi-arid areas of Kenya including Baringo, Kacheliba, Turkana, and Isiolo. The specimens were collected using CDC light-traps that were set near resting places for livestock in the afternoon and collected the following morning. The collected specimens were transported to the laboratory then sorted into pools of less than 50 specimens each, then kept in the freezer for subsequent processing. They were screened for RNA viruses using metagenomics approaches. Simultaneously, the specimens were taxonomically characterized using the metabarcoding method. We detected a total of 15 phylogenetically distinct viruses. These viruses are classified into five different families, with one virus falling under the recently proposed negevirus taxon. The 5 virus families include partitiviridae, iflaviridae, tombusviridae, solemoviridae, totiviridae and chuviridae. In addition, we identified many vector species that were possibly associated with the identified viruses. The Ceratopogonidae family contributed the majority of the vectors identified. Others included the Chironomidae and Cecidomydiae families. In conclusion, we successfully characterized 15 RNA viruses from Kenyan midges and demonstrated the simultaneous application of metagenomics and metabarcoding methods as cost-effective approaches to virus surveillance and vector characterization.

0136

COMPARATIVE TRANSCRIPTOMICS REVEALS IMMUNE REGULATION SPECIFICITIES IN TWO MAJOR MOSQUITO VECTORS

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Mosquitoes are responsible for the transmission of various tropical diseases pathogens. Decreasing their abilities to host and transmit them is a promising strategy to eradicate these diseases. Such approaches will require an extensive knowledge of the mosquitoes' immune systems, of how they interact with human pathogens and of how it affects overall host physiology. Until now, the study of mosquitoes' immunity has mainly focused on specific aspects that are directly involved in defense against major human pathogens. However, our basic knowledge of mosquitoes' immune systems and of their interactions with other physiological components remains very fragmented. I will present the first step in a more global project aiming at characterizing the response to microbial challenges of two major mosquito vectors: the arbovirus-transmitting species *Aedes aegypti* and the malaria vector *Anopheles gambiae*. It

consists in a detailed and comparative analysis of their transcriptomic responses to diverse immune challenges, including sterile wound as well as injections of live and dead microbes (fungi, Gram-negative bacteria and Gram-positive bacteria). I will describe the repertoires of immune and non-immune responses identified in each species, their respective dynamics as well as their differentiations across the conditions. These results establish key features of the two mosquitoes' responses to immune challenge and reveal unexpected and specific modes of regulation of their immune responses.

0137

YEAST INTERFERING RNA LARVICIDES TARGETING CONSERVED FEMALE SPECIFIC LARVAL LETHAL LOCI FACILITATE SEX SEPARATION IN MULTIPLE SPECIES OF DISEASE VECTOR MOSQUITOES

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Although clusters of sex-specific loci are believed to shape the boundaries of the M/m sex-determination locus of the dengue vector mosquito *Aedes aegypti,* the identities of these genes are not yet known. Female-specific larval lethal screens uncovered multiple loci adjacent to the M/m locus that are required for survival of female larvae. Larval consumption of *Saccharomyces cerevisiae* (yeast) strains engineered to express interfering RNA corresponding to these genes resulted in significant female death yet had no impact on *A. aegypti* male survival or fitness. Moreover, silencing orthologs of these M/m locus region genes in other mosquitoes, including *Aedes albopictus, Anopheles gambiae,* and *Culex quinquefasciatus,* revealed a conserved female-specific larval requirement for these genes among different species of mosquitoes. Integration of the female-specific yeast larvicides into mass culturing protocols permitted scaled production of fit adult male mosquitoes, suggesting that RNAi-based larvicides could benefit mosquito population control strategies that require mass rearing and release of adult males. These studies, which provide insight into the evolution of mosquito sex chromosomes, indicate that sex-specific yeast interfering RNA larvicides may facilitate sex separation in multiple species of disease vector mosquitoes.

0138

HERPES SIMPLEX VIRUS GENE VARIANTS AMONG ASYMPTOMATIC WOMEN IN GHANA: PROOF-OF-CONCEPT STUDY

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Herpes simplex virus infections account for a large burden of disease worldwide. HSV-1 is traditionally considered to cause orofacial infections, whereas HSV-2 is known for genital infections. Several studies have suggested an increase of genital herpes infections caused by HSV-1. As reporting of diseases caused by herpes simplex virus is not mandatory in Ghana, reliable statistics on the epidemiology of infections are not available. We took advantage of the Cervicare program in Ghana to screen for the presence of HSV variants 1 and 2 among a convenient subset of asymptomatic women presenting for cervical screening in Accra, Ghana (n=94). Genetic markers for both HSV 1 and 2 were detected in cervical swabs. There was a preponderance of HSV-1 (12.8%) genital infections in our study sample: compared to HSV-2 (4.8%). HSV-1 and 2 co-infection was detected in 4.3% of study population. Among positive cases for HSV-1 DNA, 92% had confirmed seropositive HSV-1 status and 8% were borderline result. All positive HSV-2 DNA were confirmed seropositive HSV-2 status. We have successfully demonstrated the presence of herpes simplex virus type 1 and type 2 gene variants in genital swabs. Owing to the lack of epidemiological data on genital HSV-1 infection in Ghana, the role of sexual transmission for HSV-1 is unclear: the findings of our pilot study have important public health implications. A bigger surveillance study is recommended in Ghana to identify the etiology of genital herpes and estimate the true burden of asymptomatic herpes infection in the population.

0139

A DIAGNOSTIC METHOD FOR SPECIFIC DETECTION OF ALPHAVIRAL EXPOSURE

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In Darién, Panama, arboviral diseases are on the rise and thus a target for epidemiological investigation. Alphaviruses such as Venezuelan equine encephalitis virus (VEEV) and Madariaga virus (MADV, previously known as South American equine encephalitis virus) are of particular public health interest. VEEV has been endemic as a human pathogen in the country since 1960, while is MADV actively emerging in the region of Darién. One method of alphaviral disease surveillance is through assessing population exposure to these viruses. A current method of detecting alphaviral exposure is through Enzyme-Linked Immunosorbent Assay (ELISA) that utilizes whole virus as the antigen. This method is apt at determining general alphaviral exposure but is lacking when there is a need to distinguish between viral species. Plaque reduction neutralization tests (PRNT) are the gold standard for species-specific diagnosis, but require high levels of technical expertise, biosafety, and time. We set out to develop a species-specific IgG diagnostic method to detect alphaviral exposure. We utilized 65 samples from a Panamanian cohort, comparing VEEV and MADV results for whole virus ELISAs as well as PRNTs. For the antigen, we utilized VEEV IAB and MADV recombinant envelope protein (Mapp Biopharmaceutical, Inc.). Results were plotted and a diagnostic cutoff was determined for each test, where both sensitivity and specificity were maximized. MADV assay sensitivity increased from 77.8% to 88.9%, while specificity increased from 74.5% to 91.5%. For the VEEV assay, results demonstrate a decrease in sensitivity from 100% to 95.7% and specificity from 95.1% to 92.9% These results imply superiority of the modified recombinant protein ELISA for MADV exposure detection, while the whole virus ELISA remains superior for detection of VEEV exposure. Moreover, in the modified ELISA, an overall decrease in species cross-reactivity was observed. These findings pave the way for a new, species-specific standard for detecting alphaviral exposure.

0140

SPATIOTEMPORAL OVERLAPPING OF DENGUE, CHIKUNGUNYA, AND MALARIA INFECTIONS IN CHILDREN IN KENYA

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Malaria, chikungunya (CHIKV), and dengue (DENV) are recognized as endemic causes of fever among children in Kenya. These infections have

a transmission cycle sustained by different mosquito vectors present in human settlements. The high resolution overlapping of these diseases and factors affecting their spatial heterogeneity has not been investigated in Kenya. We conducted this study to investigate the spatiotemporal pattern of mosquito-borne infections as well as factors linked with exposure risks in Kenya. From 2014 through 2018, we prospectively followed a cohort of children (1-12 years of age) every 6 months recruited from four communities in both coastal and western Kenya. After enrollment the children were followed every 6 months and demographic data such as travel, GPS data, activity, symptoms, and household factors were collected. At each visit demographic surveys were administered, and blood was collected and tested by IgG ELISA to CHIKV and DENV and by microscopy to identify malaria-positive subjects. A local hot-spot analysis test was performed to identify areas with a high number of cases for the three diseases. Kendall's concordance test was applied to compare the spatial pattern of DENV, CHIKV, and malaria across the study period. Spatiotemporal mixed model regression was performed to identify those demographic and environmental factors linked with exposure risk. Over the five years of the study, 3,521 children were tested for CHIKV, DENV, and malaria. Overall, 9.8% were CHIKV seropositive, 5.5% were DENV seropositive, and 39.1% were malaria positive. The spatial analysis identified hot-spots for all three diseases in each site and in multiple years. The location of CHIKV, DENV, and malaria hot-spots were consistent through the study period (Kendall's W> 0.7, $p<0.05$) and also overlapped in the sites (Kendall's W> 0.6, $p<0.05$). The results of the model showed that the risk of exposure was linked to demographics and demographic factors common for the three diseases and will be further explored in this analysis. These insights are of high importance to improve surveillance and targeted control of mosquito-borne diseases in Kenya.

0141

EVALUATION OF THE GROWTH KINETICS OF GETAH VIRUS IN A SCOPE OF MAMMALIAN AND MOSQUITO CELL CULTURES

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Thirty (30) years since the last reported incidence of Getah virus infection in Japan, there have been sporadic outbreaks of Getah virus (GETV) among race houses and pigs in some prefectures of Japan. This can be attributed to a disproportionate maintenance of alphaviruses and a broad-scale difference in the ecology of existing potential vectors. The detection of GETV in *Aedes vexans nipponii* and multiple isolates of a GETV12IH26 strain from *Culex tritaeniorhynchus* have led to intimations of these mosquitoes being the principal vector(s) for the equine GETV infection. Due to the risk of other potential vectors perpetuating transmission, this study bridges this gap in knowledge using an *in vitro* setup to identify a subset that would be representative of GETV *in vivo* infection life cycle of GETV, and amenable to *in vitro* model applications. We evaluated a panel of mosquito derived cell cultures, Ar-3 (*Armigeres subalbatus*), C6/36 (*Aedes albopictus*, RNAi dysfunction), NIID-CTR (*Culex tritaeniorhynchus*), and MSQ43 (*Anopheles stephensi*), to ascertain GETV activity. GETV (strain 12IH26) was inoculated at MOI of 0.01 in the cell lines and virus harvested at 12-hrs and 24-hrs intervals post-infection. Plaque assays were then performed in Vero cells to estimate virus titres. GETV induced severe cytopathic effect (CPE) in all the mosquito cell lines. Continuous cell culture of GETV in C6/36 showed highest titration of GETV replication and a severe CPE in MSQ43. Our findings showed NIID-CTR as a suitable *in vitro* culture system for GETV12IH26 replication studies. Also, early events such as RNAi of the cell and GETV induced-CPE may determine the outcome of the virus replication. Our study highlights vector-derived cell culture susceptibility studies can be a surveillance criterion for effective assessment of entomological risk of GETV transmission.

0142

PERFORMANCE OF AN ANTIBODY-BASED RAPID DIAGNOSTIC TEST FOR CHIKUNGUNYA AMONG FEBRILE PATIENTS IN RIO DE JANEIRO, BRAZIL: A PROSPECTIVE, DIAGNOSTIC ACCURACY STUDY

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There is a need to develop new strategies for Chikungunya (CHIKV) diagnosis that do not rely entirely on reverse transcriptase-polymerase chain reaction (RT-PCR). CHIKV antibody-based rapid diagnostic tests (RDTs) are available to meet this need, but little information exists regarding their performance. Thus, we evaluated the performance of an antibody based RDT for detecting CHIKV in febrile patients seeking care in Rio de Janeiro. Next, we examined the performance of the CHIKV's clinical case definition endorsed by the World Health Organization (WHO) against RT-PCR, especially in the context of an outbreak, where resources for compulsory laboratory confirmation are not practical. We prospectively enrolled non-severe febrile patients aged 2-65 years presenting as an outpatient between October 2018-July 2019. Serum samples were tested for CHIKV antibodies using the DPP® ZDC IgM/IgG (Bio-Manguinhos, Fundacao Oswaldo Cruz, Brazil) compared against the CHIKV RT-PCR and Enzyme-Linked Immunosorbent Assay (ELISA). Five hundred participants were enrolled. 232/263 (88.2%) tested ELISA-antibody positive, 100/294 (34%) CHIKV RT-PCR positive, and 117/495 (23.6%) RDT-antibody positive. The sensitivity of the IgM, IgG, and the combined IgM/IgG component ranged from 5-24%, while the specificity ranged from 75-85%. The sensitivity of the RDT components gradually increased over the days pass after the onset of illness. There was a slight agreement between RDT IgM and ELISA IgM (k coefficient = 0.01), whereas there was a substantial agreement between RDT IgG and ELISA IgG (k coefficient = 0.70). The WHO' CHIKV clinical definition had a sensitivity, specificity, positive predictive value, and negative predictive value of: 88 (79.9-93.6) %, 74 (68-80) %, 64.2 (58.2-69.8) %, and 92.3 (87.6-95.3) %, respectively. Our study revealed that the antibody-based RDT kit evaluated had a poor performance and needed significant improvement before widespread implementation. The development of accurate CHIKV RDTs is urgently needed to inform disease control strategies, strengthen the efficacy of health systems, and improve patient outcomes.

0143

CHIKUNGUNYA: SAFETY UP TO DAY 29 OF PHASE 3 CLINICAL DEVELOPMENT OF A SINGLE-SHOT LIVE-ATTENUATED VACCINE

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Chikungunya is a mosquito-transmitted outbreak disease with potentially debilitating consequences and no available causative treatments or preventative vaccines. VLA1553 is a live-attenuated chikungunya virus (CHIKV) vaccine candidate designed for active immunization as a prophylactic measure for travelers to endemic areas or areas at risk for an upcoming outbreak, as well as for the general population living in endemic regions. A double-blinded, multicenter randomized, pivotal phase 3 study comprising approximately 4,060 adult volunteers randomized in a 3:1 ratio to receive the live-attenuated CHIKV vaccine candidate VLA1553 or placebo is currently ongoing (NCT04546724). VLA1553 or placebo were administered as a single intramuscular immunization. The primary objective of the study is to evaluate the immunogenicity and safety of VLA1553 28 days after immunization. Subjects in this study were stratified into

two age strata of 18 to 64 years and 65 years of age or above. Solicited adverse events (AE) were monitored for the first ten days after vaccination and unsolicited AE throughout the study, whereas AE of special interest (AESI, defined as events potentially resembling a CHIKV-like infection) were monitored between days 2 and 21 after immunization. To date, recruitment is completed and 4,131 volunteers have been vaccinated. The study is progressing and analysis on safety up to Day 29 will be available mid 2021.

0144

DENGUE AND CHIKUNGUNYA VIRUS EXPOSURE AMONG URBAN POPULATIONS IN WESTERN AND COASTAL KENYA

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Dengue (DENV) and chikungunya (CHIKV) viruses are well-known causes of severe illnesses in Kenya, but little is known about the risk factors for their transmission in urban Kenya across all age groups. In this project, we recruited children (infants and ≥1 year old to 18) and adult participants into two urban cohorts from Ukunda [coast] and Kisumu [west], for a total of 2779 participants, 1621 adults and 1135 children respectively. Participants were followed during febrile illness and every 6 months. Questionnaire data were collected to describe demography, socioeconomic status (SES), and household environment, as well as blood samples for detection of CHIKV and DENV IgG antibodies by ELISA. Of 2779 tests results obtained, 581 participants (21%) tested positive for CHIKV, with 539 (33%) adults and 49 (4%) children respectively. In particular, 11 CHIKV seropositive children were within 0-5 years age range, 22 CHIKV seropositive within 6-12 years age range, and 16 CHIKV seropositive within 13-17 years age range. No significant differences were observed comparing the two study sites for CHIKV seropositivity (24% in the west and 19% in the coast, p=0.34). 638(23%) participants tested positive for DENV, 533 (33%) were adults and 105 (9%) were children, with 19 DENV seropositive children within 0-5 years age range, 54 DENV seropositive within 6-12 years age range and 32 DENV seropositive within 13-17 age range. DENV seropositivity was significantly higher on the coast (36%) than on the west (6%) ($p<0.001$). Strong association between increasing age and CHIKV-DENV exposure was also observed ($p\leq0.001$). Co-exposure to CHIKV and DENV was observed in both study sites (3% in the west versus 11% on the coast, $p<0.001$), with more co-exposure in adults (203 (7%)) than in children (13 (1%) ($p<0.001$)). Furthermore, exposure was highly associated with a low level of education for CHIKV and poor socioeconomic status for DENV ($p\leq0.001$). Past history of malaria in adult participants was also associated with CHIKV and DENV exposure($p\leq0.001$), highlighting the importance of social determinants of health in risk mitigation for arboviral disease threats.

0145

EFFICACY AND IMMUNOGENICITY OF A CHIKUNGUNYA VIRUS-LIKE PARTICLE VACCINE IN A HIGH-DOSE CHIKUNGUNYA INFECTION MODEL IN CYNOMOLGUS MACAQUES

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Chikungunya virus (CHIKV) causes acute illness characterized by fever, fatigue, and severe joint pain, which can lead to debilitating chronic manifestations including arthralgia. There is no licensed vaccine for prevention of CHIKV infection or disease. Emergent BioSolutions is developing a CHIKV virus-like particle (VLP) vaccine that has demonstrated a robust immune response in nonclinical and Phase 1 and 2 clinical studies and has shown protection against viremia after virulent challenge in mice and rhesus macaques. To determine whether the CHIKV VLP vaccine is protective against CHIKV-induced joint disease, a cynomolgus macaque model using intravenous administration of a high challenge dose was employed. In a non-clinical pilot study, a dose of 10^7 pfu of CHIKV LR2006-OPY1 was selected based on joint histopathology scores and clinical signs of disease. Next, groups of 5 animals were vaccinated twice via intramuscular injection, 28 days apart, with three varying doses of CHIKV VLP ± alum adjuvant. Four animals received alum adjuvant alone. Vaccinated animals all developed high serum neutralizing antibody titers, which peaked after the second immunization (all NT_{80} values > 3 log). Following CHIKV challenge 28 days after the second vaccination, all adjuvant-only control animals developed viremias that peaked 2 days after challenge and subsided to low or undetectable levels within 10 days. These control animals also developed clinical signs of infection, required supportive care, and displayed joint histopathology and high levels of viral RNA (vRNA) in joint-associated tissues upon necropsy. In contrast, animals immunized with all doses of CHIKV VLP had no detectable circulating infectious virus and significantly reduced vRNA levels in plasma, fewer clinical signs, and required less supportive care compared to control animals. Immunized animals had lower joint histopathology scores and vRNA levels in joint-associated tissues. This study demonstrated the ability of the CHIKV VLP vaccine to protect cynomolgus macaques from disease after high-dose CHIKV challenge, including joint histopathology indicative of arthritis.

0146

ELUCIDATING THE CELLULAR AND MOLECULAR DETERMINANTS OF ARBOVIRAL INDUCED CARDIOMYOPATHIES

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Chikungunya virus (CHIKV) is an arbovirus that has re-emerged in the past decade, affecting millions of people. Here, we report a patient with documented normal ECG and cardiac function who developed acute myocarditis after CHIKV infection. This patient developed ECG alterations and progressive left ventricular dysfunction early after symptom onset. Cases of myocarditis, dilated cardiomyopathy, and heart failure have been

recurrently associated with CHIKV and other arboviral infections and are linked to increased mortality. However, the underlying mechanisms driving arbovirus-induced heart disease are unknown.To study how CHIKV induces heart disease, we established a mouse model of CHIKV heart infection using C57BL/6 mice. We found that CHIKV replicates and generates infectious particles in the heart, inducing pro-inflammatory immune markers and infiltration of monocytes and neutrophils. Bulk RNA-seq of CHIKV-infected hearts showed upregulation of the interferon, innate, and adaptive immune response pathways. In addition, we found elevated levels of serum cardiac troponin-T at 2 days post infection, indicative of cardiac damage. Notably, mice deficient in components of the antiviral innate immune response (*Ifnar*$^{-/-}$ or *Mavs*$^{-/-}$) showed increased susceptibility to CHIKV cardiac infection. Particularly, *Mavs*$^{-/-}$ mice showed delayed clearance of the infection from the heart. In addition, positive staining of cleaved caspase-3 in CHIKV-infected *Ifnar*$^{-/-}$ hearts showed that CHIKV is inducing apoptosis in cardiac tissue. These results suggest that the interferon pathway plays a central role in CHIKV clearance from the heart and cardiac tissue damage. Overall, our results indicate CHIKV induces cardiomyopathies through infection of the cardiac tissue. These results underscore the importance of monitoring cardiac function in patients with CHIKV infections, and lay the foundation for the development of new approaches to prevent viral-induced cardiomyopathies. Further studies will focus on understanding the role of polymorphisms in innate immune response components and its correlation with arboviral induced-heart disease.

0147

REEMERGENCE OF CHIKUNGUNYA VIRUS AMONG THE PARTICIPANTS IN THE UNDIFFERENTIATED FEBRILE ILLNESS STUDY IN CAMBODIA, 2020

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In 2020 Cambodia experienced a resurgent Chikungunya virus (CHIKV): after many years of very low levels of detection, a widespread outbreak swept the Kingdom. An RNA arbovirus of Alphavirus genus, family Togaviridae spread and was transmitted by infected Aedes mosquitoes. CHIKV was first detected in Cambodia in 1961 and more recently, an East Central South African genotype was detected in Cambodia in larger clusters in 2012. The Naval Medical Research Unit 2 laboratory regularly conducts real-time reverse transcription PCR (RT-PCR) and serology studies of infectious disease pathogens. Samples collected in 2020 for the Global Emerging Infections Surveillance funded passive surveillance study: "Surveillance and Etiology of Acute Undifferentiated Febrile Illnesses in Cambodia" found gradually rising CHIKV cases in the months preceding the declared outbreak. In 2020, 1,193 Cambodian nationals were enrolled at referral hospitals and health centers from five provinces and provided samples for testing. Of 1,152 acute-phase serum samples tested for African CHIKV PCR and 691 samples tested for IgM, 6.9% were PCR positive, while 19.7% were IgM positive. Of those positive samples, 76.3% and 53.7% (CHIKV and IgM respectively) originated in Kandal province. One case of CHIKV had co-infection with dengue (DENV-2) and five cases had detectable dengue IgM. Seventy-nine of 80 CHIKV positive cases were identified in the months of July to December. Sixty percent of CHIKV positive cases were males while 66.2% of IgM positive were adults. The CHIKV positivity rates among the males group is significantly higher than the females (p=0.037, OR (95%CI) = 1.6 (1.1-2.6)). There were no other specific symptoms than fever (temp ≥ 38°C) for the CHIKV and IgM positive cases. After many years of apparent dormancy and limited detection in Cambodia, CHIKV made a dramatic appearance starting in 2020, with widespread and symptomatic disease. Even those datasets with consecutive years of CHIKV surveillance have deficiencies in robustness and reporting. Increased CHIKV surveillance could provide specific and early assistance in outbreak prediction and modeling.

0148

MULTIPLE BLOOD MEALS ENHANCE EARLY DISSEMINATION OF ARBOVIRUSES IN THREE MEDICALLY RELEVANT MOSQUITO GENERA

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Numerous anautogenous mosquito vectors have the propensity to acquire multiple blood meals within a single gonotrophic cycle; however, incorporation of this feeding phenotype into laboratory vector competence studies is rarely done. We have previously shown that this frequent feeding behavior can enhance the early dissemination of Zika virus, dengue virus, and chikungunya virus in *Aedes aegypti* and *Aedes albopictus* mosquitoes, yet it is unknown if arboviruses show a similar trend in non-Aedes species mosquitoes under a sequential feeding regimen. To test this, we evaluated the impact of a second non-infectious meal on the vector competence of *Ae. aegypti*, *Anopheles quadrimaculautus*, and *Culex quinquefasciatus* for Mayaro virus (MAYV) and *Cx. quinquefasciatus* for West Nile virus (WNV). Mosquitoes were offered an infectious MAYV or WNV bloodmeal and three days later the double-feed group (DSG) was offered a second non-infectious bloodmeal. Midgut infection and dissemination rates were determined by RT-qPCR between 5-10 days post infection. For MAYV, midgut infection rates were comparable between the single-feed group (SFG) and DFG for all three species; however, infection rates were extremely low in *Cx. quinquefasciatus* and, therefore, the double-feed phenotype is being evaluated in this species using WNV. Consistent with other viruses, MAYV dissemination rates were significantly higher in the *Ae. aegypti* DFG compared to the SFG at earlier timepoints. Similarly, the *An. quadrimaculatus* DFG displayed higher rates of dissemination compared to the SFG at earlier timepoints. Our results suggest that frequent blood-feeding improves MAYV dissemination in *Ae. aegypti* and *An. quadrimaculatus* and may allow for higher levels of viral transmission than previously expected. Further, these findings suggest that the shortened extrinsic incubation period of arboviruses associated with sequential blood feeding is generalizable across some, if not most, virus-vector pairings.

0149

CARDIOMYOPATHY AND DEATH FOLLOWING CHIKUNGUNYA INFECTION: AN INCREASINGLY COMMON OUTCOME

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Chikungunya virus (CHIKV) is vectored by *Aedes aegypti* and *Aedes albopictus* mosquitoes and is found throughout tropical and sub-tropical regions. Although most infections cause mild symptoms such as fever and arthralgia, there have been cases in which cardiac involvement has been reported. In adults, case reports include symptoms ranging from tachycardia and arrhythmia, to myocarditis and cardiac arrest. In children, case reports describe symptoms such as arrhythmia, myocarditis, and heart failure. Case reports of perinatal and neonatal CHIKV infections have also described cardiovascular compromise, including myocardial hypertrophy, ventricular dysfunction, myocarditis, and death. Myocarditis refers to inflammation of the heart tissue, which can be caused by viral infection, thus becoming viral myocarditis. Since viral myocarditis is also linked as a possible causative factor of other cardiomyopathies, including dilated cardiomyopathy, in which the heart muscle weakens and fails to pump blood properly, the connection between CHIKV and the heart is

concerning. We searched Pubmed, Embase, LILACS, and Google Scholar to identify case reports of CHIKV infections where cardiac symptoms were reported. We utilized NCBI Virus, and NCBI Nucleotide to explore the lineage/evolution of strains associated with these outbreaks. Statistical analysis was performed to identify which clinical features were associated with death. Phylogenetic analysis determined that CHIKV infections with cardiac symptoms are associated with the Asian, East Central South African, and the Indian Ocean lineages. Of patients admitted to hospital, death rates ranged from 26-48%. Myocarditis, hypertension, pre-existing conditions and the development of heart failure were significantly correlated with death. As such, clinicians should be aware in their treatment and follow-up of patients.

0150

THE RISK AND RISK FACTORS OF CHIKUNGUNYA VIRUS RHEUMATOLOGICAL SEQUELAE IN A FIVE-YEAR VIRTUAL COHORT OF U.S. MILITARY HEALTH SYSTEM BENEFICIARIES

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Understanding chikungunya virus (CHIKV) rheumatic sequelae across a range of populations is critical. Using the electronic medical records of U.S. Military Health System (MHS) beneficiaries, we established a 'virtual cohort' of CHIKV infections to identify the risk and risk factors of post-CHIKV rheumatic sequelae. MHS beneficiary CHIKV infections diagnosed 2014-2018 were identified from the Disease Reporting System Internet, TRICARE Encounter Data Non-Institutional, and Comprehensive Ambulatory Professional Encounter Record systems. Controls were matched (1:4) by age, gender, beneficiary status, and encounter date. The frequency of comorbidities and incident rheumatic diagnoses were derived from ICD codes. Logistic regression models derived the association of CHIKV infection with rheumatic sequelae, compared to controls, and risk factors for post-CHIKV sequelae. 195 CHIKV cases were diagnosed between July 2014 and December 2018. The mean age was 42 years, and 43.6% were active duty. 63/195 (32.3%) of CHIKV cases had an incident rheumatic diagnosis, including arthralgia, polyarthritis, polymyalgia rheumatica, and/or rheumatoid arthritis. Those with rheumatic CHIKV sequelae had a median 7 healthcare encounters (IQR 3 -15). Compared to controls, CHIKV infection was associated with short-term (≤ 3 month) and long-term (> 3month) rheumatic sequelae (aOR = 6.41, p< 0.001; aOR = 1.43, p = 0.09 respectively) after adjusting for prior rheumatic disease, age, sex, and race. Among CHIKV infections, we found no association between post-CHIKV rheumatic sequelae and age, sex, race, active-duty status, service branch, or comorbidities. In conclusion, CHIKV infection is associated with rheumatic sequelae among US Military Beneficiaries. 25% of post-CHIKV rheumatic sequelae cases required at least 15 healthcare visits, indicating a substantive burden of disease. No demographic, clinical, or occupational variables were associated with post-CHIKV rheumatic sequelae, suggesting that prediction of these complications is challenging in those presenting with a CHIKV diagnosis.

0151

A SYSTEMATIC REVIEW AND META-ANALYSIS OF THE POTENTIAL NON-HUMAN ANIMAL RESERVOIRS AND ARTHROPOD VECTORS OF THE MAYARO VIRUS

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Mayaro Virus (MAYV) is an *Alphavirus* endemic to Latin America that is transmitted through a sylvatic transmission cycle. Improving our understanding of MAYV ecology is critical to guide disease/vector surveillance and risk assessment. We conducted a PRISMA-adherent systematic review of the literature to identify potential arthropod vectors and non-human animal reservoirs of MAYV. We searched PubMed, Embase, Web of Science, SciELO and grey-literature sources including PAHO databases. Original research studies were included if they assessed MAYV occurrence in field-caught, domestic, or sentinel animals or in field-caught arthropods. We conducted an animal seroprevalence meta-analysis using a random effects model and the I^2 statistic as a measure of heterogeneity. Of 1024 literature results, 55 studies were eligible. Nineteen studies reported MAYV in wild mammals or birds, and five studies reported MAYV in domestic animals. MAYV positivity was reported in 55 wild-caught vertebrate species overall, including 16 in the order Charadriiformes, 15 in the order Primate, and six in the order Rodentia. Sixteen studies detected MAYV in wild-caught mosquito genera including *Haemagogus, Aedes, Culex, Psorophora, Coquillettidia,* and *Sabethes*. Animals or arthropods with MAYV were detected in Brazil, Panama, Peru, French Guiana, Colombia, Trinidad, Venezuela, Argentina, and Paraguay. Among mammals, the primate order had the highest pooled prevalence (PP) at 0.11 (95% CI: 0.05-0.21, I^2= 95%, p<0.01). From the most studied primate genera (*i.e.*, N > 100 across all studies) we noted the highest prevalence in *Alouatta* (PP: 0.41, 95% CI: 0.24-0.60, I^2: 69%, p=0.01), followed by *Callithrix* (PP: 0.12, 95% CI: 0.00-0.83, I^2= 44%, p=1.00), and *Cebus* (PP: 0.08, 95% CI: 0.03-0.18, I^2= 77%, p=0.02). MAYV occurs in a wide range of vectors beyond *Haemagogus* sp. MAYV is most frequent in non-human primates but has a potentially broad range of vertebrate reservoirs. These findings support further risk emergence prediction, guide field surveillance efforts, and prompt further *in-vivo* studies to better define the ecological drivers of MAYV emergence.

0152

CHIKUNGUNYA VIRUSES CONTAINING THE A226V MUTATION DETECTED RETROSPECTIVELY IN CAMEROON FORM A NEW GEOGRAPHICAL SUBCLADE

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Chikungunya virus has been associated with sporadic outbreaks throughout many of the combatant command areas of responsibility in West Africa. The debilitating impact of CHIKV on deployed forces detrimentally impacts force health protection especially in tropical environments. CHIKV is classified into three main evolutionary genotypes: West African, East/Central/South African, and Asian. Recent viral WGS efforts have revealed the emergence of evolutionary distinct sub-lineages prompting the need for constant genomic surveillance. This study generated viral whole genomes to analyze the origins of recent Cameroon CHIKV outbreak strains; whole blood samples collected between 2016-2019 during CHIKV outbreaks in Cameroon were analyzed. Three coding complete CHIKV genomes were obtained from samples originating from the urban capital of Yaoundé and from the rural town of Mfou. Phylogenetic analysis revealed that all three strains belong to an emerging sub-lineage of the ECSA genotype. The new sequences formed a monophyletic taxon with strains previously isolated in Gabon, Congo,

and a 2006 Cameroon isolate, which we have named the new Central African clade and that appears to be evolving at 3.0 x 10^{-4} nucleotide substitutions per site per year (95% HPD interval of 1.94 x 10^{-4} and 4.1 x 10^{-4}). Comparative genomic analysis revealed 87 amino acid substitutions across the coding regions of the genomes, with many of these being described for the first time. Mutations in the envelope proteins (E1-A226V, E2-L210Q, and E2-I211T) that are known to enhance CHIKV adaptability and infectious potential in *Aedes albopictus* were present in all strains; Yaoundé strains mapped to established high-density *Aedes albopictus* populations in both urban and suburban areas. These new CHIKV strains constitute a conserved genomic pool of an emerging sub-lineage of the ECSA genotype, reflecting a putative vector host adaptation to *Aedes albopictus*, which has practically displaced *Aedes aegypti* from Yaoundé and many smaller towns in the Guinean sub-equatorial region of Cameroon.

0153

IMMUNOLOGICAL INSIGHTS BASED ON ANTIBODY BINDING EPITOPES ON THE CHIKUNGUNYA VIRUS ENVELOPE

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To identify the Chikungunya virus (CHIKV) structures that elicit a protective immune response, we have epitope mapped over 70 monoclonal antibodies (MAbs) against the CHIKV envelope glycoprotein E2/E1, using a comprehensive shotgun mutagenesis library of 910 E2/E1 alanine-scan mutants. Published studies used epitope maps to characterize broadly cross-reactive and ultrapotent neutralizing MAbs that blocked post-attachment steps, and whose binding mapped to functionally important E2 domains A or B, suggesting that MAbs inhibit virus-host membrane fusion by preventing exposure of the E1 fusion loop. Other studies characterized MAbs that induce structural changes on E2 domains A and B, or that target the E1 protein. We also isolated 7 human MAbs against CHIKV E2/E1, including previously unreported cross-reactive, but non-neutralizing, MAbs against the highly conserved E1 fusion loop. Neutralizing epitopes are confined to the exposed topmost and outer surfaces of the E2/E1 trimer, providing a rationale for using the trimer for vaccine design and therapeutic MAb development. Our most potent MAb, IM-CKV063, was highly neutralizing (50% inhibitory concentration, 7.4 ng/ml), showed high-affinity binding (320 pM), and gave therapeutic and prophylactic protection in animal models up to 24 h post-exposure. Epitope mapping identified an inter-subunit conformational epitope on E2 domain A. Subsequent studies demonstrated that IM-CKV063 blocks both virus entry and virus release, and that optimal therapeutic activity required interaction of MAb Fc region with Fcγ receptor. We also used CHIKV E2/E1 mutants to map the binding site of cell adhesion molecule Mxra8, identified as an entry mediator for CHIKV, and other alphaviruses, by infectivity screens of cells targeted by CRISPR/Cas9 gene knockouts. Mxra8 enhanced virus attachment and internalization into cells, and mapping suggests that Mxra8 binds to E2 A and B domains. Mxra8-Fc protein or anti-Mxra8 MAbs blocked CHIKV infection in multiple human cell types and in mice, suggesting Mxra8 as a target for therapeutics against CHIKV and other arthritogenic alphaviruses.

0154

THE DIGITAL TRANSFORMATION OF THE HIV CARE AND TREATMENT\PROGRAM AT JUBA MILITARY HOSPITAL IN SOUTH SUDAN

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The goal of the Department of Defense HIV/AIDS Preventing Program (DHAPP) in South Sudan is to strengthen the capacity of the South Sudan Peoples Defense Forces (SSPDF) HIV Secretariat to implement its HIV prevention, care, and treatment program and to cover the unmet need of soldiers, their dependents, and the communities. As the implementing partner, RTI International monitors status across 8 program areas and regularly reports program results into two seperate reporting systems: including DATIM, the U.S. President's Emergency Plan for AIDS Relief's (PEPFAR's) online reporting database and DC2, DHAPP's online reporting database. To comply with the data reporting requirements of these two systems the RTI team was expending significant effort to ensuring consistency and accuracy across these two platforms. To address this challenge, the RTI turned to the Principle for Development in designing a solution. The Principles for Digital Development are nine living guidelines designed to help digital development practitioners establish best practices into technology enabled programs. The result is the HIV Care and Treatment Database for SSPDF, a database built on the popular DHIS2 platform. This database provides a centralized repository for all program data, interoperability with partner systems, and data entry capabilities. In this case study we discuss some emerging best practices, operational approaches, challenges and opportunities modernizing monitoring and evaluation in the age of interconnected data systems.

0155

EPITOPE BIOMARKERS FOR THE DETECTION OF ANTI-DENGUE ANTIBODIES IN HUMAN SERA

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In a world with an increasing population at risk of arthropod-borne Flaviviruses infection, access to timely and accurate diagnostic tests impact profoundly on the management of cases. Unfortunately, there is a poor deployment of laboratory-based diagnostic capacity in low-resource settings (LRS) that can identify and discriminate the infectious agent when needed. The relative simplicity and affordability of serological assays make them good candidates to be implemented in LRS. However, flavivirus serology-based diagnosis is hindered by low test specificity, as consequence of the high genetic and antigenic similarities among the flavivirus family eliciting cross-reactive (CR) antibodies (Abs) upon infection. Replacement of conventional antigens such as viral lysates or recombinant proteins by epitope biomarkers could have the potential to discriminate type-specific from CR Abs. Previously, using a microarray peptide covering the entire proteome and diversity of dengue (DENV), Zika (ZIKV) and yellow fever (YFV) viruses, we identified 20 DENV derived peptides widely recognized by IgM or IgG Abs from dengue infected individuals. The 9 selected peptides recognized by IgM Abs are located in the E, NS1, NS2a, NS2b, NS3 and NS4b proteins, while the 11 peptides recognized by IgG Abs are located in the prM, E, NS1, NS2b, NS3, NS4b and NS5 proteins. To further investigate the discriminatory potential of these peptides, we set-up a bead-based multiplex peptide immunoassay. The peptides were coupled to Luminex paramagnetic beads to determine Ab binding levels to each peptide/antigen over a panel of 224 human serum samples. These are derived from individuals either infected with different flaviviruses,

including the four DENV serotypes, ZIKV, YFV, tick-borne encephalitis (TBEV), West Nile and Japanese encephalitis (JEV) viruses, or receiving vaccination against YFV, TBEV or JEV. This screening will demonstrate the capability of the peptides to differentiate dengue infected individuals from other flavivirus-exposed subjects and will be instrumental for future development of diagnostic tests with better specificity characteristics.

0156

DENGUE AND CHIKUNGUNYA SURVEILLANCE IN CAMBODIA, 2015-2020

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The U.S. Naval Medical Research Unit No. 2 (NAMRU-2) maintains a network of nine surveillance sites in Cambodia, comprising urban locations in Kampong Speu and Kandal and rural sites in Battambang, Kratie, Preah Vihear, Retanakiri, Sihanoukville, Stung Treng, and Svay Rieng. Cambodian nationals experiencing symptoms of acute undifferentiated febrile illness (AUFI) arrive for treatment and are offered the opportunity to participate in this study. Anyone eligible for inclusion must be two years of age or over and present with symptoms of febrile illness at least 24 hours prior to the time of enrollment with no longer than 10 days of duration with or above an oral or tympanic temperature of 38.0°C or axillary temperature of 37.5°C. After providing consent, blood samples were collected for laboratory testing using RT-PCR and IgM/IgG ELISA targeting dengue (DENV) and chikungunya (CHIKV) infections. Between 2015 and 2020, blood samples were collected from 14,244 participants (54.3% male, 60.7% adults 18 y/o or over, mean = 25 y/o); in total, 4,078 samples were tested for CHIKV and 11,341 samples were tested for DENV using a standard testing algorithm, which also includes Zika virus, Leptospira, and rickettsia. Our DENV surveillance data during this period indicate a spike in percent positivity in Quarter 2 (Q2, April - June) and/or Quarter 3 (Q3, July - September) each year. Similarly, DengueNet and WHO data indicate similar increases in DENV cases during the April - September timeframe. These findings align with National Center for Medical Intelligence (NCMI) and CDC assessments that indicate DENV seasonality, with rainy season peak transmission (June - August). Our data also shows an increase in percent positivity between 2017 and 2019, with a peak in Q2 2019. Trends in CHIKV data 2015 - 2020 generally spike in percent positivity in Q3 (July-September) or Q4 (October - December) each year, except for a Q1 spike (January - March) in 2018. DENV and CHIKV continue to present a health risk to Department of Defense personnel in Cambodia, and surveillance networks such as this are critical to capturing disease trends in regions where military forces operate.

0157

DEVELOPMENT AND CHARACTERIZATION OF A MULTIPLEX ASSAY TO QUANTIFY COMPLEMENT-FIXING ANTIBODIES AGAINST DENGUE VIRUS

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The complement system (CS) is an arm of innate immunity that functions as an effector mechanism of the humoral response. Antibodies against dengue virus (DENV) and other flaviviruses can mediate CS activation. For over two decades, a complement-fixing antibody test has been used to measure the ability of DENV-specific serum antibodies to activate the CS. As originally developed the test is time-consuming, cumbersome and uses reagents that are difficult to standardize. In addition, it has lower sensitivity for DENV diagnosis relative to neutralizing or hemagglutination inhibiting antibody assays. An improved method is needed for quantifying complement-fixing antibodies against all 4 DENV serotypes. We have developed and characterized a novel multiplex anti-DENV complement-fixing assay based on the Luminex platform to quantitate serum antibodies against structural proteins of all 4 serotypes (DENV1-4) that can activate CS based on their ability to fix the complement component C1q. The assay has good precision and linearity as well as high sensitivity and specificity relative to a microneutralization assay. In non-human primates, antibodies produced in response to primary DENV1-4 infection induced CS activation against homologous and heterologous serotypes. Higher complement-fixing antibody titers were found against the homologous serotype. Moreover, inter-serotype cross-reactivity was associated with homology of the envelope protein. Interestingly, antibodies produced following vaccination against Zika virus, but not yellow fever virus, Japanese encephalitis virus, West Nile virus and tick-borne encephalitis virus activated CS against DENV structural proteins. The anti-DENV complement fixing antibody assay represents an alternative approach to determine the quality of antibodies produced following DENV natural infection and a biomarker for dengue serostatus, while providing insights about flavivirus-specific antibody cross-reactivity.

0158

VARIATION IN DENGUE SEVERITY ACROSS PREGNANCY STATUS FROM 2017-2018 IN MEXICO: AN INTERACTION BETWEEN PREGNANCY AND DENGUE SEROTYPE

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Infection with dengue fever during pregnancy is associated with miscarriages, intrauterine fetal death, and maternal mortality. There is, however, a gap in literature portraying how the severity of dengue in pregnancy and its occurrence in individuals with other comorbidities is modified by dengue serotype. The aim of this study was therefore to determine if the severity of dengue was dependent on the interaction between, pregnancy and/or other comorbidities and dengue serotype, in Mexico. The association between pregnancy and specific serotypes will guide policies related to dengue prevention in Mexico. Dengue surveillance data was acquired from the nationally notifiable disease reporting system of Mexico's Ministry of health. The dataset contains non-identifiable health information collected from notifying health units in 2,469 Mexican municipalities. Since the main variable of interest was pregnancy, the analysis was restricted to women (N= 15608). SAS 9.4 by SAS Institute Inc., Cary, NC, USA was used to conduct all analysis. Overall, 21.4% of our sample experienced signs of dengue hemorrhagic fever. Of those who had signs of dengue hemorrhagic fever, 65.2% were infected with dengue serotype 2, 33.1% were associated with serotype 1, 1.69% were associated with serotype 3 and none were associated with serotype 4. Of those with dengue serotype 1, being pregnant was associated with 40% (CI: 17% - 68%) higher prevalence odds of dengue hemorrhagic fever compared to non-pregnant women. Among those infected with dengue serotype 3, being pregnant was associated with 2.5 times (CI: 1.04 – 6.06) the prevalence odds of developing hemorrhagic fever compared to non-pregnant women. Most women with dengue hemorrhagic fever were found to have either dengue serotype 1 or 2. However, among pregnant women, serotypes 1 and 3 were associated with more severe presentations compared to non-pregnant women. Hence, while preventive efforts should be geared towards eradicating dengue in Mexico, targeting dengue serotypes 1 and 3 in vaccine development or adjusting to a more aggressive management in pregnant women infected with serotypes 1 and 3 seems prudent.

0159

CORRELATION OF THROMBOCYTOPENIA WITH GALL BLADDER WALL THICKNESS: A PREDICTIVE ANALYSIS FOR BLEEDING RISK AND NEED OF PLATELET TRANSFUSION IN DENGUE INFECTION

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Severe dengue is characterized by an increase in capillary permeability. Increased gallbladder wall thickening (GBWT) is one manifestation of increased capillary permeability and can be detected by a bedside abdominal ultrasound. This study was carried out to link the severity of GBWT with the bleeding risk and the need for platelet transfusion. The study was conducted as a retrospective design including all the patients with a diagnosis of dengue infection either via Dengue nonstructural protein-1 (NS-1) antigen or IgM antibody. Pearson's correlation, multiple linear regression and receiver operating characteristic (ROC) curves were used for predictive analysis of GBWT with an event of bleeding and need for transfusion of platelets during the hospital stay. A total of 177 dengue-infected patients were included in the analysis with a mean age of 33.17 ± 13.63 years. Mean GBWT was found to be 0.37 ± 0.15 cm, with 46.3% of patients had a thickness greater than 0.30 cm. A total of 16 patients were documented with bleeding events out of which 7.3% had minor bleeding and 1.7% had a major bleeding event. Increased GBWT was found correlating with decreased platelet count on admission ($p=0.006$) and lowest platelet counts during hospital stay ($p=0.001$). While lowest platelet counts were also found correlating with increased length of hospital stay ($p=0.020$). Multiple linear regression analysis showed increased GBWT was found associated with decreased platelet count on admission ($p=0.002$) and lowest platelet counts ($p=0.004$). ROC curves showed GBWT at a cut-off value of 0.385 cm was predictive of bleeding event with an area under the curve of 0.821, sensitivity of 92.3% and specificity of 66.5% ($p<0.001$). The same cut-off was predictive of platelet transfusion with an AUC of 0.932, sensitivity of 100% and specificity of 71.9% ($p<0.001$). GBWT was found predictive of bleeding event and transfusion of platelets at higher sensitivity and specificity than platelet count on admission and lowest platelet counts during hospital stay. Hence, GBWT should also be assessed in every dengue patient to predict the risk of bleeding and a prompt need for platelet transfusion.

0160

CHARACTERIZATION OF THE NEUTRALIZING ANTIBODY RESPONSE IN BASELINE SERONEGATIVE CHILDREN, ADOLESCENT AND ADULT RECIPIENTS OF A TETRAVALENT DENGUE VACCINE

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Takeda's live attenuated tetravalent dengue vaccine (TAK-003) is comprised of structural proteins from each of the four serotypes in an attenuated dengue virus 2 (DENV-2) genomic backbone. Data from the pivotal phase 3 clinical trial (DEN-301) showed no important safety risk observed and that TAK-003 demonstrated overall efficacy against dengue infection and associated hospitalizations by any DENV serotype regardless of baseline dengue serostatus in children and adolescents from dengue endemic regions. The production of neutralizing antibodies (nAbs) consisting of both cross-reactive (CR) and serotype-specific (TS) nAbs are thought to contribute to vaccine-elicited protection against dengue, although a correlate of protection against dengue has not been identified so far. In this study, we characterized the frequency and magnitude of TS and CR nAb responses to DENV-1, DENV-3 and DENV-4, post-depletion of DENV-2 nAbs, using the dengue reporter virus particle (RVP) neutralization assay. Post-vaccination serum samples from TAK-003 recipient children and adolescents from dengue endemic regions in DEN-301, and US-based adults from another phase 3 trial (DEN-304) were used in these assessments. The data from these evaluations revealed that TAK-003 elicits a mixture of CR and TS nAbs against DENV-1 and DENV-3, and a predominantly TS response against DENV-4 in baseline seronegative children, adolescents and adults. The magnitude and frequency of TS nAb responses against DENV-1 and DENV-3, were comparable, and higher for DENV-4 across trials and age groups. Our results indicate that each component of TAK-003 contributes to the serotype-specific response elicited by the vaccine, and the frequency and magnitude of TS nAb responses may be independent of the magnitude of overall nAb response. To understand the roles for CR and TS nAb responses elicited by TAK-003 in protection against dengue, characterization of nAb responses in the context of vaccine efficacy is currently underway.

0161

TAK-003 VACCINE-DRIVEN NEUTRALIZATION OF DIVERSE DENGUE GENOTYPES CIRCULATING IN ASIA PACIFIC AND LATIN AMERICA REGIONS

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Dengue disease is caused by four related, but distinct, virus serotypes. The virus envelope (E) protein, the main target of the neutralizing antibody (NAb) response, has accumulated intra-serotype genetic diversity with heterogeneous distribution worldwide. Substitutions in E protein amino acids could potentially allow specific strains to escape neutralization, become more virulent and cause severe dengue disease. Therefore, it is critical to develop a dengue vaccine that provides protection against diverse genotypes across dengue serotypes. Takeda's live attenuated tetravalent dengue vaccine (TAK-003) is comprised of structural proteins from each serotype in an attenuated dengue virus type 2 (DENV-2) genomic backbone. Data from a pivotal, phase 3 efficacy trial of TAK-003 (DEN-301, NCT02747927) showed no important safety risk and demonstrated overall efficacy against virologically-confirmed dengue, and associated hospitalizations irrespective of dengue serotype or serostatus. Due to dengue virus (DENV) evolution, an evaluation of cross-genotype immunity is essential to assess vaccine coverage in different parts of the globe. We evaluated the ability of post-vaccination serum from baseline seronegative TAK-003 recipients from DEN-301 to neutralize diverse genotypes using a panel of dengue reporter virus particles (RVPs) carrying E genes from contemporary dengue strains representing viruses circulating in Asia-Pacific and Latin American countries during 2002-18. Preliminary results indicate that TAK-003-elicited antibodies are capable of neutralizing both vaccine-matched DENV-3 sequences and DENV-3 genotypes responsible for recent outbreaks in the Asia Pacific region. Neutralization assays using DENV-1, -2 and -4 RVPs representing the E genes of contemporary, circulating strains is underway, and similar studies with viruses causing dengue cases during the trial will be undertaken in the future. Continued evaluation will help predict the ability of TAK-003 to elicit NAbs capable of neutralizing contemporary dengue strains of diverse genotypes circulating in areas of high endemicity around the world.

0162

SAFETY AND IMMUNOGENICITY OF TAKEDA'S TETRAVALENT DENGUE VACCINE ADMINISTERED DURING THE SECOND HALF OF ITS 24-MONTH SHELF-LIFE

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Illness owing to infection by any of the four co-circulating dengue virus serotypes ranges from asymptomatic disease to fatality. Infection with one serotype produces life-long homologous immunity but increases the risk of severe disease following a secondary heterotypic infection, so a dengue vaccine should ideally generate protective responses against all four serotypes. Takeda's tetravalent live-attenuated dengue vaccine candidate, TAK-003, comprises four dengue virus strains: an attenuated dengue serotype 2 strain (DENV-2), and three recombinant strains with the DENV-2 backbone and the pre-Membrane (prM) and Envelope (E) genes of DENV-1, DENV-3, and DENV-4. This open-label single-arm phase 3 trial evaluated the effect of administering TAK-003 vaccine after storage at 2°C to 8°C for >1 year (ie, >half of its anticipated 2-year shelf-life): two TAK-003 doses, 3 months apart, were administered to 200 healthy adults (18–60 years), from non-endemic areas of the USA. Robust increases in microneutralization titers [MNT_{50}] were observed for all 4 serotypes: Day 120:Day 270 geometric mean titer ratios were consistent with those observed in other TAK-003 clinical trials. Seropositivity rates by Day 120 were 99.2%, 100%, 97.7%, and 99.2% for DENV-1, DENV-2, DENV-3, and DENV-4, respectively, and 97% of participants had tetravalent seropositivity. By Day 270, 78.9% and 91% of participants still had tetravalent and at least trivalent seropositivity, respectively. TAK-003 was well-tolerated: solicited local and systemic adverse events (AEs) were mostly mild and transient, and no severe unsolicited AEs or medically attended AEs were observed. None of the serious AEs or AEs leading to TAK-003 withdrawal and/or trial discontinuation was assessed as related to TAK-003 or trial procedure. The immunogenicity and safety data from this trial are consistent with those observed in other TAK-003 trials that involved administration of TAK-003 after shorter storage times, including the DEN-301 efficacy trial (ClinicalTrials.gov NCT02747927) involving >20,000 participants that demonstrated efficacy against dengue infection in endemic regions.

0163

PHYLODYNAMICS VERSUS EPIDEMIOLOGICAL DYNAMICS OF DENGUE IN BANGKOK, 1994-2014

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Dengue virus is endemic in Thailand, and all four serotypes have been co-circulating for decades. It constitutes an ideal setting to explore the dynamics of dengue genetic diversity, and how it relates to the epidemiology. In this study, we sequence the whole genomes of 1814 viruses (DENV1: 617; DENV2: 415; DENV3: 424; DENV4: 358) isolated in Bangkok, Thailand between 1994 and 2014. We reconstruct the phylodynamics of each serotype to characterise how their genetic diversity has changed over the two decades and relate these changes to concurrent dynamics in dengue cases by leveraging two other independent sources of data. The first source is the passive surveillance data for Bangkok, which while not serotype-specific, provides the most reliable indicator of the dynamics of dengue cases. The second are case counts from Queen Sirikit National Institute of Child Health (QSNICH), which constitute a subset of the cases reported in the passive surveillance and captured 1.5 to 29.3% of the cases reported during the study period, but are characterised to the serotype-level. Using various schemes, we adjust the QSNICH time series to account for the total number of cases reported in the passive surveillance data. Using time series analyses, we compare and contrast the serotype dynamics of case counts with the genetic diversity time series. In the process, we highlight the strengths and weaknesses of the datasets, their utility, and help build a better understanding of the dynamics of dengue in Thailand.

0164

CHYMASE AND LIPOPOLYSACCHARIDE BINDING PROTEIN: POTENTIAL BIOMARKERS FOR SEVERE DENGUE

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Dengue is the most common vector-borne viral disease worldwide. Most cases are mild, but some will evolve into severe dengue (SD), with high lethality. Therefore, it is important to identify which patients will develop severe disease. The aim of this study was to quantify chymase and lipopolysaccharide binding protein (LBP) levels in serum or plasma from dengue patients with varying degrees of severity and analyze the potential of these molecules as severity biomarkers. A descriptive cross-sectional study was performed. Patients from Paraguay with confirmed dengue (NS1 and/or rRT-PCR positive) and a duration of symptoms ≤8 days were included. Median age was 38 years old (IQR=31,5) and DENV-4 was the predominant serotype. Cases were classified according to 2009 WHO criteria in 3 groups: dengue without warning signs (DWS-), dengue with warning signs (DWS+) and SD. Chymase levels were analyzed in 145 samples and median concentration was significantly higher in patients with SD compared to the DWS- and DWS+ groups (p=0.0003 and p=0.0005, respectively). For LBP analysis, 108 positive dengue patients and 55 healthy controls were included. Mean LBP level were significantly higher when comparing SD with all other groups (p≤0.0001 for all comparisons). Mean LBP concentration in DWS+ group was also significantly higher than that of healthy controls (p=0.0004). Levels of chymase and LBP increased according to severity of dengue cases. These results, in accordance with previous studies, indicate that chymase and LBP have potential as severity biomarkers, and their performance as prognostic tests warrants further evaluation.

0165

NEUTRALIZING AND ENHANCING EFFECTS OF DENGUE ANTIBODIES IN INDIVIDUALS OVER TIME WITH DIFFERENT DISEASE OUTCOMES

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Heterotypic secondary dengue infection is a risk factor for the development of severe dengue. The humoral immune response during heterotypic secondary DENV infection is complex and dynamic and consists of a polyclonal response of pre-existing antibodies against the previous infecting serotype and newly generated antibodies. In order to assess the contribution of the developing humoral immune response to the course of acute infection, we have determined antiDENV antibody titers, neutralizing antibodies, percentages of antibodies binding to DENV-infected cells and *in vitro* antibodydependent enhancement (ADE) to the infecting serotype in two independent patient cohorts of DENV-infected Cambodian children (n=54). Individuals were either asymptomatic infected (n=10, asymptomatic dengue, ASD) or hospitalized (n=44, clinical dengue, CD) and classified according to WHO classification criteria. The results showed that DENV-IgG titers and neutralizing titers were higher in secondary dengue cases compared to primary dengue cases, but no difference was observed between ASD and CD cases. Percentages of antibodies bound to DENV-infected cells are higher at the time of inclusion in secondary infected cases and are associated with disease outcome after DENV-2 infection and severity in hospitalized patients after DENV-1 infection. ADE-mediating antibodies evolved over time and were highest against the infecting serotype. ADE, IgG titers, and titers of neutralizing antibodies were highly correlated to each other but less to percentages of antibodies binding to DENV-infected cells. Taken together, these data indicate that binding antibodies might contribute to protection or pathogenesis via other mechanisms not related to neutralization or enhancement of infection. These findings are important for the understanding of the measurement of the totality of the humoral response to natural infection and vaccine candidates that may identify novel correlates of protection.

0166

THE BURDEN OF DENGUE IN LATIN AMERICA AND ASIA -EPIDEMIOLOGICAL DATA FROM A PHASE 3 TRIAL

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One of the challenges faced by global dengue disease surveillance is the lack of comparability across surveillance systems. Since September 2016, we have been conducting TIDES (ClinicalTrials.gov NCT02747927), a phase 3 trial of Takeda's tetravalent dengue vaccine candidate (TAK-003) among 20,099 healthy children aged 4-16 years at enrolment across 26 study centers in the Philippines, Sri Lanka, Thailand, Brazil, Colombia, Dominican Republic, Nicaragua and Panama. Here we present data collected over approximately 39 months in the placebo group (n=6,687 as safety population) to provide comparable burden of disease information across countries and regions. During 21,142.5 person-years of active surveillance, there were 8,254 febrile illness cases recorded at study centers (IR=39/100 person-years). Of these, overall, 6% (503/8,254) were virologically confirmed dengue (VCD) using a central laboratory RT-PCR, 7.8% (334/4,287) in Asia and 4.3% (169/3,967) in Latin America. The majority (66.4%) of VCD cases occurred within Asian study sites. The overall incidence of VCD decreased with age group, 3.2/100 Person-Years in those aged 4-5 years at enrolment, 2.5/100 Person-Years in those aged 6-11 years at enrollment, and 1.8/100 Person-Years in those aged 12-16 at enrolment. Overall, 126 VCD cases required hospitalization (IR=0.6/100 person-years), 83% of which were in Asia. Ten VCD cases in Asia and three VCD cases in Latin America met the WHO 1997 Dengue Hemorrhagic Fever case definition. Overall, DENV-1 was the most prevalent serotype seen among VCD cases (39%), followed by DENV-2 (36%), DENV-3 (22%) and DENV-4 (3%). The data confirms a higher burden of dengue in Asia. We will present more detailed analysis including country-specific data.

0167

TRENDS AND CHARACTERISTICS OF HUMAN RABIES IN NORTHWESTERN BORNEO ISLAND, 2017 - 2020

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Since 2017, rabies has been an ongoing public health concern in Northwestern Borneo, specifically in the Malaysian state of Sarawak. This investigation provides the first insight into the trend and epidemiological characteristics of human rabies in the region. Data on laboratory-confirmed human rabies from 2017 to 2020 were gathered from official online press statements reported by the Director General of Health Malaysia. A total of 31 cases of human rabies has been admitted for the past four years with an average of 7.75 cases per year and a case-to-fatality rate of 93.5%. Initially clustered in the South, human rabies cases have now spread northwards. Overall, male cases outnumbered the females with a male-to-female ratio of 2.1:1. The human rabies victims were primarily adult males within the age group 45-64. The majority of cases among children and adolescents were from rural areas. In contrast, most adult patients were found to be urban residents. Dogs were responsible for all animal exposures with 60% of them being stray. The rate of rabies post-exposure prophylaxis (PEP) was low with only two victims completed the full regime. The median incubation period for human rabies was 65 days, while the median duration of disease was 12 days. The most common clinical symptoms were weakness in the limbs, hydrophobia, fever and aggressiveness. Interestingly, the only two rabies survivors were both children with history of immediate and complete PEP treatment. All in all, we identified the culture of adopting stray dogs and poor treatment-seeking behaviour as the risk factors for human rabies in Sarawak. Hence, emphasizing awareness, improving PEP accessibility and intensifying mass dog vaccination may help to mitigate rabies in Sarawak.

0168

GLYCATED CHITOSAN REDUCES MORTALITY IN A LETHAL MOUSE MODEL OF SARS-COV-2

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The rapid emergence and global dissemination of the novel coronavirus, SARS-CoV-2, has led to an ongoing global health burden of severe morbidity and mortality, particularly in aged-populations. SARS-CoV-2, which was first detected in late 2019, has infected more than 124 million people worldwide and caused over 2.8 million deaths in a year. While multiple vaccine countermeasures have been approved for emergency use, additional non-specific treatments continue to be needed as a sluggish vaccine rollout, vaccine refusal, and neutralization-escape variants threaten to prolong the pandemic. Immunoadjuvant compounds delivered intranasally can shape non-specific innate immune responses during the critical early stages of viral infection, reducing morbidity and mortality as well as physically interfering with virus-receptor interactions. Glycated chitosan (GC), a polymer of β-0-4-linked N-acetylglucosamine solubilized by the conjugation of galactose glycans, has previously been described as a water-soluble immunoadjuvant for anti-tumor therapy. GC administered intranasally before and after otherwise lethal SARS-CoV-2 exposure diminished morbidity and mortality in humanized ACE2-receptor-expressing mice by up to 75% and reduced infectious virus levels in the upper airway and lungs. Coinciding with lower viral levels in lungs,

a reduction in both the severity of lung lesions and total inflammation was observed in GC-treated compared to mock-inoculated SARS-CoV-2 infected mice. Our findings demonstrate a potential new application for soluble immunoadjuvants such as GC for preventing and possibly treating SARS-CoV-2.

0169

KNOWLEDGE AND RISK PERCEPTION ABOUT ZIKA AMONG REPRODUCTIVE-AGED WOMEN IN THE ZIKA VIRUS OUTBREAK IN PERU

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The aim of the study was to identify the knowledge and risk perception about Zika among women of childbearing age in Peru in 2017. Cross-sectional study, in which a secondary database analysis was carried out from three cities in Peru (Lima, Piura and Iquitos). Of the 716 women interviewed, only 85.5% heard of Zika although they were in the Zika virus outbreak, only 72.4% of women identified that they can get Zika in their community and 65.6% properly recognize that all people who can get Zika virus. Knowledge of the different mechanisms of transmission of Zika is greater in the northern of Peru. Women mostly recognized the transmission of mosquitoes (79.9%) in contrast to sexual transmission (30.2%), blood transfusion (36.6%) and vertical transmission (37.0%). Comparing these knowledge according sociodemographic variables, it was found that there were no differences according to the number of children, occupation, number of housemates, or time of residence, only having undergraduate studies increased it($p<0.05$). In addition, there were no differences identifying these transmission mechanisms whether they were pregnant or not ($p\geq0.05$). They obtained the information mostly from television (55.9%) and health workers (33.7%). They identified the following risks: 51.3% for pregnant women (abortion) and 40.6% for the fetus risk (microcephalus). To conclude, there is a need to increase the women's knowledge about the other ways of transmission different from mosquito and the use of television combine with health workers' educational campaigns could be useful in new interventions.

0170

RANDOMIZED CLINICAL TRIAL, PHASE 2, TO EVALUATE THE EFFICACY OF ARTESUNATE IV ALONE OR COMBINED WITH VITAMIN C IV FOR THE TREATMENT OF COVID-19

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There is currently no effective, proven, and safe treatment for severe illness caused by SARS-CoV-2. Artesunate showed its anti-inflammatory and antiviral *in vitro* activity against SARS-CoV-2. Vitamin C, known for its antioxidant propriety is reported to be effective on the immune system response, has antiviral properties and reduces cytokine storm in acute respiratory distress syndrome. A total of seventy (70) patients were screened and forty- four patients (44) with confirmed COVID-19 were enrolled in the study and divided into three groups: artesunate IV (n = 15), artesunate IV+Vit C (n=14) and control (n = 15). The primary outcome was the time taken to reach undetectable levels of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) and the percentage of participants with undetectable SARS-CoV-2 on days 7, 14, 21 and 28. The computed, adverse events, and abnormal laboratory parameters were corrected interval QT changes the secondary outcomes. The median time to reach undetectable viral RNA was 15 days for the artesunate group, 14 days for artesunate IV plus Vit C and 21 days for the control group. The percentages of patients with undetectable viral RNA on days 7, 14, 21, and 28 were 20%, 6.7%, 13.3%, and 0%, respectively, in the control group; 40%, 33.3%, 13.3%, and 0%, respectively, in the artesunate IV alone group and 21.4%, 21.4.%, 14%, and 7.1%, respectively, in the artesunate IV plus VitC group. Electrocardiography adverse events were more common in each group. Premature discontinuation of treatment in placebo occurred because of serious adverse events. No biological failure was noted. In conclusion, there is a trend on the fact that patients in artesunate alone group has a better treatment success. However, a large-scale study should be conducted for evidence based and immunological parameters will be done.

0171

DETECTION OF SARS-COV-2 IN EXHALED AIR USING NON-INVASIVE EMBEDDED STRIPS IN MASKS

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SARS-CoV-2 emerged in 2019 and resulted in a pandemic causing millions of infections and deaths. Early in the pandemic, testing was severely limited and has only recently caught up with demand. The gold-standard for SARS-CoV-2 detection uses quantitative RT-PCR on respiratory specimens (nasopharyngeal swab, nasal swab, saliva, etc.) to detect viral RNA (vRNA). Acquiring these samples is invasive, can be painful for those with xerostomia and other health conditions, and sample quality varies greatly based on sample source, collection method, and the skill of the collector. Frequently, only symptomatic individuals are tested, despite the fact that asymptomatic individuals can have comparable viral loads and efficiently transmit virus. Therefore, we sought to utilize a non-invasive approach to detect SARS-CoV-2 in individuals regardless of symptom status, using polyvinyl alcohol (PVA) strips embedded in KN95 masks. In laboratory experiments, we show recovery of vRNA, viral protein and infectious virus from virus-spiked PVA strips with detection limits comparable to nasal swab samples. We then recruited 24 individuals from healthcare settings in the Denver area that had recently tested positive for SARS-CoV-2 to wear masks embedded with PVA strips. Seven participants (29%), including both symptomatic and asymptomatic individuals, had detectable SARS-CoV-2 vRNA on their PVA strips. Additionally, we were able to culture infectious virus and sequence vRNA from these PVA strips. Varying levels of human RNA were detected on strips, indicating a range in the amount of material participants exhale. The longer the mask was worn, the more host and vRNA was detected. Participants were surveyed on their activities while wearing the mask. Talking was significantly associated with detecting SARS-CoV-2 on the PVA strip. Together these results demonstrate the feasibility of using masks embedded with PVA strips as a non-invasive platform for detecting SARS-CoV-2 in exhaled air in both symptomatic and asymptomatic individuals. We are expanding this study to a larger cohort of index cases and their contacts to track and evaluate transmission.

0172

DIRECT DETECTION OF GUAICO CULEX VIRUS IN CULEX SPP. MOSQUITOES OF COSTA RICA

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Guaico Culex Virus (GXCV) is a multicomponent Jingmenvirus that infects mosquitoes. It was isolated in 2014 from *Culex declarator* in Trinidad, and later from *Culex coronator* and/or *Culex interrogator* in Peru, Brazil, and Panama. Considering that all previous detections of GCXV were done after isolation and replication of viruses in cell lines, our aim was to implement a one-step reverse trascriptase PCR (RT-PCT) and a real-time RT-PCR protocol to detect GCXV RNA directly from field-caught mosquitoes in Costa Rica. Before analyzing mosquitoes, both protocols were tested to confirm no unspecific amplification of arboviruses, including dengue (DENV), Zika (ZIKV), and yellow fever (YFV) viruses. A total of 387 *Culex* spp. mosquitoes were collected from different regions of the country using CDC (with CO_2) and gravid female traps. They were pooled by location, species, and date of collection into 87 pools of up to 10 individual mosquitoes. RNA was extracted and analyzed by RT-PCR and real-time RT-PCR using primers that amplify a 240 bp fragment of genome segment 1 of GCXV. The following controls were included in each PCR run: GCXV cDNA from cell culture isolate, purified GCXV Segment 1, cDNA from uninfected *Aedes aegypti* (laboratory colony), and a reagent control. GCXV was detected by both protocols in only one pool (4 females) of *Cx. coronator* mosquitoes from the Carribean region of the country. The product obtained was sequenced and it showed 98.9 % (87/88) similarity to sequences of GCXV obtained from *Cx. coronator* in Peru. Of interest, similarity with GCXV sequences from Panama was 93.2 to 95.5 %. Results confirm that GCXV is present in Costa Rica, and that this virus can be detected directly in mosquitoes by one-step and real time RT-PCR. Although GCXV has not been associated with human infection, continuing studies on distribution, ecology and mosquito-virus interactions are necessary to determine possible implications of infection on mosquito fitness and vector competence for pathogenic arboviruses.

0173

SEROLOGICAL EVIDENCE OF LOW PATHOGENIC AVIAN INFLUENZA SEROTYPE H9 IN LIVE BIRD MARKETS, JOS, PLATEAU STATE NIGERIA

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Avian influenza is a zoonotic disease that can adversely affect public health and the economy. Nigeria reported outbreak of avian influenza for the first time in 2006, Virological and serological survey thereafter revealed the importance of local birds in live bird markets in the epidemiology of avian influenza in Nigeria. In the present study, 276 serum samples were collected over 5 months from apparently healthy local birds at live bird markets in two Local Government Areas in Plateau State. The Detection of influenza A antibody was carried out using Enzyme linked immunosorbent assay and further tested by haemagglutination inhibition to determine the specific serotype of the influenza A virus. The result showed a prevalence of 30.4% (n=84) of antibody to influenza A, 26% (n=72) of serotype H9, 1.4% (n=4) of serotype H7, and none was confirmed to be H5 serotype. Comparatively Jos-North had higher relative risk of the disease as compared to Jos-South. This study indicated the presence of avian influenza virus in live bird market within the study area with the dominance of antibody to H9. To our knowledge, this is the first serological indication of serotype H9 in Plateau State and Nigeria. Evidence of influenza A/H9 in an ecological niche known for the circulation of subtypes H5Nx may complicate the epidemiology and control of avian influenza in region and Nigeria at large. Live-bird market operators and poultry farmers should increase or maintain a good level of biosecurity and limit co-mingling with local birds to prevent the transmission of the virus which may have adverse effects on poultry production, national and international trade, the economy and public health.

0174

CO-CIRCULATION OF CHIKUNGUNYA VIRUS, ALL FOUR DENGUE VIRUSES AND ZIKA VIRUS IN GUERRERO, MEXICO, 2019 AND EVIDENCE OF GROSS UNDER-REPORTING OF ARBOVIRAL DISEASES IN THE REGION

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A clinical investigation was performed to test the hypothesis that arboviral diseases are grossly underreported in Mexico. Sera were collected from human subjects from low-income households in Guerrero, southwest Mexico. Guerrero has a high (66.5%) poverty rate and therefore, many of its residents have restricted access to healthcare and diagnostic testing. We specifically targeted individuals from households poorly represented in the national arbovirus diagnosis and surveillance program. The sample population consisted of 639 patients who developed febrile illness in 2019. Patients were serologically assayed for chikungunya virus (CHIKV) and select flaviviruses, including dengue virus (DENV) and Zika virus (ZIKV). Plaque reduction neutralization test (PRNT) revealed that 181 (28.3%) patients were seropositive for CHIKV. To identify patients with acute CHIKV infections, a subset of sera from seropositive patients were tested for CHIKV-specific IgM. Sera from 21 of 189 (11.1%) patients were positive. These patients met the chikungunya case definition. The number of chikungunya cases identified in this study is over twofold higher than the total number of cases reported across all of Mexico by the public health authorities that same year. To identify patients with acute flavivirus infections, sera from select patients (n = 263) were tested for flavivirus NS1. Sera from 48 (18.3%) patients contained viral antigen. All NS1-positive sera were titrated and tested by PRNT using DENV-1 to -4, St. Louis encephalitis virus, West Nile virus and ZIKV. Seven patients were seropositive for DENV-1, five patients were seropositive for DENV-2, one patient was seropositive for DENV-3 and two patients each were seropositive for DENV-4 and ZIKV. The remainder had secondary flavivirus infections or antibodies to an undetermined flavivirus. In summary, we provide evidence for the concurrent circulation of CHIKV, all four DENV serotypes and ZIKV in Guerrero, Mexico. The public health authorities reported no cases of CHIKV, DENV-3, DENV-4 and ZIKV in Guerrero in 2019 and therefore, we provide evidence of gross under-reporting in the region.

0175

AN EPIDEMIOLOGICAL STUDY OF A SARS-COV-2 OUTBREAK INVOLVING A RURAL CHURCH SERVICE

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We performed an outbreak investigation of SARS-CoV-2 – the virus causing the novel coronavirus disease (COVID-19) – among 57 cases associated with a church service that took place in March 2020 in a rural and predominantly elderly community in Sumter County, Florida. As part

of the investigation, we interviewed 151 church members to identify cases of SARS-CoV-2 associated with a church service. Of the 151 church members interviewed, 90 reported attending the service in question and 46 reported symptoms consistent with SARS-CoV-2 infection within 14 days of attending, corresponding to an attack rate of 51%. Including secondary cases, the investigation uncovered 32 confirmed and 25 probable cases associated with this church service. Of these cases, 46 reported attending the church service and 11 were close contacts of those who attended. Four deaths were linked to this outbreak, revealing a case fatality rate of 5.26%. The estimated attack rate of this church service was higher than previous studies, suggesting that this particular service describes a potential "super-spreading" event. Factors likely contributing to the high attack rate observed in this outbreak included frequent hand shaking, singing, communal meals, and a lack of masks and social distancing. In addition to the high attack rate, this outbreak also had a high case fatality rate likely owing to the high median age (64 years) and prevalence of comorbidities in this population. This study provides important information to help understand the factors contributing to the high SARS-CoV-2 attack rates observed in faith-based settings.

0176

DO NOROVIRUS AND SAPOVIRUS GASTROENTERITIS EPISODES IN EARLY CHILDHOOD PROTECT AGAINST FUTURE EPISODES?

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Human caliciviruses are among the leading causes of acute gastroenteritis (AGE) in children. Little is known about whether natural norovirus and sapovirus episodes evoke protection against future episodes, information needed for vaccine development. We estimated the protective efficacy of first episodes on future episodes using a Nicaraguan population-based birth cohort. Starting in June 2017, 444 children were followed weekly for AGE episodes until 36 months of age; stools collected during AGE episodes were tested by RT-qPCR. As of June 2020, 83, 109, and 97 children experienced a first norovirus GI, norovirus GII, and sapovirus AGE episode, respectively. Of these, 15, 20, and 16 children experienced subsequent norovirus GI, norovirus GII, and sapovirus AGE episodes, respectively. Because children who experience a first episode are likely to have characteristics that place them at higher risk of future episodes, we used two different statistical approaches to estimate hazard ratios (HR), accounting for confounding: 1) an adjusted Andersen-Gill (AG) Model, and 2) a case-only approach comparing the event rates of norovirus and sapovirus episodes to the rates of other AGE episodes with similar risk factors (negative control method). The adjusted AG Model found that first episodes did not protect against future episodes, however, the hazard for future norovirus episodes decreased after second episodes: HR: 0.29 (95% CI: 0.11, 0.78) after two norovirus GI episodes, and HR: 0.11 (95% CI: 0.03, 0.44) after two norovirus GII episodes. In contrast, the negative control method found a lower hazard of subsequent episodes even after the first episode of sapovirus (HR: 0.31, 95% CI: 0.06, 0.83), norovirus GI (HR: 0.67, 95% CI: 0.32, 1.31), and norovirus GII (HR: 0.20, 95% CI: 0.04, 0.44). Although results differed between methods used, these findings suggest that norovirus and sapovirus AGE episodes in early childhood confer some protection against future episodes. Further analyses to include additional confounders in the AG Model and to estimate the protective efficacy against genotype-specific norovirus and sapovirus AGE episodes are underway.

0177

DETECTION OF ANTIBODIES TO LOKERN, MAIN DRAIN, ST. LOUIS ENCEPHALITIS AND WEST NILE VIRUSES IN VERTEBRATE ANIMALS IN CHIHUAHUA, GUERRERO AND MICHOACÁN, MEXICO

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We conducted serologic surveillance for flaviviruses and orthobunyaviruses in vertebrate animals in Mexico in 2018-2019. Sera were collected 856 vertebrate animals, including 323 dogs, 223 horses and 121 cows, from 16 species. The animals were from three states: Chihuahua in northwest Mexico (704 animals) and Guerrero and Michoacán on the Pacific Coast (27 and 125 animals, respectively). Sera were assayed by plaque reduction neutralization test using four flaviviruses (dengue type 2, St. Louis encephalitis, West Nile and Zika viruses) and six orthobunyaviruses from the Bunyamwera (BUN) serogroup (Cache Valley, Lokern, Main Drain, Northway, Potosi and Tensaw viruses). Antibodies to West Nile virus (WNV) were detected in 154 animals of nine species, including 89 (39.9%) horses, 3 (21.4%) Indian peafowl and 41 (12.7%) dogs. Antibodies to St. Louis encephalitis virus (SLEV) were detected in seven animals, including three (0.9%) dogs. Antibodies to Lokern virus (LOKV) were detected in 22 animals: 19 (8.5%) horses, 2 (1.7%) cows and a dog (0.3%). Antibodies to Main Drain virus (MDV) were detected in three (1.3%) horses. WNV and LOKV activity was detected in all three states, SLEV activity was detected in Chihuahua and Michoacán, and MDV activity was detected in Chihuahua. None of the animals were seropositive for Cache Valley virus, the most common and widely distributed BUN serogroup virus in North America. In conclusion, we provide serologic evidence that select flaviviruses and BUN serogroup viruses infect vertebrate animals in Chihuahua, Guerrero and Michoacán. We also provide the first evidence of LOKV and MDV activity in Mexico.

0178

DETAILED VIRAL SHEDDING KINETICS PRIOR TO SYMPTOM ONSET IN PATIENTS WITH SARS-COV-2 INFECTION

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There are limited data on the detailed kinetics of pre-symptomatic viral shedding of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Through the pre-admission screening and epidemiological investigation, we had a unique opportunity to acquire the PCR results of patients with SARS-CoV-2 infection in their pre-symptomatic period. We retrospectively reviewed patients with SARS-CoV-2 infection who were linked with nosocomial clusters in a tertiary referral center (Asan Medical Center, Seoul) in South Korea between March 1, 2020 and December 15, 2020. Patients with a positive PCR result at least 2 days before symptom onset were categorized as pre-symptomatic viral shedders, and those with a negative PCR result at 1 or 2 days before symptom onset were categorized as symptomatic viral shedders. Patients who did not meet either criterion were categorized as indeterminate cases. During the study period, a total of 19 patients who had one or more PCR results within 7 days prior to symptom onset were identified. Of them, 6 patients were classified as pre-symptomatic viral shedders and 10 patients were classified as symptomatic viral shedders; the remaining three patients were classified as indeterminate cases. In the pre-symptomatic shedders, the median duration of viral shedding prior to symptom onset was 3 days (range,

2-5 days). Four of the 6 pre-symptomatic viral shedders were linked to 7 secondary infection cases that they had contact with during the pre-symptomatic phase, whereas none of the 10 symptomatic viral shedders were linked to any secondary cases. While more than half of the patients had no evidence of pre-symptomatic viral shedding prior to symptom onset, about one-third of the patients shed SARS-CoV-2 around 3 days prior to symptom onset and led to secondary infection cases during the pre-symptomatic period. These findings reaffirm the possibility of pre-symptomatic shedding and infectivity of SARS-CoV-2; in contrast, the virus excretion does not precede symptoms in a considerable proportion of the patients.

0179

RHABDOMYOLYSIS AS PRESENTING FEATURE OF COVID-19: CASE SERIES AND LITERATURE REVIEW

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Rhabdomyolysis has been increasingly recognized as a complication of COVID-19. We describe four cases of rhabdomyolysis as presenting feature of SARS-CoV-2 infection and conduct a review of the current literature. Four patients between 26 and 80 years were hospitalized with COVID-19 in a New York City Hospital system during March and April 2020. All patients presented with myalgias and had evidence of rhabdomyolysis on admission. Only two patients had respiratory symptoms. Creatinine kinase (CK) ranged from 15,000 to 400,000 U/L. Three patients required renal replacement therapy and died during the hospitalization. We identified 41 case reports with 70 patients and one observational study with 140 patients that were published between June 2020 and March 2021 with a total of 210 cases of rhabdomyolysis in COVID-19. Among those patients, 148 (71%) were male. The majority were Black (45%), followed by Hispanic (16%) and Non-Hispanic White (8%) patients. The most common co-morbidities were hypertension (61%), diabetes mellitus (39%), chronic kidney disease (21%) and dementia (11%). Patients presented most often with dyspnea (59%), fever (54%) and cough (50%). Respiratory symptoms were absent in one third of cases and 20% complained of myalgias. Acute kidney injury developed in 75% of cases and 23% required renal replacement therapy. CK levels differed widely between the observational study and the pooled case reports with a median peak CK of 2,209 U/L and 13,581 U/L, respectively. The most commonly used COVID-19 specific therapies described in the case reports were hydroxychloroquine (30%), azithromycin (24%) and steroids (21%). The in-hospital mortality for all combined cases was 45%. In conclusion, patients with COVID-19 and rhabdomyolysis can present with severe myalgias in the absence of respiratory symptoms. Acute kidney injury is common and may require renal replacement therapy. More research is needed to understand the underlying mechanism.

0180

MOLECULAR BARRIERS TO SARS-COV-2 REPLICATION IN BAT CELLS

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Bats are natural reservoirs for numerous coronaviruses, including the potential ancestor of SARS-CoV-2. Knowledge concerning the interaction of coronaviruses and bat cells is, however, sparse. There is thus a need to develop bat cellular models to understand cell tropism, viral replication, and virus-induced cell responses. Here, we report the first molecular study of SARS-CoV-2 infection in chiropteran cells. We investigated the susceptibility to SARS-CoV-2 infection of a panel established bat cell lines, belonging to the species *Myotis myotis*, *Eptesicus serotinus* and *Tadarida brasiliensis*, as well as novel *Nyctalus noctula cells*, which was completed with primary cells from *Rhinolophus ferrumequinum* and *Myotis spp.* bats. None of these cells were sensitive to infection, not even the one expressing detectable levels of angiotensin-converting enzyme 2 (ACE2), which serves as the viral receptor in many mammalian species including humans. Following transduction with human ACE2, six bat cell lines expressed comparable or higher level of hACE2 than a permissive human cell line. In three out of these six cell lines, the resistance to infection was overcome by hACE2 expression, suggesting that restriction to viral replication was due to lack of bACE2 expression or absence of bACE2 binding. By contrast, multiple restriction factors to viral replication exist in the three *N. noctula* cells since hACE2 expression was not sufficient to permit infection. Notably, viral replication was efficiently controlled in *E. serotinus* cells and correlated with a potent induction of interferon-stimulated genes. Despite high level of replication, infectious virions were not released from *M. myotis* cells. Together, our data highlight the existence of species-specific molecular barriers to viral replication in bat cells. Our newly developed chiropteran cellular models are useful tools to investigate the interplay between viruses belonging to the SARS-CoV-2 lineage and their natural reservoir, including the identification of factors responsible for viral restriction.

0181

SEROPREVALENCE OF RIFT VALLEY FEVER VIRUS IN URBAN KENYA: A POTENTIAL PUBLIC HEALTH BURDEN HIDING IN PLAIN SIGHT

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Rift Valley fever virus (RVFV) is a mosquito transmitted bunyavirus that can also transmit directly to humans from animals and can cause severe infections in both. Previous studies have shown that consuming raw milk and preparing meat are risk factors for RVFV exposure, but it is difficult to disentangle those risk factors from other livestock rearing activities. Urban areas have an increased demand for animal source foods, different vector distributions, and various arboviruses are understood to establish localised urban transmission cycles. Thus far, RVFV is an unevaluated public health risk in urban areas. We tested our ongoing urban cohort study on dengue (DENV) and chikungunya (CHIKV) virus for RVFV exposure and found 34/2,779 (1.2%) of adults in two urban areas in Kenya had anti-RVFV antibodies. Higher seroprevalence was seen in the larger western city, Kisumu (1.7%), compared to Ukunda (0.9%) on the coast, and 88% also had co-exposure to DENV, CHIKV, or both. Unlike rural areas, 92% of the cohort did not own animals, but RVFV exposure was significantly associated with seeing goats around the homestead ($p=.02$) which could represent viral amplifiers. Consumption of raw milk($p=.05$) and not boiling milk ($p=.002$) were also significant risk factors for exposure. To further characterize food-related risk factors, we conducted a nested case-control study and those buy unpasturized milk, from milk vendors or private purchasing from dairy owners, were three times as likely to have RVFV exposure ($OR=3.3$, $p=.07$). 98% of all participants consumed beef at least

monthly and 2/105 reported tasting the raw beef before cooking and had RVFV exposure. Using animal blood for cooking (OR=3.3, *p=.08)* was also associated with an increased odds of RVFV exposure. None of the seropositive participants knew they had been exposed to or been tested for RVFV, yet were more likely to report occassional sudden decreases in vision compared to seronegative participants (OR=6.25, *p=.00*). Overall this study provided baseline evidence that there may be an urban burden of RVFV tied to food consumption practices and risk factors for disease may differ from rural areas.

0182

PREVALENCE AND VERTICAL TRANSMISSION OF SARS-COV-2 INFECTION AMONG PARTURIENTS FROM LUANDA, ANGOLA

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SARS-CoV-2 emerged in China in December 2019. Currently, the infection constitutes the main public health problem. Although some studies have identified SARS-CoV-2 in pregnant women, the possibility of vertical transmission to newborns remains uncertain. In the present study, we investigated the prevalence and vertical transmission of SARS-CoV-2 among 3610 parturients from Luanda, the capital city of Angola. The parturients were screened to SARS-CoV-2 antigen using the COVID-19 Ag Rapid Test Device kit. The age of parturients ranged from 13 to 57 years. A total of 0.3% (10/3610) parturients tested positive on the day of delivery and of these, 2/10 (33.3%) remained positive until the seventh day after the delivery. Moreover, a total of 2 parturients died from the SARS-CoV-2 infection. None of the newborns tested positive in the first 24 hours after birth, however, one of the newborns tested positive seven days after birth. Our results showed a low prevalence of SARS-CoV-2 among the parturients in Luanda. The low rate of transmission observed in this study suggests that the care of the mother infected with SARS-CoV-2 is crucial for continuing breastfeeding. On the other hand, our findings suggest that is necessary to increase SARS-CoV-2 screening in pregnant women, in order to reduce the maternal or newborn mortality rate in Angola.

0183

KNOWLEDGE, ATTITUDES, AND PRACTICES REGARDING SARS-COV-2 IN A UNIVERSITY COMMUNITY, MASSACHUSETTS, USA AND THE OPTIMIZATION OF COVID-19 RESPONSE PLANNING: A QUALITATIVE STUDY

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Mitigation of the spread of SARS-CoV-2 within a population is impacted by the degree to which individuals adhere to recommended infection control measures. An evaluation of individuals' knowledge, attitudes, and practices (KAP) regarding SARS-CoV-2 can provide information regarding the most effective ways to tailor a COVID-19 response program for maximum adherence. Few studies have evaluated knowledge, attitudes, and practices regarding SARS-CoV-2 in university campus communities. We used the information gathered during these interviews to inform COVID-19 response efforts for the Spring 2021 semester. We performed focus groups with undergraduate students, graduate students, faculty members, and staff members at the University of Massachusetts Amherst to investigate COVID-19-specific KAP. Focus group discussions involved sources of COVID-19 related information, definitions of isolation/quarantine, perceptions of contact tracing, and risk perceptions regarding socialization activities. We asked about experiences in utilizing the campus asymptomatic testing center and gathered any improvement suggestions. Interviews were conducted individually with those who had experienced isolation or quarantine during the Fall 2020 semester. Interview questions included sources of information regarding COVID-19, experiences while in isolation/quarantine, resources utilized or desired while in isolation/quarantine, impacts on academic performance and mental health, and overall perceptions of the contact tracing program. We gathered feedback regarding improvements for the asymptomatic testing center, contact tracing program, and on-campus isolation/quarantine coordinators. Suggestions for improvement were incorporated into Spring 2021 planning efforts as appropriate. Qualitative data analysis will utilize Dedoose software for thematic analysis. Results are expected in August 2021. This study is innovative in being one of the only studies performed in a university community to investigate COVID-19-specific KAP and to incorporate feedback from these studies into future COVID-19 response planning efforts.

0184

THREE YEARS OF INSECTICIDE RESISTANCE MONITORING IN FIVE SENTINEL SITES IN CAMEROON: CONTRIBUTION TO VECTOR CONTROL DECISION MAKING

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The U.S. President's Malaria Initiative VectorLink Cameroon project has conducted annual insecticide resistance monitoring in five sentinel sites (Bonabéri, Gounougou, Nyabessang, Mangoum, and Simatou) from 2018-2020. Bioassays were carried out using World Health Organization (WHO) susceptibility test kits and CDC bottle bioassays depending on the insecticide. Larvae collected in the field and reared to 2-5-day-old adult female *Anopheles gambiae* s.l. were tested against alpha-cypermethrin (0.05%), deltamethrin (0.05%), permethrin (0.75%), pirimiphos-methyl (0.25%), bendiocarb (0.1%), clothianidin (2%), and chlorfenapyr (100 and 200 µg/bottle). Resistance status was determined using WHO criteria. When pyrethroid resistance was found, resistance intensity (5x and 10x the diagnostic doses) was assessed and synergist assays using 4% piperonyl butoxide (PBO) were done. Following susceptibility testing, a subset of mosquitoes (dead and alive) was also screened for genetic markers of resistance using PCR assays: knock down resistance (*kdr*)-west, *kdr*-east, acetylcholinesterase (*Ace-1*) and N1575Y. Results showed persistent pyrethroid resistance and varying susceptibility to chlorfenapyr and clothianidin. *An. gambiae* s.l. were resistant to all pyrethroids in all sites, with an average mortality of less than 78% for alpha-cypermethrin and deltamethrin, and about 29% for permethrin. Pirimiphos-methyl and bendiocarb resistance was recorded only in Mangoum. Susceptibility to clothianidin and chlorfenapyr was recorded in all five sites. High pyrethroid resistance was recorded in all sites except Bonabéri, where moderate permethrin resistance and low alpha-cypermethrin resistance was found.

Pre-exposure of *An. gambiae* s.l. to PBO restored susceptibility to alpha-cypermethrin in Bonabéri and partially restored susceptibility to pyrethroids at all other sites. All four mutations were detected in varying frequency by site. These results can inform the country's vector control decision making, particularly in the selection and strategic deployment of insecticide-treated nets for the planned 2022/23 mass campaign

0185

BIOMARKERS OF ACUTE KIDNEY INJURY AND PERSISTENT KIDNEY DISEASE IN CHILDREN WITH SEVERE MALARIA

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Acute kidney injury (AKI) is an important complication in children with severe malaria associated with increased mortality and chronic kidney disease (CKD) in survivors. AKI may appear to resolve in the short-term but can lead to acute kidney disease (AKD) or injury may be sub-clinical and result in the gradual development of AKD. The objective of this study was to evaluate established and next-generation biomarkers of AKI (admission) and AKD (1-month). We conducted a prospective observational study enrolling 600 children with severe malaria between 2014 and 2017. Children had AKI defined during the first 24 hours of hospital admission and AKD at 1-month follow-up using the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines. We tested biomarkers (BUN, Cystatin C, NGAL, IL-18, L-FABP, Angpt-2, sICAM-1, sFlt-1, CHI3L1, CXCL10, sTNFR1, sTREM-1) at admission and 1-month follow-up by ELISA or Luminex. Biomarker performance was evaluated using receiver operating characteristic curve analysis and comparing the area under the curve (AUC). On enrollment, 45.3% of children had AKI and 15.6% of survivors had AKD at one month follow-up. All biomarkers were elevated in children with AKI on admission and increased across AKI stages ($p<0.001$ for all). To determine whether prolonged increases in biomarkers were related to AKD, we measured 10 of the biomarkers at 1-month follow-up. We found 8 of the 10 biomarkers (Cystatin C, NGAL, Angpt-2, sICAM-1, sFlt-1, CHI3L1, sTNFR1, and sTREM-1) remained elevated in children with AKD ($p<0.05$ for all). The top performing biomarkers to identify children with AKD at 1-month follow-up were sTNFR1 (AUC: 78, 95% CI 0.73 to 0.83), sTREM-1 (AUC: 0.84, 95% CI 0.78 to 0.89) and NGAL (AUC: 0.76, 95% CI 0.70 to 0.82). These findings suggest that persistent inflammation (sTNFR1, sTREM-1) and tubular injury (NGAL) at one-month follow-up in children with ongoing kidney disease. Future studies need to assess biomarkers of AKI and AKD in urine, as a more direct measure of kidney injury, and to evaluate how markers of AKI and AKD relate to long-term risk of CKD.

0186

STUDIES OF THE PARASITE-MIDGUT INTERACTION REVEAL PLASMODIUM PROTEINS IMPORTANT FOR MALARIA TRANSMISSION TO MOSQUITOES

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Malaria transmission relies on parasite-mosquito midgut interaction. Targeting on the interactive proteins is hypothesized to block malaria transmission to mosquitoes. We chose 76 genes that contain signal peptide-coding regions and are upregulated and highly abundant at sexual stages. Forty-six of these candidate genes (60%) were cloned and expressed using the baculovirus expression system in insect cells. Six of them, e.g., PF3D7_0303900, PF3D7_0406200 (Pfs16), PF3D7_1204400 (Pfs37), PF3D7_1214800, PF3D7_1239400, and PF3D7_1472800 were discovered to interact with blood-fed mosquito midgut lysate. Among these interactive proteins, previous works showed that knockout the orthologs of Pfs37 or Pfs16 in *Plasmodium berghei* reduced oocysts in mosquitoes. Here we further found that anti-Pfs16 polyclonal antibody significantly inhibited *P. falciparum* transmission to *Anopheles gambiae*. Investigating these candidate proteins will improve our understanding of malaria transmission and discover new targets to break malaria transmission.

0187

INCREASED LEVELS OF HYPOXIA INDUCIBLE FACTOR1-ALPHA: IMPLICATIONS FOR THE PATHOGENESIS OF PEDIATRIC SEVERE MALARIAL ANEMIA

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The molecular basis of *Plasmodium falciparum* severe malarial anemia [SMA, hemoglobin (Hb) <5.0gdL^{-1} with any parasite density] pathogenesis remains only partially understood. Our previous global gene expression profiling revealed that the microRNA (miRNA), hsa-miR-186, was significantly down-regulated in patients with SMA compared to those with non-SMA. Moreover, *in-silico* analysis revealed that hsa-miR-186 potentially targets the mRNA encoding human hypoxia inducible factor 1-alpha (HIF1α) and its consequent expression levels. The current study, therefore, explored this putative pathway. Children of both sexes (n=100, with *P. falciparum,* based on *a priori* classification into SMA (Hb<5.0g/dL; n=26) and non-SMA (Hb≥5.0g/dL; n=74) were recruited at Siaya County Referral Hospital. Peripheral blood samples were obtained for measurement of HIF1α and hsa-miR-186 using ELISA and qRT-PCR, respectively. Bivariate analysis revealed that plasma HIF1α levels were higher in children with SMA [(mean±standard error of the mean (SEM)]; 3.31±0.11 pgmL^{-1}] compared to the non-SMA group [mean (SEM); 2.23 ±0.08 pgmL^{-1}; P=2.697^{e-09}]. Levels of HIF1α were inversely correlated with Hb concentrations (r=-0.540, P<0.001). However, hsa-miR-186 transcripts did not differ between the SMA (1.09±0.25 arbitrary units) and non-SMA (0.88±0.12 arbitrary units) groups (P=0.407) and were not correlated with either HIF1α levels (r=-0.006, P=0.954) or Hb levels (r=-0.030, P=0.766). Collectively, results presented here suggest that HIF1α may play a role in the pathogenesis of SMA and serve as a correlate of anemia, whereas hsa-miR-186 does not appear to influence either HIF1α levels or disease.

0188

IDENTIFICATION OF A LIGAND AND A RECEPTOR THAT MEDIATE PLASMODIUM SPOROZOITE LIVER INFECTION.

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After inoculation by the bite of an infected mosquito, *Plasmodium* sporozoites enter the blood stream and infect the liver, where each infected cell produces thousands of merozoites. These in turn, infect red blood cells and cause malaria symptoms. To initiate a productive infection, sporozoites must exit the circulation by traversing the blood lining of the liver vessels after which they infect hepatocytes with unique specificity. We screened a phage display library for peptides that structurally mimic

(mimotope) a sporozoite ligand for hepatocyte recognition. We identified HP1 (hepatocyte-binding peptide 1) that mimics a ~50 kDa sporozoite ligand (identified as phospholipid scramblase). Further, we determined that HP1 interacts with a ~160 kDa hepatocyte membrane putative receptor (identified as carbamoyl-phosphate synthetase 1). Importantly, immunization of mice with the HP1 peptide protects them from infection by the rodent parasite *P. berghei*. Moreover, an antibody to the HP1 mimotope inhibits human parasite *P. falciparum* infection of human hepatocytes in culture. The sporozoite ligand for hepatocyte invasion is a novel pre-erythrocytic vaccine candidate.

0189

CHARACTERIZATION OF PLASMODIUM MALARIAE RETICULOCYTE BINDING PROTEIN 1A

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The advent of highly sensitive molecular diagnostic methods has demonstrated *Plasmodium malariae* infection is more prevalent than previously thought. Although it is considered to cause mild malaria, it has been implicated in nephrotic syndrome, inflammation of the gall bladder and anaemia-related deaths. These complications are because of its unique biology of a relatively longer life cycle, typical low parasitemia and chronic-promoting factor(s) which allows its persistence in the human host. Furthermore, the current neglect by malaria elimination programmes could lead to the persistence and expansion of *P. malariae* population. With the publication of P. malariae reference genome, Rutledge et al. (2017) also found *P. malariae* reticulocyte binding protein 1a (*Pm*RBP1a) to be the most divergent among the PmRBPs. With computational analysis, nucleotide-binding and erythrocyte-binding domains of PmRBP1a were identified which suggests it is involved in both sensing and anchorage to erythrocytes during erythrocyte invasion. The predicted tertiary structure of PmRBP1a's erythrocyte-binding domain is structurally similar to *P. falciparum* reticulocyte binding-like homologue 5 (*Pf*Rh5) and *P. vivax* reticulocyte binding homologue 2b (*Pv*RBP2b). PmRBPs genetic analysis revealed limited genetic sequence variation in the sampled strains with evidence of the genes under purifying selection. Relatively high titre of naturally acquired antibodies specific to the N-terminus of *Pm*RBP1a was quantified, a region that overlaps with the erythrocyte-binding domain. The measured anti-*Pm*RBP1a positively correlated with increasing age and exposure levels.

0190

QUANTIFICATION OF PLASMODIUM FALCIPARUM CYCLOPHILIN 19B TRANSCRIPTS VIA QPCR IN NORMAL AND SICKLE-TRAIT HEMOGLOBIN GENOTYPES

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The sickle-cell trait hemoglobin genotype (HbAS) protects against severe *Plasmodium falciparum* malaria. However, the biological mechanisms behind this protection are not well understood. A comparative transcriptomics study of asexual *P. falciparum* parasites found cyclophilin 19B (*Pf*CyP-19B) to be significantly downregulated in HbAS red blood cells (RBCs) compared to normal hemoglobin (HbAA) RBCs. *Pf*CyP-19B is a parasitic gene that produces the protein cyclophilin 19B, a member of the unfolded protein response that is important in parasitic protein folding and trafficking. A separate study in Southeast Asia found *Pf*CyP-19B to be up-regulated in artemisinin resistant *P. falciparum* parasites, posing a threat to malaria elimination efforts there. We measured the transcript expression level of *Pf*CyP-19B to investigate its potential role in the mechanisms that confer protection for HbAS individuals. RNA was extracted from both *in vivo* samples collected from Malian children with *P. falciparum* malaria as well as *in vitro* samples of parasite strain 3D7 collected throughout a 48-hour life cycle. RNA extracts were reverse transcribed and transcript expression was measured via qPCR. Wilcoxon rank sum and bootstrapping methods were used to compare transcript units between parasites grown in HbAA and HbAS RBCs. *In vitro* time series results showed no significant difference in *Pf*CyP-19B transcript expression levels between genotypes but did display a 24-hour pattern of peak expression for both HbAA and HbAS genotypes. The results from our *in vivo* data revealed a reduction in expression of *Pf*CyP-19B among individuals with the HbAS genotype compared to those with the HbAA genotype (Wilcoxon rank sum p=0.006). The under expression of *Pf*CyP-19B among HbAS individuals could be linked to impaired protein trafficking, interfering with the parasite's ability to display surface proteins vital for cytoadherence and severe disease manifestation. The differential expression of *Pf*CyP-19B observed in HbAS RBCs highlights further need for *Pf*CyP-19B research to understand its diverse role within the parasite and in relation to artemisinin resistance.

0191

GENETIC VALIDATION OF THE FUNCTION OF PFEMP1 IN PLASMODIUM FALCIPARUM ROSETTE FORMATION

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Malaria is a disease of global concern, responsible for a significant burden of mortality and morbidity, especially in developing countries of tropical and sub-tropical regions. Rosetting, the binding of *Plasmodium falciparum* infected erythrocytes (IEs) to uninfected erythrocytes to form clusters (rosettes), is thought to contribute to severe malaria. Rosetting phenotypes are mediated through the adhesive properties of *P. falciparum* Erythrocyte Membrane Protein 1 (PfEMP1), encoded by the var gene family (~60 distinct copies per parasite genome). Two *var* genes "IT4var60" and "IT4var09" have been identified as encoding rosette-mediating PfEMP1 variants in the well-characterised culture-adapted *P. falciparum* line IT4. However, the role of PfEMP1 in adhesion in live IEs remains poorly understood, mainly due to the difficulty in genetically manipulating *P. falciparum*. We aim to investigate the hypothesis that specific motifs within PfEMP1 mediate rosetting, utilizing CRISPR-Cas9 genome editing technology. This will contribute to the rational design of anti-rosetting interventions with the ultimate goal of reducing deaths from severe malaria. So far, we have successfully constructed CRISPR-Cas9/mutant plasmids for the mutagenesis of rosetting *var* genes and currently in the process of generating transgenic parasites.

0192

AXONAL INJURY MARKER TAU IN PLASMA IS ELEVATED IN ALL FORMS OF PEDIATRIC SEVERE MALARIA AND ASSOCIATED WITH IN-HOSPITAL MORTALITY AND COGNITIVE IMPAIRMENT AT 12-MONTH FOLLOW-UP

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The axon microtubule binding protein tau is predominantly expressed by neurons. Increased tau concentrations in circulation are a well validated marker of diffused axonal injury and disease progression in several neurodegenerative disorders. We previously reported elevated levels of tau in cerebrospinal fluid and plasma from Ugandan children with cerebral malaria (CM), compared to community children (CC). In children with CM, who present in coma, elevated cerebrospinal fluid and plasma tau concentrations on admission predict long-term neurocognitive impairment (NCI). In the present study, we assessed whether plasma tau concentrations are elevated in other forms of severe malaria, including those without overt neurologic symptoms, and if they are associated with NCI in these children. Plasma tau concentrations were measured in a single molecule, highly sensitive assay. The study cohort consisted of children 6 months to 4 years of age with one or more of the 5 most common forms of severe malaria: 1) CM; 2) respiratory distress (RDS); 3) severe malarial anemia (SMA); 4) malaria with multiple seizures (M/S); and 5) prostration (PRS). Plasma tau concentrations in children with severe malaria (n=383) were elevated when compared to CC (n=123) (median [95% confidence interval (CI)], 7.51 pg/mL [4.67, 11.59 vs. 2.63 [1.44, 3.96], p<0.001). Plasma tau concentrations on admission were significantly higher in children who died than in survivors (median [95% CI], 13.55 [7.62, 42.45], vs. 7.30 [4.59, 11.26], p<0.001), and in children with malaria retinopathy compared to those without retinopathy (median [95% CI] 9.28 [6.39, 15.11]) vs. 7.12 [4.63, 11.4], p=0.02). Elevated plasma tau concentrations among children with severe malaria were associated with a lower z-score for overall cognition at 12-month follow-up (β -0.34 [-0.67, -0.004], p=0.05). Plasma tau shows promise as a reliable, minimally invasive biomarker of brain injury associated complications in survivors of all forms of severe malaria that could be used to identify children susceptible to long-term neurocognitive impairment.

0193

DIFFERENCES BETWEEN ORTHOLOGOUS GENES OF RODENT AND HUMAN MALARIA PARASITES IN THEIR ROLE DURING SPOROZOITE FORMATION

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Genes that are only essential for late liver stage development during the complete life cycle of malaria are considered target genes for generation of genetically attenuated parasites (GAP) that arrest growth in late liver (LL) stages. These genes can therefore be used to improve GAP-vaccination approaches. Rodent parasites have been used for screening for essential LL-stage genes in gene-deletion studies. These screens resulted in identification of several genes that play a critical role in LL-stage development, including several genes involved in the FAS-II fatty acid (FA) synthesis pathway, such as *Fabi*, *Fabb/f*. However, the use of these FA-synthesis genes for generation of human *P. falciparum* GAP have proven to be impossible because of their essential role for sporozoite formation. Based on published rodent parasite gene-deletion studies, we have selected three other genes with a role in LL-stage development for deletion of the orthologous *P. falciparum* genes using CRISPR/Cas9 gene-editing. These genes were *palm*, a gene with unknown metabolic function and two genes encoding enzymes; NADH-cytochrome b5 reductase (*cbr*), that has multiple functions including in fatty acid elongation and biological oxidation-reduction processes, and biotin-protein ligase 1 (*hcs1*), with a role in biotin metabolism. We will report on the generation of the three *P. falciparum* gene-deletion mutants and their phenotype characterization throughout the life cycle, revealing i) their essential role in *P. falciparum* life cycle ii) differences between the rodent and human malaria orthologs and iii) insights into their (metabolic) functions.

0194

MARKED REDUCTION OF MALARIA TRANSMISSION WITH SEASONAL MALARIA CHEMOPREVENTION IN CHILDREN UNDER FIVE YEARS OF AGE DURING TES IN SÉLINGUÉ, MALI

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The seasonal malaria chemoprevention (SMC) is a WHO recommendation to reduce the burden of malaria infection in children of 3 to 59 months of age with sulfadoxine-pyrimethamine plus amodiaquine (SP-AQ) treatment during the malaria transmission season. Therapeutic efficacy studies (TES) are performed bi-annually to monitor the efficacy of antimalaria drugs. The objective of this study was to assess the impact of SMC on malaria incidence rates in children under five years of age before and during SMC campaign. Data were collected in the health district of Sélingué between July and December in 2016 during the malaria season, one year before SMC was initiated in Selingue, as a baseline and between July 2017 and January 2018 while SMC was implemented. *Plasmodium falciparum* genotypes were examined using *Msp-1*, *Msp-2*, and *Glurp* markers. The impact of SMC was assessed by comparing malaria incidence in children enrolled in the TES before and after SMC was initiated. The proportion of confirmed malaria cases enrolled in the TES study at baseline, 15.22% (449/2951) was higher than the year when SMC was initiated, 4.12% (180/4370). The reduction rate was 59.91% with an Odds ratio = 0.24, 95% CI (0.20 - 0.29), p-value < 0.0001. The PCR corrected reinfection rate at baseline without SMC was 15.49% (66/426) and with SMC campaign, 8.52% (15/176). The odds ratio = 0.50 (0.28 - 0.91) the difference between these two reinfection rates was statistically significant, p-value = 0.025. Our results indicate that SMC may have contributed to reductions in malaria incidence and malaria reinfections in Sélingué.

0195

A NATURAL VARIANT IN PLASMODIUM FALCIPARUM ACYL CO-A SYNTHETASE 10 CONFERS RESISTANCE TO NOVEL ANTIMALARIALS

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The emergence and spread of drug resistance to current antimalarial therapies remains a pressing concern, escalating the need for compounds that demonstrate novel modes of action and overcome the development of drug-resistance. We are characterizing the acyl Co-A synthetase (ACS) enzyme family as new potential drug targets. Mutations in ACS10 and ACS11 were identified from selections of two structurally unrelated

compounds (MMV665924 and MMV019719) by the Malaria Drug Accelerator (MalDA) consortium, which has adopted a chemogenomic approach to identify targets of the most promising compounds from chemically diverse libraries. ACSs activate fatty acids (FA) scavenged from the host, which can then be used for protein modification, phospholipid biosynthesis, and FA elongation. By introducing the observed mutations into the 3D7 parental line using CRISPR/Cas9, we demonstrated that the mutations in ACS10 and ACS11 are indeed sufficient to phenocopy the resistance phenotype of the selected lines. We generated conditional knock-down lines and confirmed that ACS10 is essential while ACS11 is non-essential in asexual growth *in vitro*. Reducing the protein levels of ACS10 and ACS11 lead to changes in the FA composition of the parasites confirming their role in FA metabolism. Thermal-shift assays followed by Western blot did not show a direct interaction between the compounds and ACS11. Thermal-shift assays followed by mass spectrometry are on the way to determine potential interaction partners for the two compounds. This will further our understanding if ACS10 and ACS11 are the direct targets of the compounds or are part of a resistance mechanism. ACS genes are highly polymorphic and surprisingly, the ACS10 M300I mutation identified here was present at 78% in a Malawi parasite population. We obtained Malawian isolates and found that an isolate containing the M300I polymorphism was five-fold more resistant to MMV665924 than a matched ACS10 wild type Malawi isolate. Regardless, if ACS10 and ACS11 are the target or involved in a resistance mechanism, natural occurring polymorphisms might reduce the efficacy of these compounds.

0196

DECREASED SUSCEPTIBILITY TO PFDHFR INHIBITORS ASSOCIATED WITH GENETIC POLYMORPHISMS IN UGANDAN PLASMODIUM FALCIPARUM ISOLATES

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The *Plasmodium falciparum* dihydrofolate reductase (PfDHFR) inhibitor pyrimethamine is combined with sulfadoxine for malaria chemoprevention, and proguanil, which is metabolized to the PfDHFR inhibitor cycloguanil, is combined with atovaquone for chemoprophylaxis in travelers. However, drug resistance, mediated by PfDHFR mutations, challenges the efficacies of these inhibitors. P218 is a novel PfDHFR inhibitor designed to overcome this challenge. However, data are available only for a small number of *P. falciparum* laboratory strains. We studied *ex vivo* susceptibilities to P218 and other PfDHFR inhibitors of 559 *P. falciparum* isolates collected in Tororo and Busia districts, Uganda from 2016-2020. Median IC_{50}s were 42,100 nM for pyrimethamine, 1,200 nM for cycloguanil, 13,000 nM for proguanil, and 0.6 nM for P218. Among 383 isolates, three PfDHFR mutations, 51I (100%), 59R (93.7%), and 108N (100%), were very common, as previously seen in Uganda, and another mutation, 164L (12.8%), had moderate prevalence. Increasing numbers of mutations were associated with decreasing susceptibility to pyrimethamine, cycloguanil, and P218, but not to proguanil, which does not directly inhibit PfDHFR. Importantly, differences in P218 susceptibilities between haplotypes were modest, with susceptibility to quadruple mutant (51I/59R/108N/164L) parasites (median IC_{50} 1.4 nM for mixed isolates and 5.7 nM for pure mutants at position 164) at a level expected to maintain excellent treatment or preventive efficacy for the compound. Previous studies reported duplication of the GTP cyclohydrolyse I (*pfgchI*) gene in mutant (particularly 164L) parasites. For 58 Ugandan isolates with various genotypes and drug susceptibilities, associations were not seen between *pfgchI* copy number and I164L genotype or susceptibility to PfDHFR inhibitors. Overall, PfDHFR mutations associated with high level resistance to pyrimethamine and cycloguanil were common in Ugandan isolates; activity of P218 decreased with increasing mutations, but the compound retained potent activity against all Ugandan isolates, warranting continued research on PfDHFR inhibitors.

0197

VARIED SUSCEPTIBILITY TO PROTEASOME INHIBITORS AND ASSOCIATIONS WITH GENETIC POLYMORPHISMS IN PLASMODIUM FALCIPARUM ISOLATES FROM UGANDA

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The proteasome is a multi-protein complex responsible for protein degradation in eukaryotes. Proteasome components are promising targets for antimalarial chemotherapy. In considering these targets, it is important to characterize naturally occurring variation in sensitivity of *P. falciparum* from malaria-endemic regions to proteasome inhibitors under study. We assessed *ex vivo* susceptibilities of fresh clinical isolates of *P. falciparum* collected in Tororo and Busia districts in Uganda from 2017-2020 to nine asparagine ethylenediamine and three macrocyclic peptide proteasome inhibitors. IC_{50} values were determined using a 72-h microplate growth inhibition assay with SYBR Green. We observed a range of proteasome inhibitor potencies, with median IC_{50}s from 1.1 - 1942 nM (28 - 170 isolates tested per compound), with 10 of the compounds having median IC_{50}s < 100 nM. TDI8304, a lead compound with drug-like properties, had a median IC_{50} of 16 nM (range 3.0 - 50 nM). Positive associations (r = 0.36 - 0.94) were seen between IC_{50} values for all asparagine ethylenediamine compounds. We sequenced the β5 (catalytic) proteasome subunit, which is the target of these inhibitors, and the β6 (structural) proteasome subunit, in Ugandan isolates. We observed two mutations in the β5 subunit, A142S and D150E, in two isolates. We observed no mutations in the β6 subunit. Considering the β2 (catalytic) subunit, we observed two mutations, I204T and S214F, in three isolates. Mutations in the β2 and β5 subunits were not associated with decreased susceptibility to the twelve proteasome inhibitors tested. *In vitro* screening of susceptibilities to the peptide proteasome inhibitors WLW and WLL, which target the β2 subunit and β2 and β5 subunits, respectively, demonstrated low nM activity, and did not show decreased susceptibilities to β2 S214F mutant parasites. Our results showed that a number of proteasome inhibitors had potent activity against *P. falciparum* isolates circulating in Uganda. Genetic variation in presumed proteasome targets was uncommon, and this variation was not clearly associated with changes in inhibitor susceptibility.

0198

ASSESSMENT OF PLASMODIUM FALCIPARUM RESISTANCE TO PIPERAQUINE IN WESTERN KENYA USING PIPERAQUINE SURVIVAL ASSAY AND MOLECULAR MARKER ANALYSES

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Malaria is a significant public health burden, especially in sub-Saharan Africa (sSA). Chemotherapy is central in the control of malaria. Dihydroartemisinin-piperaquine (DHA-PPQ) is the recommended second-line antimalarial in Kenya. The shift to this regimen was driven by increased reports of quinine resistance & declining artemether-lumefantrine (AL) efficacy, a first line drug. However, the widespread use of DHA-PPQ in Kenya may be a short-lived solution if PPQ resistance, such as that observed in South Eastern Asia (SEA) spreads to Kenya. It is therefore important to establish PPQ susceptibility in Kenya using piperaquine survival assay (PSA) & molecular marker analyses. Clinical isolates collected from *Plasmodium falciparum* naturally infected individuals were tested for *ex-vivo & in-vitro* PPQ susceptibility using PSA. A subset of the isolates was analyzed for *in-vitro* susceptibility using SYBR Green 1 method. Further, each isolate was genotyped for piperaquine resistance markers in *Pfcrt, Pfmdr1, Pfpm2, Pfpm3, Pfdhps Pfexo* & *Pfk13* genes using qPCR & MassARRAY platform. A total of 40 clinical isolates showed PSA median (interquartile range) of 0% (0-11.02%), n=40, at 95% CI, of these, 34/40 clinical isolates had PSA <10% depicting sensitivity to PPQ. Six isolates had PSA >10% consistent with PPQ resistance. PPQ median IC_{50} (interquartile range) for the subset of clinical isolates *in-vitro* was 20.81 nM (17.33-42.26), n=20. A statistically significant positive association was observed between PPQ IC_{50} and *Pfcrt* K76T (p=0.0007)*, Pfdhps* A437G (p=0.0167) and A613S (p=0.0043) respectively. Re-culture for *in-vitro* testing & analyses of polymorphisms in PPQ resistance genes for these samples are underway. These findings show that circulating Kenyan malarial parasites are sensitive to PPQ. PPQ resistance putative markers finding provide baseline status for continued monitoring of PPQ susceptibility as DHA-PPQ continues to be widely embraced in the country. Our previous studies showing extensive genotype changes in this region herald the need for genetic studies focusing on samples with PSA survival rate >10% for timely detection of changes.

0199

SANGER SEQUENCING AND DECONVOLUTION OF POLYCLONAL INFECTIONS: A QUANTITATIVE APPROACH TO MONITOR DRUG-RESISTANT PLASMODIUM FALCIPARUM

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Molecular markers are used in epidemiological surveillance of drug-resistant pathogens to monitor their emergence and spread. Molecular markers are constituted of either single nucleotide substitution (SNP) or concatenated SNPs in microbial genes that interact with drugs. Although various technical improvements in current sequencing methods have been introduced to identify SNPs, current tools commonly used for the measurement of molecular markers of antimalarial resistance do not allow discrimination of mixed infections and need to be improved for more sensitive surveillance of antimalarial resistance and improved control strategies. We developed a new method to quantify molecular markers of drug resistance in PfDHPS and PfDHFR genes by standard sequencing of amplicons and bioinformatic estimation of the proportions of different genotypes in individual samples. The method is based on Sanger sequencing of resistance gene fragments and deconvolution of chromatograms to quantify the molecular marker variants. This computational approach uses a combination of R scripts and the DuffyTools package on GitHub. We assessed the performance of our sequencing and computational approach in a pilot study, using mixtures of FCR3 and V1/S parasites at varying proportions between 0-100%. Our results demonstrated quantitative discrimination of varying proportions of wild-type versus mutant-type PfDHPS and PfDHFR alleles. We are now applying this method to field samples and will report changes in mean levels of drug-resistant parasites. We will compare this new approach to the standard approach that assigns a binary call to each parasite sample (resistant vs. sensitive). In conclusion, this new method is a cost-effective sequencing approach for population-based surveys that characterize infections at the individual level and thereby provide an earlier signal so that public health officials might sooner plan and implement treatment policy changes.

0200

THE INFLUENCE OF BIOLOGICAL, EPIDEMIOLOGICAL, AND TREATMENT FACTORS ON THE ESTABLISHMENT AND SPREAD OF DRUG-RESISTANT PLASMODIUM FALCIPARUM

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The evolution of *Plasmodium falciparum* parasites less susceptible to artemisinin derivatives and their partner drugs threatens the effectiveness of Artemisinin Combination Therapies (ACTs). Identifying the factors that promote the establishment and spread of drug-resistant malaria parasites is essential to delay these processes and develop more sustainable therapies. We adapted a stochastic, individual-based model of malaria epidemiology and transmission dynamics to systematically quantify the influence of biological, transmission settings, health systems, and pharmacokinetics/pharmacodynamics factors on the establishment and spread of drug-resistant parasites. We did this via a global sensitivity analysis by exploring a range of drug properties of artemisinin derivatives and partner drugs for various epidemiological settings and treatment levels. Our analysis confirms that the establishment and spread of drug-resistant parasites is more likely in low, seasonal transmission settings due to the reduced level of human immunity and the lower level of within-host competition between genotypes. Furthermore, our study demonstrates that the spread of partially artemisinin-resistant parasites greatly accelerates when the efficacy of the partner drug decreases. Therefore, proper adherence to treatments should be ensured, alongside molecular surveillance to detect the emergence of resistance to partner drugs and change first-line treatments accordingly. In addition, we found that prolonged exposure to artemisinin during treatments reduced the spread of partially artemisinin-resistant parasites. Consequently, future ACTs should optimize or increase parasites exposure to artemisinin. Finally, the spread of parasites resistant to a partner drug depended strongly on the length of the selection window of the partner drug. To minimize this selection window, future ACTs should include an additional long-acting drug. This would reduce the risk of resistance evolution to each of the long-acting partner drugs and thereby further protect artemisinin derivatives.

0201

THE THERAPEUTIC EFFICACIES OF ARTEMETHER-LUMEFANTRINE AND CHLOROQUINE FOR THE TREATMENT OF UNCOMPLICATED PLASMODIUM FALCIPARUM AND P. VIVAX INFECTIONS, RESPECTIVELY IN TWO SENTINEL, ETHIOPIA 2021

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Regular monitoring of antimalarial drugs is recommended for early detection of drug resistance and to maintain effective national malaria treatment guidelines. In Ethiopia, the national malaria diagnosis and treatment guidelines employ a species-specific approach for malaria treatment. Artemether-lumefantrine (AL) and chloroquine (CQ) are the first-line schizonticidal treatments for *Plasmodium falciparum* (Pf) and *P. vivax* (Pv), respectively. AL is used for mixed infection treatment. In this stud, we report the clinical and parasitological efficacy of AL against uncomplicated *P. falciparum* and CQ against uncomplicated *P. vivax*

infections. The WHO guideline, Surveillance of antimalarial drug efficacy protocol with 28 days follow up was followed. The study was conducted between December 2020 and March, 2021 in two sentinel sites. A total of 348 patients were followed for 28 days in two arms (n = 152 in the AL/Pf arm, n = 232 in the CQ/Pv arm). High cure rate was observed for the 28 day follow up (PCR uncorrected): AL/Pf 97.7% (95% CI: 93.5-99.5), CQ/Pv 95.2% (95% CI: 95.2-97.7). In both arms 100% of patients cleared parasites on the third day. No early treatment failure was detected in all arms. Three late clinical failures (LTF) were detected on the AL/Pf arm and a total of 10 failures (6 late clinical failures (LTF) and 4 late parasitological failures (LPF)) were detected on CQ/Pv arm before the 28 days follow up. Our study on progress is showing similar results for Pyramax (Artesunate-pyronaridine) against uncomplicated *P. falciparum* and *P.vivax* on patients above 18 years and 42 days follow up, full data collection is not completed for reporting.The study showed the current anti-malaria drugs used by the malaria control program in Ethiopia are efficacious. In addition, the Pyramax (Artesunate-pyronaridine) study on progress showing promising results.

0202

ASSESSING THE REAL-WORLD STABILITY OF ARTESUNATE RECTAL CAPSULES

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Artesunate rectal capsules (ARC) were first developed and tested in large-scale clinical trials by WHO-TDR. They comprise 100mg of artesunate in an inert lipid fill, encapsulated in a soft gel capsule. Two manufacturers, Cipla and Strides, supported by MMV, have developed products which were prequalified in 2018 and now play a vital part in the pre-referral management of severe malaria in over 15 African countries. As part of routine product development, the stability of ARC under controlled storage conditions (25°C/60%RH, 30°C/75%RH, 40°C/75%RH) has been rigorously assessed. Globally, the data indicate that ARC is a reasonably robust product, but specifically, the data do not support a 24-month shelf life under Zone IVb (30°C/75%RH) conditions. In the absence of any additional data, the shelf-life is set at 24-month under Zone II (25°C/60%RH) conditions. For clinical use, WHO-PQ recommends a cautious approach, whereby stocks of ARC should remain in the field on a short-term basis, for between 4 and 6 months. The need for frequent discard and resupply of ARC has proven to be logistically difficult. It risks stock-outs at the community health worker level, affects trust in the product and wastes limited resources. Encouraged by the data from the controlled storage studies, we will retrieve and test ARC that has been in field storage in the DRC, Nigeria and Uganda for defined periods, including between 9 and 12 months. Although the storage conditions in these settings are not controlled, they are not unknown. Site-specific temperature logger values, as well as local and regional maximum, minimum and average temperatures provide a comprehensive dataset against which to interpret the results of the testing. The testing itself will be done by an independent laboratory in Switzerland, using the same tests and acceptance specifications as the original capsule suppliers. The data from the testing of these real-world samples will complement the existing data from the controlled storage studies and may allow end-users and regulatory agencies to make more informed decisions regarding the appropriate intervals for product replacement in the field.

0203

INHIBITION MECHANISMS AND STRUCTURES OF PLASMODIUM-SELECTIVE PROTEASOME INHIBITORS DETERMINE IN VITRO RESISTANCE PROFILES

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Recent research has identified the *Plasmodium falciparum* proteasome as a promising new antimalarial drug target. This multi-subunit proteolytic enzyme complex is essential for maintaining cellular homeostasis, regulating diverse processes such as cell cycle progression and removal of damaged proteins. Large-scale screening efforts combined with the elucidation of differences in substrate preferences and the *Plasmodium* proteasome structure, relative to its human counterpart, have enabled the design of highly selective inhibitors. These inhibitors differ substantially in their binding properties (covalent vs. noncovalent, reversible vs. irreversible) and chemotypes. We assessed the resistance liabilities of a panel of inhibitors comprising boronates, epoxyketones, vinyl sulfones, asparagine ethylenediamines and N,C-capped peptides. We screened these compounds against *P. falciparum* lines harboring mutations in the 20S proteasome core particle or in the 19S regulatory particle, and performed selection experiments to determine the minimum parasite inoculum required to yield *in vitro* resistance. Vinyl sulfones (covalent, irreversible peptide inhibitors) exhibit minor shifts in their half-maximal effective concentrations (EC50 values) when tested against proteasome mutant parasites, while asparagine ethylenediamines (noncovalent, reversible binders) exhibit the largest changes in EC50 values. Several classes of *Plasmodium* proteasome inhibitors do not readily select for resistance *in vitro*, with select epoxyketone and vinyl sulfone inhibitors yielding no resistance even with large starting inocula (1E8 parasites). Moreover, we observe infrequent cross-resistance between compounds; in fact, partial resistance to one compound can yield hypersensitivity to another, suggesting that resistance to *Plasmodium* proteasome inhibitors is largely mechanism- and occasionally compound-dependent. These data underscore the potential of targeting the *P. falciparum* proteasome with small-molecule inhibitors to treat multidrug-resistant parasites.

0204

NOVEL MOLECULES TO OVERCOME EXISTING RESISTANCE AND PREVENT THE EMERGENCE OF RESISTANCE TO ARTEMISININ AND INHIBITORS OF PROTEIN DEGRADATION PATHWAYS

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Malaria, an ancient infection that still causes 200 million cases and nearly half million deaths globally. Worldwide emergence and spread of *Plasmodium falciparum* resistance to first-line artemisinins and artemisinin-combination therapies has created an urgent need of therapeutics with novel mechanisms that suppress the ability of parasites to develop resistance. We will be presenting novel molecules that overcome existing artemisinin resistance and prevent the emergence of the resistance to artemisinin and protein degradation pathways. These molecules exert a novel mode of action that differs between each individual parasite cells. We propose that this aspect of parasite killing will increase selectivity of our compounds over human cells. We also identify that our novel

compounds can overcome mechanisms of resistances and retain potency against a variety of global isolates with varied background resistance patterns.

0205

TRACKING ANTIMALARIAL DRUG RESISTANCE USING MOSQUITO BLOOD MEALS

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Strong surveillance systems are needed to monitor the emergence and spread of antimalarial drug resistance. Yet approaches are often limited by costly infrastructure, regulatory oversight, and significant reporting delays. In this study, we hypothesized that mosquito blood meals could be used as an efficient method to monitor antimalarial resistance in *Plasmodium* populations and also provide data on vector metrics relating to malaria transmission. We conducted a series of cross-sectional surveys to compare resistance-associated molecular markers in humans and mosquitos in Bama, Burkina Faso. We surveyed in two high-transmission seasons (10/2018, 09/2019) and one low-transmission season (03/2019). Household clusters in six village sub-sectors were sampled proportionately to sector size. Following consent, we obtained 1,481 dried blood spots via capillary finger-prick. We simultaneously collected 2,349 blood-fed mosquitos via vacuum aspiration from the same households and then pressed abdominal contents onto FTA cards. Parasite DNA was extracted with the KingFisher Flex System and *P. falciparum* (*Pf)* infections were detected with qPCR for *var*ATS. Drug resistance-associated mutations were identified in *pfmdr1* N86Y, D1246Y, and *pfcrt* K76T using High Resolution Melting. Over the three surveys, we found that 54, 43 and 34% of humans were infected, respectively, and 22, 17, and 21% of mosquito blood meals were *Pf*+. In the first survey, humans and mosquito midguts exhibited statistically equivalent rates of *pfmdr1* 86Y and 1246Y mutations. However, we observed significantly more mixed infections at *pfcrt* K76T in blood meals compared to human blood sampling. We will present full statistical comparisons of the prevalence of all alleles in humans and mosquito blood meals for each survey, at household and community cluster spatial scales, as well as comparative frequency data on multiplicity of infection. Our study is sufficiently powered to validate or invalidate the use of mosquito blood meals as a low-cost and rapidly deployable tool to monitor changes in the distribution of antimalarial resistance.

0206

THE EFFECT OF COVID-19 PANDEMIC ON THE PERFORMANCE OF ESSENTIAL MALARIA CONTROL INTERVENTIONS IN UGANDA: A CASE STUDY OF AMURU, AGAGO, DOKOLO AND OTUKE DISTRICTS OF NORTHERN UGANDA

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Uganda confirmed her first COVID 19 case in March 2020 and the government acted quickly to institute total lockdown in the same month. This study was conducted to assess the effect of COVID-19 on performance of essential malaria control services in four high malaria endemic districts. Eight district health team (DHT) members, 40 health workers, 160 village health teams (VHTs) and 618 households (HHs) participated. DHIS 2 data was analyzed and significance tests performed at 5% to establish significance of the observed change. Data from interviews was also analyzed. Outpatient attendance decreased from 187,737 before COVID-19 to 164,132 during lockdown. Confirmed malaria cases significantly (Fisher's value=0.038<5%) increased from 70,369 before COVID-19 to 80,924 during lockdown and to 110,716 when the lockdown was eased. VHTs and HHs also reported a significant increase of malaria cases in the communities during COVID-19 lockdown (p-value=0.000<5%). Malaria in pregnancy significantly (Fisher's value=0.040<5%) increased from 1,936 before COVID-19 to 2,220 during lockdown and then to 3,045 when the lockdown was eased. VHTs also reported a significant increase of malaria in pregnancy during COVID-19 fear and lockdown in place (p-value=0.013 and 0.001, <5% respectively). Malaria deaths increased from zero before COVID-19 to six during COVID-19 scare, dropped to five and to four deaths during lockdown easing. VHTs also reported a significant decrease during and after lockdown (p-value=0.035<5% and p-value=0.044<5% respectively). IPT3+ coverage significantly (Fisher's exact=0.000<5%) increased from 24.8% before COVID-19 to 63.2% during lockdown easing. The study confirmed that COVID-19 significantly affected malaria service delivery as shown by indicator performance. The Ministry of health and districts should strengthen health education systems on malaria and COVID-19 prevention and environmental management. Health facilities should ensure adherence to standard operating procedures (SOPs) and distinctively separate COVID-19 patients and suspects from other patients and effectively deliver adequate services.

0207

EFFECT OF COVID-19 ON TREATMENT-SEEKING AMONG PEOPLE LIVING IN A MALARIA-ENDEMIC AREA OF COASTAL KENYA

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In malaria-endemic countries, prompt testing of fevers and treatment of positive individuals are the main pillars of interventions to reduce the malaria burden. However, COVID-19 has affected people's treatment-seeking behavior due to reduced mobility and fear of attending health facilities. This study aimed to evaluate the impact of COVID-19 on hospital visits and malaria burden in coastal Kenya during 2020. We gathered data of fever cases tested for malaria at Msambweni sub-County Referral Hospital, Kwale County, Kenya, from January 2014 to December 2020. Per each visit, gender, age, the village of residence, and malaria test results were recorded. Trend analysis of hospital visits and malaria positive rate among tested people were performed using generalized additive mixed models (GAMMs). Spatial analyses were performed to identify the heterogeneity of changes in treatment-seeking behavior among villages during the year 2020. Among the 84,607 tested febrile cases, 24.4% were positive for *Plasmodium* infection. Malaria positivity rates showed a marked reduction from 2014 to 2019, followed by an increase of positive fevers during 2020. The total number of tested fevers in 2020 (9,685) was 20.8% lower compared to 2019 (12,238). A higher reduction of tested fevers was recorded among males (-28.1%) compared to females (-15.8%). However, only children under five years (U5) of age recorded an evident reduction of tested fevers with 43.5% fewer tested fevers in 2020 (3,426) compared to 2019 (6,064). The time trend analyses estimated that 290 (95% CI: 283-315) U5 malaria cases could have missed treatment at the hospital during 2020. The reduction of tested fevers showed a marked spatial heterogeneity associated with the distance from the hospital. This study showed that during the COVID-19 epidemic, malaria cases have increased in coastal Kenya. Furthermore, treatment was not sought for

a high fraction of U5 children. Our results highlight the importance of adapting malaria interventions during COVID-19 epidemics to mitigate its effect on malaria burden and protect highly vulnerable age groups.

0208

THE RELATIONSHIP BETWEEN COVID-19 AND PERCEIVED ACCESS TO MALARIA CARE IN UGANDA

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During the COVID-19 pandemic, malaria remained the leading cause of death among all ages (13.3%) in Uganda. The USAID Social and Behavior Change Activity explored the relationship between COVID-19 and perceived access to malaria care in Uganda. A nationally representative mobile phone survey was conducted among 1,400 men and women aged 18-49 years. Multivariable regressions explored the relationship between the perception that the COVID-19 pandemic affected access to malaria services and sociodemographic factors. More respondents reported the COVID-19 pandemic influenced access to malaria services (65%) compared to HIV (51%) and maternal health (56%). Perceived access to malaria care was significantly different among residents of Eastern (AOR=2.5, 95% CI 1.8, 3.5) and Western (AOR=2.1, 95% CI 1.5, 3.0) regions, educated (AOR=1.6, 95% CI 1.2, 2.2), those with 5 or more children (OR=1.9, 95% CI 1.2, 3.0), and those exposed to two or more COVID-19 prevention messages (AOR=1.6, 95% CI 1.1, 2.3). Community members who perceive barriers to accessing malaria care due to the COVID-19 pandemic may be less likely to seek care when they have symptoms. The impact of COVID-19 on access to malaria services may be due to restrictions on travel to health facilities, risks of exposure to COVID-19, overlapping symptoms, and potentially perceptions of low quality of care. Interventions are needed to promote COVID and malaria preventive measures, uptake of non-pandemic health services, and address challenges to health services access.

0209

IMPACT OF COVID-19 PANDEMIC ON SEASONAL MALARIA CHEMOPREVENTION 2020 CAMPAIGN IN WEST AND CENTRAL AFRICA

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As the world came together to tackle the COVID-19 pandemic, it was important to ensure access to core malaria preventive measures such as Seasonal Malaria Chemoprevention (SMC). It was anticipated that COVID-19 pandemic could create disruptions during 2020 SMC campaign for which countries would need to put in place new strategies to mitigate its impact. Through the OPT-SMC project, a cross sectional survey using mixed methods (qualitative and quantitative) measurements was conducted to evaluate the level of disruptions due to COVID-19 on 2020 SMC planning and delivery activities, and to document mitigation strategies put in place by the countries. An evaluation tool was developed using a systematic approach with risk assessment of disruption because of COVID-19 for each SMC activity and definition of adequate indicators to measure disruptions. Additional information was gathered through interviews of partners supporting National Malaria Programmes and during the 2021 SMC working group meeting. Twelve out of 13 countries implementing SMC participated in the survey. Half of the countries had to adapt their micro-planning activities and nine faced transport and supply chain management disruptions. All countries adapted their training methods either in increasing the number of training sessions for smaller groups or with using virtual platform. Social mobilisation was disrupted in all countries with challenges to reach houses due to rumours and fear of community drug distributors wearing masks. The use of digital technology for training, supervision and coordination activities was instrumental. Despite COVID-19 pandemic, countries didn't scale back their SMC implementation plan for 2020 and over 33 million children were reached with SMC. Only some activities were partially disrupted by the COVID-19 pandemic and none were cancelled. This is because of mitigation strategies put in place by countries since the preparatory phase and thanks to the substantial financial and technical support provided by partners and WHO. Lessons learned from this survey should inform 2021 SMC preparation phase.

0210

IMPACT OF THE COVID-19 PANDEMIC ON ACCESS TO OUTPATIENT MALARIA CARE IN UGANDA: AN INTERRUPTED TIME SERIES ANALYSIS

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In March 2020, the Ugandan government implemented several policy measures to prevent the spread of COVID-19. The effect of these interventions on outpatient malaria care is not well documented. We assessed the impact of the COVID-19 outbreak and corresponding government-mandated restrictions on indicators of routine malaria care at 17 public health facilities in rural Uganda. We analyzed routine health management information system (HMIS) data from outpatients seen at 17 facilities as part of an enhanced malaria sentinel site surveillance network from March 2017 to February 2021. Interrupted time series analyses (ITSA) were conducted to quantify the impact of COVID-19 lockdown policies on counts of outpatient visits, non-malaria visits, and malaria cases. We extrapolated the pre-policy trend, estimated using Poisson regression with generalized estimating equations, into the post-policy period and compared this counterfactual trend to the observed post-policy trend. Incidence rate ratios were calculated by summing the outcomes across the one-year post policy period and dividing the observed by predicted values. A total of 1,438,517 patients visited the 17 facilities over the 4-year observation period. More than half (55%) of the patients were suspected to have malaria, of which 98.7% had a malaria diagnostic

test performed; there was no change in these measures pre- and post-lockdown. ITSA showed no difference between observed and predicted total outpatient visits (IRR=0.96, 95%CI=0.86,1.07), non-malarial visits (IRR=1.01, 95%CI=0.92,1.11), or number of malaria cases (IRR=1.02, 95%CI=0.69,1.51) in the post-lockdown period. In conclusion, despite restrictions to public transit, closure of non-essential businesses, and diversion of personnel/resources to support response to the pandemic, the number of patients seeking care at these 17 health facilities was unaffected. Additionally, there were no significant differences in number of malaria cases diagnosed or measures of malaria case management in the pre- versus post-lockdown period.

0211

THE IMPACT OF COVID-19 PANDEMIC ON OUTPATIENT AND MALARIA SERVICES: EXPERIENCE FROM EIGHT STATES IN NIGERIA

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While health systems are being challenged by increasing demand for care of COVID-19 patients, it is critical to maintain basic health services, especially for the most vulnerable populations (WHO 2020). In Nigeria, the government instituted a total lockdown (with the exception of health service delivery) between April-May 2020. An analysis of routine national health management information systems (NHMIS) data was conducted in eight states for uptake of outpatient services including malaria. Data for March - August 2020 was compared with the same period for the previous year. COVID-19 incidence data was also analyzed for 2020. Seven states reported a decline in the number of patients who visited the health facilities for general outpatient services. Nasarawa and Oyo reported the largest declines, 45% and 31%, respectively, while Ebonyi reported <1% decline. Uptake of malaria services also declined in six states. The analysis showed that as COVID-19 cases increased, uptake of services declined. This pattern was particularly evident in Oyo, which had the highest number of COVID-19 cases among the eight states, and the largest percentage decline of outpatient and malaria services uptake. Health facility services utilization began to increase after the lockdown was eased across the states and reached pre-COVID-19 figures by August 2020. While the decline in care seeking could have been due to physical restrictions associated with the lockdown, fear and uncertainties brought about by the pandemic, along with possible stock out of some medicines due to supply chain effects may have also contributed. Access to health facilities was never restricted during the lockdown, revealing a pattern of changed health seeking behavior and concerns around sources of treatment for malaria and other febrile diseases during the period.

0212

FUNGAL METABOLITE ASPERACULANE B INHIBITS MALARIA INFECTION AND TRANSMISSION

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Mosquito-transmitted *Plasmodium* parasites cause millions of people worldwide to suffer malaria every year. Drug-resistant *Plasmodium* parasites and insecticide-resistant mosquitoes make malaria hard to control. Thus, the next generation of antimalarial drugs that inhibit malaria infection and transmission are needed. We screened our Global Fungal Extract Library (GFEL) and obtained a candidate that completely inhibited *Plasmodium falciparum* transmission to *Anopheles gambiae*. The candidate fungal strain was determined as *Aspergillus aculeatus*. The bioactive compound was purified and identified as asperaculane B. The concentration of 50% inhibition on *P. falciparum* transmission (IC50) is 7.89 μM. Notably, asperaculane B also inhibited the development of asexual *P. falciparum* with IC50 of 3 μM, and it is nontoxic to human cells. Therefore, asperaculane B is a new dual-functional antimalarial lead that has the potential to treat malaria and block malaria transmission.

0213

CLINICAL PHARMACOLOGY OF EXTENDED DURATION ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF PLASMODIUM FALCIPARUM MALARIA IN EFAVIRENZ-TREATED HIV-INFECTED CHILDREN IN UGANDA

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There is substantial overlap of HIV and malaria infections in sub-Saharan Africa. Efavirenz (EFV)-based antiretroviral therapy (ART) is a recommended first-line ART for HIV-infected children >3 years and amongst the most widely used ART in malaria-endemic regions. For malaria, artemether-lumefantrine (AL) is the most widely used ACT. Previous studies have shown significant drug interactions between EFV and AL, with EFV reducing exposure to artemether (ARM), dihydroartemisinin (DHA), and lumefantrine (LUM) in children. To compensate for the effect of EFV on AL exposure, we conducted a prospective PK/PD study to see if an extended 5-day regimen could improve AL exposure in children on EFV, compared to a 3-day regimen. We also enrolled HIV-uninfected children for comparison (n=100). Children ages 3-17 years were randomized to 5-day (10-dose) or 3-day (6-dose) AL in a high transmission setting in Uganda. PK sampling for ARM, DHA, and LUM was performed for 21 days with 42 day follow-up. HIV-infected children were randomized into 5-day (n=37) or 3-day (n=39) AL. Day 28 treatment outcomes for 5-day vs 3-day were: ACPR (71% vs 62%), LPF (26% vs 26%), and LCF (3% vs 12%). Day 42 treatment outcomes were: ACPR (36% vs 32%), LPF (44% vs 44%), and LCF (19% vs 24%). Total ARM exposure ($AUC_{0\text{-}8hr}$) post-last dose indicates a geometric mean $AUC_{0\text{-}8hr}$ of 70 and 63 ng·hr/mL for the 5-day and 3-day regimens, respectively. Total DHA exposure indicates a geometric mean $AUC_{0\text{-}8hr}$ of 99 and 112 ng·hr/mL for the 5-day and 3-day regimens, respectively. Total LUM exposure measured from the time of the last dose over 21 days indicates a geometric mean $AUC_{0\text{-}21d}$ of 216 and 149 ug·hr/mL, for the 5-day and 3-day regimens, respectively. Terminal LUM concentrations were significantly increased in the 5-day vs 3-day regimen on days 8 and 14 for HIV-infected children (460, 130 ng/mL vs 150, 68 ng/mL, respectively; all p-values <0.005), and similar to the day 8 and 14 LUM concentrations in HIV-uninfected children on 3-day AL (330, 131 ng/mL). In HIV-infected children, extended duration AL compensates for the EFV-induced reduction of LUM. Data is preliminary but final PK/PD analysis will be presented.

0214

IN SILICO PRIORITIZATION OF LIBRARY COMPOUNDS FOR ANTIMALARIAL DRUG DEVELOPMENT

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Persistent malaria transmission and increasing cases of drug resistance to current antimalarials necessitates the urgent development of new antimalarial drugs. In this study we employed a chemogenomics target-similarity approach to prioritize drugs with antimalarial activity. Protein targets of drugs with potential for repurposing were searched for and retrieved from Drugbank and Therapeutic Target Database (TTD). Using the Basic Alignment Local Search Tool (BLAST), we searched *Plasmodium falciparum* proteome for similar proteins (orthologs) using protein sequences of the known drug targets. We analysed for similarity in functional amino acids between known drug targets and predicted *P. falciparum* protein targets (for protein pairs that were at least 30% identical in the BLAST analysis). We performed molecular docking using Autodock to compare binding orientation and binding affinities of the drug compounds to the known targets and to the predicted *P. falciparum* protein targets. We retrieved essentiality and druggability indices of the predicted *P. falciparum* protein targets from Tropical Disease Research (TDR) database. Currently, we are assessing the association between the similarity of known drug targets and corresponding predicted *P. falciparum* proteins target (BLAST percentage similarity, BLAST bit score and functional amino acid similarity) with *in vitro* antimalarial activity (EC50s). BLAST search revealed 735 *P. falciparum* proteins that are likely targets to 143 drugs. A total of 161 *P. falciparum* proteins (out of 308 with data) had druggability indices above 0.5, and 251 (out of 545 with data) were essential for the survival of the parasite. Preliminary results indicate that the target similarity approach used in this study can be successfully used to prioritize compounds for antimalarial drug discovery and development.

0215

CONSENSUS LIGAND AND STRUCTURE-BASED SCREENING FOR IDENTIFICATION OF PFDXR INHIBITORS

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The malaria parasite *Plasmodium falciparum* is a life-threatening pathogen that incurs a high death and economic burden, especially in Africa and Southeast Asia. Chemotherapy is the most effective strategy in disease burden reduction. Despite a trend toward elimination in the last decade, malaria still remains a health concern with the parasite developing resistance to drugs. Virtual screening is both time and cost-effective in the early stages of drug discovery. In this study, 3 million leads from the ZINC database were screened against. *P. falciparum* 1-deoxy-D-xylulose-5-phosphate reductoisomerase using a combined ligand and structure-based approach, combined with consensus scoring. Multiple molecular fingerprints were used to compare ZINC compounds to 17 known DXR inhibitors and to select 50000 compounds. These were docked to the receptor using Qvina-w and rescored using 11 scoring functions. Further, Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA), steered molecular dynamic (SMD) and umbrella sampling (US) were performed to select final hits. Four compounds outperformed the potent 280 nM PfDXR inhibitor LC5 in US. ZINC000050633276 showed a promising -20.43 kcal/mol binding free energy corresponding to Ki of 1.934 fM. Comparing the methods ranking correlations, in both LVBS and SBVS, some techniques remained uncorrelated while others agreed. In LBVS, two main clusters (ES, USR, USRCAT, OBSPEC) and (MHFP, RDKit_3dpharm) are noted. In SBVS (Vina, Idock, and Smina), (AutoDock, DSX, Cyscore, Xscore) and the Rf-score group (Rf-score_V1 to V4) formed distinct clusters. At the residue level, GLU233, CYS268, SER270, TRP296, and HIS341 had a significant contribution to binding free energy in MM-PBSA. Breaking of interactions with these residues in steered MD correlated with higher values of rupture force.

0216

NATURAL PRODUCT INSPIRED NOVEL ANTIMALARIALS WITH RADICAL CURE POTENTIAL

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Prodiginines are a family of intriguing pyrrolylpyrromethane alkaloid antibiotics produced by actinomycetes and other eubacteria. Our investigations of the antimalarial activity of prodiginines were inspired by previous reports, dating back to 1967. The intriguing structural and antimalarial properties of the prodiginines motivated us to reassess the activity of the natural prodiginines and encouraging preliminary results led us to expand the structural and functional diversity toward the development of prodiginines as potent antimalarials. In our study, a number of the natural and synthetic prodiginines were equally effective at low nanomolar (IC_{50} < 10 nM) concentrations against a panel of multidrug-resistant (MDR) *Plasmodium falciparum* strains. Several of these prodiginines exhibited excellent oral efficacy in erythrocytic *Plasmodium yoelii* murine model without any evident weight loss or overt clinical toxicity. These prodiginine analogues also exhibited optimal metabolic stability and PK profiles. Significantly, prodiginines displayed antimalarial activity in two *in vitro* liver-stage assays: schizonticidal assay using luciferase-expressing *Plasmodium berghei* sporozoite infected human hepatocyte HepG2 cells and hypnozoitocidal assay using non-human primate *Plasmodium cynomolgi*. Our compounds also demonstrated the transmission blocking potential against sexual blood-stage parasite. Moreover, the prodiginines likely operate by a novel mechanism(s) of action given the pan-sensitivity against a large panel of MDR malaria parasites. Detailed structural optimization, multiple-stage antimalarial activities, safety, and metabolic stability studies will be presented.

0217

LATE LEAD OPTIMIZATION OF SECOND-GENERATION NOVEL ANTIMALARIAL ACRIDONES

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Malaria remains one of the deadliest diseases in the world today and accounts for over 200 million clinical cases worldwide each year along with roughly half a million deaths, mostly children under the age of five and pregnant women. With increasing multi-drug resistance (MDR) and absence of a clinically proven vaccine, there is an urgent need and continuous search for novel, effective, affordable and safe antimalarial drugs for both treatment of active bloodstream infections as well as preventing the disease at the liver stage. Recently, we have developed a novel antimalarial acridone chemotype with dual stage activity against both liver stage and blood stage malaria, as well as single-dose cure ability and potential to prevent relapsing infections. Here, we disclose the detailed late lead optimization and structure-activity relationships of the second-generation novel antimalarial acridones.

0218

THE USE OF A HUMANIZED MOUSE MODEL TO ASSESS ANTIMALARIAL EFFICACY AND IDENTIFY OPTIMAL DRUGS COMBINATIONS

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New antimalarial medicines are urgently needed to counter the emergence of resistance and contribute to malaria's global elimination. These medicines should be developed as fixed-dose combinations of at least two drugs, each with different mechanisms of action and compatible PK profiles. Unlike in the past, these medicines are now deliberately developed as fixed-dose combinations of two or more agents, where at least one of the agents is novel. However, the selection and ranking of optimal combination candidates is a complex scientific challenge because of the high number of biological, pharmacological and harmacodynamics variables involved. Moreover, there is a high number of potential drug combinations of the set of antimalarials currently in preclinical development. In our current drug development model, new drug combinations are prioritized based on their efficacy in the *P. falciparum* infected humanized mouse model (NOD*scid*IL2Rγ*null*, NSG) in which mice are engrafted with human erythrocytes. This model allows *in vivo* growth of human *Plasmodium* providing a means of assessing the efficacy of novel agents to kill the human parasite. *In vivo* drug combination studies are conducted to investigate, understand, and gauge the individual contribution and mechanistic interactions of the partner drugs. So far, thirty combinations have been studied using this animal model. Our results indicate that the data generated in the *P. falciparum* infected NSG model effectively translates to the human volunteer infection study model. Therefore, the development of humanized mouse models has emerged as a powerful enabling technology to assess new candidate drug combinations.

0219

SURVEILLANCE OF EFFICACY AND SAFETY OF ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN MAINLAND TANZANIA

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The World Health Organization (WHO) recommends regular surveillance of efficacy and safety of artemisinin-based combination therapies (ACT) for the treatment of uncomplicated malaria, which are the currently recommended antimalarial drugs. A prospective one-arm study was conducted to assess the efficacy and safety of artemether-lumefantrine (AL), the first line ACT for uncomplicated malaria in Tanzania at four outpatient health facilities in Bagamoyo, Kigoma, Kilombero, and Muheza districts. This study was conducted from June to December 2020 and recruited children aged 6 months to 10 years with microscopy confirmed uncomplicated *P. falciparum* malaria who met the inclusion criteria. The inclusion and exclusion criteria was based on WHO protocol. Children were treated with AL twice daily for three days and followed-up on days 3, 7, 14, 21, and 28, and on any day of recurrent illness for clinical and parasitological assessments. Blood was also collected on Whatman filter paper on day 0 and at the time of recurrent infection for polymerase chain reaction (PCR) analysis. The primary outcome measure was PCR-corrected adequate clinical and parasitological response (ACPR) on day 28. A total of 672 children were screened; among these, 356 (53.0%) were enrolled, and 350 (98.3%) completed 28 days of follow-up or attained the treatment outcomes according to the protocol. There was no early treatment failure; late clinical failure occurred in 20 (5.6%) patients, and late parasitological failure occurred in 36 (10.1%) patients. The day 28 PCR-uncorrected ACPR ranged from 82.7% at Muheza district to 94.8% at Kilombero district. The overall PCR-corrected cure rate was high (98.3%). There were 56 (15.7%) recurrent infections, with the majority (32.5%) occurring in Muheza district. The drug was well tolerated, and no serious adverse events were reported. The most commonly reported adverse event was cough in 13.7% of enrolled patients. The findings show that AL is still highly efficacious and was well tolerated with minimal adverse events in Tanzania. The high proportion of Recurrent infections were seen in a district with high transmission rate.

0220

DEVELOPMENT OF AN ECTOPIC HULIVER MODEL FOR PLASMODIUM LIVER STAGE INFECTION

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The first stage of *Plasmodium falciparum* and *P. vivax* infection involves parasite replication within the host hepatocytes, and is referred to as the liver stage (LS). Current knowledge regarding human LS infection dynamics is limited due to species-specificity challenges. Here we report a moderate-throughput, moderate-cost, and reproducible *in vivo* model to study *P. falciparum* and *P. vivax* LS using NOD/SCID/ *IL2rg*^{null} mice, herein referred to as the ectopic huLiver model. In this model, ectopic huLiver tumors were generated following subcutaneous injection of HC-04 cells. HuLivers were highly vascularized and expressed essential hepatic receptors, including the hepatic asialoglycoprotein receptor 1 (ASGPR1)—an essential receptor for targeted delivery of therapeutics. After investigating various methods of delivery, inoculation with infected *P. falciparum* sporozoites via mosquito bite was determined to be the most effective. The ectopic huLiver model was shown to support complete LS development and to allow for subsequent transition to blood-stage infection in mice repopulated with human red blood cells. Blood stage infection was quantified by measuring *P. falciparum* 18S expression levels using qRT-PCR. The ability of the model to enable testing of compounds for malaria chemoprophylaxis was demonstrated with primaquine, which was able to penetrate the ectopic liver and act as an effective prophylactic in this model. Furthermore, we established that our ectopic huLivers could be infected with *Plasmodium vivax* sporozoites isolated from infected mosquitoes suggesting the utility of this model for other *Plasmodium* species. As such, the ectopic huLiver model presented here provides an additional system for studies of human *Plasmodium* to elucidate parasite biology and aid in the development of anti-malarial therapeutics.

0221

INHIBITION OF THE PLASMODIUM FALCIPARUM ACETYL-COA SYNTHETASE BY MULTIPLE CHEMOTYPES DISRUPTS PROTEIN ACETYLATION AND EPIGENETIC REGULATION IN BLOOD STAGE PARASITES

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Global malaria control and elimination efforts rely on new generations of antimalarial drugs with novel modes of action against the malaria parasite *Plasmodium falciparum*. Using *in vitro* resistance evolution experiments, we previously identified mutations in the parasite's Acetyl-CoA Synthetase (PF3D7_0627800; PfAcAS) that confer resistance to structurally distinct compounds, including MMV084978 and MMV019721. Allelic exchange using the CRISPR/Cas9 system confirmed that the A597V or T648M mutations in PfAcAS phenocopied the resistance phenotype. Conditional knockdown using the Tet-DOZI-RNA-aptamer system demonstrated that PfAcAS is essential for parasite growth, and partial knockdown sensitized parasites to both compounds. MMV019721 and MMV084978 directly inhibited recombinant PfAcAS activity in a substrate-competitive manner, with Ki values of 73 and 369 nM respectively, and the A597V mutation reduced inhibitor affinities by >90-fold. Orthologues of PfAcAS in eukaryotes catalyze the formation of the central metabolite acetyl-CoA from acetate, coenzyme A and ATP, and participate in a range of essential processes including epigenetic regulation. Metabolomic analyses revealed that exposure of trophozoite parasites to PfAcAS inhibitors reduced cellular acetyl-CoA levels by ~4-fold. To examine the biological implications of PfAcAS disruption further, we have explored the effects of PfAcAS inhibition on histone acetylation throughout the blood stage of the parasite. Together these findings suggest that PfAcAS may play a role in maintaining nucleo-cytosolic acetyl-CoA pools that are necessary for epigenetic regulation of parasite gene expression. These findings highlight PfAcAS as a promising drug target with inhibitors that exert their effects on the parasite via a unique mode of action.

0222

COLLATERAL SENSITIVITY AS A STRATEGY TO SUPPRESS THE EVOLUTION OF RESISTANCE IN IN VITRO AND IN VIVO CONTEXTS

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Resistance has emerged to frontline antimalarial combination therapies, making the development of new treatment strategies for malaria a public health priority. In addition to safety and efficacy, a key target profile for novel therapeutics is a reduced probability of resistance emergence. Previously, we characterized the rapid evolution of resistance both *in vivo* and *in vitro* to the clinical antimalarial candidate DSM265, a *Plasmodium* dihydroorotate dehydrogenase (DHODH) inhibitor. Interestingly, we found that mutant lines resistant to DSM265 showed increased sensitivity to other DHODH inhibitors, a phenomenon known as 'collateral sensitivity'. Here we explore the potential of using collateral sensitivity to develop a combination therapy that would suppress the evolution of resistance. We report that parasites resistant to DSM265 display collateral sensitivity to the structurally-distinct DHODH inhibitor Genz669178. Work in other systems has shown that when two compounds confer collateral sensitivity to each other, resistance to combination treatment with both compounds is less likely to emerge. This led to the hypothesis that a combination treatment of DSM265 and Genz669178 would suppress the emergence of resistant parasites. We test this hypothesis using both an *in vitro* cell culture system and an *in vivo* mouse model of resistance selection. We find that while resistance to DSM265 + Genz669178 arises rapidly *in vitro*, cross-resistant parasites do not emerge *in vivo*. The different results from in our *in vitro* and *in vivo* models highlight the importance of the *in vivo* environment, including the pharmacokinetics of drug exposure, on the evolution of resistance. Our findings also suggest that combination strategies based on collateral sensitivity may be effective in an *in vivo* context. The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents. All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EEC and the GSK Policy on the Care, Welfare and Treatment of Animals.

0223

MALARIA TRANSMISSION DYNAMICS IN A HUMANITARIAN CRISIS IN SOUTHERN VENEZUELA, 2016-2020

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Since 2010, malaria transmission has increased significantly in Venezuela, and currently more than 50% of cases in all the Americas region being reported by Venezuela. Health data has been censored by the Venezuelan government since 2016, hence civil society organizations have played an important role in monitoring malaria data. This study aims to describe the changes in malaria transmission, geo-distribution, and the impact of COVID-19 on malaria transmission in Bolivar state, Venezuela from 2016 to 2020. During this period, Bolivar state reported on average 64% of the malaria cases in the country. A total of 2,754,606 blood samples were taken with 1,047,437 reported malaria cases. Overall, 40% of those tested for malaria were positive with test positivity rates ranging from 31% in 2016 to 67% in 2018 with a significant decrease (28%) in 2020. Annual parasite incidence (API) declined 41% overall with a high API from 2016 to 2019 (102-147 per 1000 population at risk) and sharply declined to 60 per 1000 par in 2020. Key and vulnerable populations included mainly young adults and indigenous groups. Illegal mining remains the main occupational risk factor. The causes of this malaria resurgence were multifactorial and, occurred as part of the complex humanitarian emergency: internal/external migration, illegal mining, limited funding for operations, and inadequate implementation of interventions. Stock-outs of antimalarial drugs were common in 2016. At the beginning of 2018; supplies of all antimalarials drugs were widely distributed. LLINs have been distributed in main hotspots since late 2018 with high coverage (>98%). The COVID-19 pandemic has hit Venezuela hard with >390 deaths among health workers. Gasoline shortages and strict quarantine rules have shrunk domestic activities. Venezuela has been driving Latin America severely off track in meeting WHO's 2016-2030 milestones. Venezuela lacks resilient infrastructure and is clearly ill-equipped for effective COVID-19 and malaria national responses. The consequences of Venezuela's crisis spillover will be also presented.

0224

HIGH PREVALENCE OF ASYMPTOMATIC MALARIAL ANEMIA AND ASSOCIATION WITH EARLY CLINICAL CONVERSION IN A PLASMODIUM FALCIPARUM HYPER-ENDEMIC SETTING IN CAMEROON

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Asymptomatic malaria is highly prevalent in *Plasmodium falciparum* endemic areas and often associated with increased prevalence of mild-to-moderate anemia. The aim of this study was to assess the prevalence of anemia during asymptomatic malaria parasite carriage and the interplay with persistent infection in highly exposed malaria-endemic residents. A household-based longitudinal survey was undertaken in a malaria hyper-endemic area in Cameroon using multiplex nested PCR for malaria diagnosis. Residents with *P. falciparum* asymptomatic parasitemia were monitored over a three-week period with the aid of structured questionnaires and weekly measurements of axillary temperatures. Of the 353 individuals included in the study (median age: 26 years, range 2-86 years, male/female sex ratio 0.9), 328 (92.9%) were positive for malaria parasitemia of whom 266 (81.1 %) were asymptomatic carriers. The prevalence of anemia in the study population was 38.6%, of which 69.2% were asymptomatic for *P. falciparum* infections. Multivariate analyses identified elevated blood parasitemia and female gender as associated risk factors of asymptomatic malarial anemia in the population. 15.4% of the asymptomatic participants reported with a fever before the end of the study. Furthermore, risk analyses revealed anemia at the time of enrolment as a key predictor of early development of febrile illness in the population. Together, the data reveal an extremely high prevalence of asymptomatic malaria parasitemia and anemia in the study area, unveiling for the first time the association of asymptomatic malarial anemia with early clinical conversion. These findings underscore the negative impact of asymptomatic malaria parasitemia on individual health, necessitating the development of adequate control and preventive measures.

0225

MALARIA INFECTION BURDEN AND ASSOCIATED RISK FACTORS IN AREA OF PYRETHROID-RESISTANT VECTORS TO LONG-LASTING INSECTICIDAL NETS IN BENIN

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Recent reductions in the malaria burden, mainly due to long-lasting insecticidal nets (LLINs), are currently threatened by the wide-scale selection of insecticide-resistant malaria vectors. In the present study, we aimed to determine the overall burden of malaria and its associated factors in a rural area of Benin with high pyrethroid-resistant vectors. As part of the pre-intervention activities of the New Nets project, a cluster randomised controlled trial assessing the efficacy of two dual-active ingredients LLINs, a community-based cross-sectional survey was conducted in three districts in southern Benin. More than 4,320 participants all ages were randomly selected using two-stage cluster sampling. Malaria rapid diagnostic tests were performed on consented participants. Risk factors for malaria infection were evaluated using multi-level mixed logistic regression adjusted within clusters. LLIN possession and the population net use were very high in the population (97% and 96%, respectively). However, malaria infection prevalence was 43.5% (cluster range: 15.1-72.7%). High household density, low socioeconomic status, young age (<5y; 5-10y; 10-15y), high education level, poor LLIN condition, fever in the past 48 hours and at the time of the survey were the risk factors significantly associated with malaria infection. Despite important population net use, malaria prevalence remain high in this area of intense pyrethroid-resistance. New classes of LLINs effective against resistant vectors are therefore crucial to sustain malaria reduction.

0226

THE EFFECTS OF METEOROLOGICAL FACTORS AND GEOGRAPHICAL ELEVATION ON MALARIA INCIDENCE IN ELIMINATION TARGETED DISTRICT OF ETHIOPIA

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Ethiopia has made significant strides in the fight against malaria. The country has now adopted & is implementing the global malaria elimination program, which is expected to be completed by 2030. The success of the elimination attempt, on the other hand, can be influenced by multiple factors. Climate & environmental conditions can be one of the most significant factors affecting the progress of elimination. As a result, the study aimed to investigate the burden & transmission drivers of malaria in one of the country's elimination targeted districts. From 2010 to 2017, all febrile patients in the district's health facilities were diagnosed for malaria using a microscope & Rapid Diagnostic Test. The malaria data were collected from the malaria registers, the meteorological data from the country's National Meteorological Agency, & the geographic coordinates from each village. The data were entered using EpiData 3.1, & analyzed by R version 4.0.0. During the study period, a total of 135,607 patients were diagnosed, with 29,554 (21.8%) of them being confirmed malaria cases. Only two species, *Plasmodium falciparum* & *Plasmodium vivax*, were identified, with 56.3 % & 38.4 %, respectively. The rest 5.2 % was a mixed infection. A time series plot revealed a significant reduction of malaria, with a clear downward trend in case numbers. In a Negative Binomial Regression, the transmission season, rainfall, temperature, elevation, & the patient's sex & age were found to be predictors of disease incidence & spatial distribution. An ARIMA (2, 1, 2), the best fit model for point prediction of potential malaria incidence in 2030, the target year for elimination, projected that the monthly incidence will fluctuate around 88 cases. Finally, the results showed a significant reduction in malaria morbidity in the district. The results of the predictive model, on the other hand, raised doubts about whether the elimination goal will be reached within the time frame. Thus, achieving the elimination goal would necessitate an equitable distribution & efficient use of current interventions, as well as the development of evidence-based interventions.

0227

MALARIA PARASITEMIA AND ANEMIA AMONG PREGNANT WOMEN ATTENDING GENERAL HOSPITAL, ENUGWU UKWU, ANAMBRA STATE, NIGERIA

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The physiological changes associated with pregnancy make women more susceptible to malaria infection. Malaria infection is a cause of severe anemia in pregnancy which is an important contributor to maternal morbidity and mortality, in addition to low birth weight in many parts of

the world including Nigeria. A study to determine malaria parasitemia and hemoglobin concentration among pregnant women attending General hospital Enugwu-Ukwu, Anambra State, Nigeria, was conducted between July and December, 2019. Two milliliter of venous blood was collected from 408 pregnant women during antenatal visits. Thick and thin blood films were prepared. stained with 10% Giemsa stain and examined microscopically for malaria parasites. Hemoglobin (Hb) concentration was estimated using cyanmethemoglobin method. Hemoglobin concentration below 11.0g/dl was regarded as anemia in pregnancy. Of 408 blood samples collected, 112 (27.5%) were positive for malaria parasites. The age group 15–20 years had the highest prevalence 8 (40%) while the age group 36–40 years had the least 24 (23.1%). The primigravidae had the highest malaria prevalence 72 (36%), while the multigravidae had the least 40(19.2%). Malaria prevalence among the pregnant women in relation to gravidity was not statistically significant (P>0.01). The pregnant women in their first trimester had the highest malaria prevalence 48 (50%), while those in third trimester had the least 24 (16.7%). Malaria parasitemia in relation to trimester was statistically significant (P<0.01). A total of 248 (60.8%) pregnant women had Hb value less than 11g/dl. The multigravidae 128(61.5%) had an average Hb of 9g/dl, while the primigravidae 120(60.0%) had an average Hb of 10g/dl. Health education and proper administration of Intermittent Preventive Therapy (IPT) during pregnancy is recommended for malaria prevention and control in pregnancy.

0228

A QUICK LOOK INTO MALARIA AMONG THE AMERINDIAN PEOPLE OF VENEZUELA

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Venezuela accounts for more than half the cases of malaria in the Americas, most of which occur in mining areas south of the Orinoco river, and the north-east shore of the country, where several Amerindian groups live. These communities have traditionally had limited access to healthcare, and the real magnitude of their malaria burden is unknown. We reviewed published and unpublished epidemiological records collected by field workers to determine the risk of contracting malaria among Amerindian patients of Amazonas, Bolivar, and Sucre, three states that accumulate 90% of all cases registered in the country. We determined the geographic distribution of cases, and the risk factors associated to infection with *Plasmodium falciparum.* We registered 116,097 cases of malaria among Amerindian patients between 2014 and 2018. Despite an increased incidence between 2014 and 2016, the risk of contracting malaria among Amerindian patients compared to the general population has reduced in later years. Hot spots were identified in municipalities of the three states, although more clearly in Bolivar. Two groups, the Yanomami, and the Hoti, showed higher odds for infection with *P. falciparum.* Cases of malaria among Amerindian patients in Venezuela increased between 2014 and 2018, although not as quickly as in the rest of the population. These cases are clustered in different geographic regions than those in the general population, and their control will likely require tailored approaches. Interventions should initially prioritize the Yanomami and the Hoti, given their increased risk of infection with *P. falciparum*, associated to cases of severe malaria.

0229

AGE AND SEASONAL DISTRIBUTION OF MALARIA HOSPITAL ADMISSIONS IN THE CONTEXT OF SEASONAL MALARIA CHEMOPREVENTION IN OUELESSEBOUGOU, MALI

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Seasonal Malaria Chemoprevention (SMC) was approved as a policy for malaria control in children under the age of 5 years by WHO in 2012. Nationwide implementation in Mali was achieved in 2016 including the health district of Ouelessebougou. This study assessed age and seasonal distributions of malaria hospital admissions to the district hospital of Ouelessebougou in 2018 after SMC was fully deployed. A total of 943 children aged 0-15 years were admitted for malaria disease confirmed by rapid diagnostic test or blood smear microscopy. Of these, 453 had severe malaria according to WHO criteria. Children under 5 years of age represented 77.9% (735/943) of laboratory confirmed malaria admissions and 83.2% (377/453) of severe malaria admissions. 89.4% (843/943) of the laboratory confirmed malaria admissions and 90.3% (409/453) of severe malaria admissions occurred during the malaria transmission season between July and December. Despite the wide implementation of SMC, malaria remained the major cause of the hospital admissions especially in children under 5 years of age. Most of the cases are concentrated over a six-month period from July to December. Additional tools are needed to reduce the burden of malaria especially in children under 5 years of age.

0230

ENHANCING COUNCIL HEALTH TEAMS ON USING MALARIA RISK MAPS FOR SUPPORTING DECENTRALISED MALARIA CONTROL PLANNING IN MAINLAND TANZANIA

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Control efforts in mainland Tanzania have led to a decline in the prevalence of malaria infection ($Pf PR_{6\text{-}59mo}$) from 18.1% (DHS/MIS-2008) to 7.5% (MIS-2017). This decrease in transmission was accompanied by an increasing trend in the heterogeneity of transmission. Hence, the country recognized that a more strategic allocation of resources to the sub-national epidemiological context was needed. In line with the World Health Organization High Burden to High Impact initiative, mainland Tanzania employed a country-led data-driven approach to develop a national malaria risk stratification. This approach was originally developed during the revision of the previous national malaria strategic plan. The current plan (2021-2025) makes use of available evidence and advocates for tailored interventions. As the country moves towards implementation of targeted intervention, a more granular micro-stratification of malaria risk becomes valuable for even further targeting of community-based interventions. The country's routine health management information

system provides numerous data that can be used to define the risk strata of each ward within a council. As a first step, combinations of survey and routine indicators were collated at ward level for the period 2017-2019. The council prevalence estimated through school surveys served as a guide to set appropriate cut-offs for the routine indicators through a sensitivity analysis. This allowed for allocating the 3,311 wards to one of four risk groups: very low, low, moderate and high. As a next step, capacitating council health teams to translate the risk map for micro-planning of suitable intervention strategies becomes crucial in the process towards decentralization. To this end, the country is currently employing a top-down approach, whereby trained national malaria program staff are enabling the council teams to take charge of community-case management and focal vector control initiatives such as indoor residual spraying and larviciding down to ward level. Such a detailed and dynamic framework can lead to better allocation efficiency to maximize future impacts on disease burden.

0231

SYSTEMATIC REVIEW OF PLASMODIUM FALCIPARUM AND P. VIVAX POLYCLONAL INFECTIONS: IMPACT OF PREVALENCE, STUDY POPULATION CHARACTERISTICS, AND LABORATORY PROCEDURES

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Multiple infections of genetically distinct clones of the same *Plasmodium* species are common in many malaria endemic settings. Mean multiplicity of infection (MOI) and the proportion of polyclonal infections are often reported as surrogate marker of transmission intensity, yet the relationship with traditional measures such as parasite prevalence is not well understood. We screened published articles and assessed the impact of population prevalence, genotyping method, number of genotyping markers, method for diagnosis (microscopy/RDT vs. PCR), presence of clinical symptoms, age, geographic region, and year of sample collection on multiplicity indices. For *P. falciparum* (275 data points, 33526 genotyped individuals) the proportion of polyclonal infections ranged from 0-96%, and mean MOI from 1-6.1. For *P. vivax* (115 data points, 13325 genotyped individuals) the proportion of polyclonal infections ranged from 0-100%, and mean MOI from 1-3.8. A weak correlation between prevalence and the proportion of polyclonal infections was observed (Pf: 0.34% increase per percentage increase in prevalence, P<0.001); Pv: 0.78% increase per percentage increase in prevalence, P<0.001). In multivariable analysis, higher prevalence, typing multiple markers, diagnosis of infections by PCR, and sampling in Africa were found to result ina higher proportion of *P. falciparum* polyclonal infections. For *P. vivax,* prevalence, year of study, typing multiple markers, and geographic region were significant predictors. In conclusion, polyclonal infections are frequently present in all settings, but the association between multiplicity and prevalence is weak.

0232

LOW PREVALENCE OF PLASMODIUM MALARIAE AND P. VIVAX, AND HIGH PREVALENCE OF P. OVALE DETECTED AMONG TANZANIAN SCHOOL CHILDREN WITHIN THE 2017 SCHOOL MALARIA PARASITEMIA SURVEY (SMPS)

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Characterization of non-*falciparum* malaria species across Tanzania is necessary for informing national control efforts toward elimination. Evidence from large-scale studies is currently limited, and non-*falciparum* malaria is thought to be under-diagnosed due to lower accuracy of rapid diagnostics for these species. We leveraged dried blood spots from a large, cross-sectional survey encompassing 8 geographically diverse regions and 198 primary schools across Tanzania to quantify prevalence of non-*falciparum* infections among school children in 2017. A sub-population was selected via stratified random sampling by region for real-time PCR to identify species and estimate prevalence and parasitemia levels. Mapping was performed by school district to assess geographic distribution of non-*falciparum* infections. Among 3,456 school children, 23.6% (n=814) were infected with *Plasmodium ovale spp.*, 3.9% (n=136) with *P. malariae*, and 0.3% (n=11) with *P. vivax,* including single and mixed species infections; *P. falciparum* infection was detected in 18.8% (n=651) of children as single or co-infections. Single-species infections were identified in 67.3% (n=548), 24.3% (n=33), and 36.4% (n=4) of all *P. ovale, malariae, and vivax* infections, respectively. The majority of infections were low parasitemia (*P. ovale*: median [IQR] 1.8 [0.3 - 6.2] p/µL; *P. malariae*: 2.9 [0.7 - 13.7] p/µL; *P. vivax*: 0.1 [0.1 - 0.2] p/µL). *P. ovale spp.* infections were detected in all 8 regions, and *P. malariae* was detected in all but 2 regions (northern and central). *P. vivax* infections were predominately clustered near the northern-western border (54.5%, n=6). Findings confirm non-*falciparum* species are prevalent across Tanzania among school children, with *P. ovale* prevalence considerably higher than *P. malariae*. *P. ovale* and *P. malariae* infections are widely distributed across Tanzania, and would generally be detected as malaria due to co-infection with *P. falciparum*. As malaria control continues and *P. falciparum* parasitemia is reduced, testing specific to non-*falciparum* species will become increasingly important for supporting further malaria elimination efforts.

0233

USE OF A CONTINUOUS MALARIA INDICATOR SURVEY (CMIS) FOR EVALUATION OF MALARIA INDICES AND INTERVENTION COVERAGE IN WESTERN KENYA, 2015-2020

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Cross-sectional Malaria Indicator Surveys are timed to occur every 2-5 years during peak prevalence seasons to provide discrete snapshots of malaria indices. However, these indices vary greatly by month and year, and survey timing may result in inaccurate estimates of intervention coverage and impact. In 2015, we began a continuous malaria indicator survey (cMIS) in western Kenya. Following a sub-county-wide census, households were randomly selected yearly for inclusion. Each working day, staff administered questionnaires and malaria rapid diagnostic tests (RDTs) to consented, eligible household members. A total of 21,754 surveys were administered from April 07, 2015 to March 20, 2020 across 3,753 households. Using a negative binomial model, we found that malaria prevalence increased at a rate of 1.13 times (95% CI: 1.07-1.20) annually, insecticide-treated net (ITN) ownership decreased at a rate of 0.97 times (95% CI: 0.95-0.98) annually, and ITN use decreased at a rate of 0.93 times (95% CI: 0.91-0.95) annually. Annual peak malaria prevalence varied between June and September, ranging from 34% (95% CI: 27-41%) to 51% (95% CI: 43-58%). Annual malaria prevalence troughs occurred in April, May, October, or December, ranging from 17% (95% CI: 12-23%) to 40% (95% CI: 32-48%). Monthly ITN ownership ranged from 80%

(95% CI: 70-87%) to 99% (95% CI: 95-100%) and use ranged from 62% (95% CI: 53-71%) to 95% (95% CI: 90-97%). In July 2017, prior to an ITN campaign, ITN ownership was 90% (95% CI: 83-95%), ITN use was 77% (95% CI: 69-84%), and malaria prevalence was 48% (95% CI: 42-54%). After the campaign, in September 2017, ITN ownership rose to 98% (95% CI: 94-100%), ITN use rose to 91% (95% CI: 86-95%) and malaria prevalence fell to 33% (95% CI: 27-39%). In 2018, malaria prevalence was 34% (95% CI: 28-40%) in July and peaked at 40% (95% CI: 34-46%) in September; an MIS done in 2018, a year after the mid-2017 ITN campaign, may have inaccurately estimated the impact of the campaign. In 3 of the 5 years, a traditional MIS in July would have missed the annual peak. Data from these analyses will be used to inform National Malaria Control Program intervention and evaluation timing.

0234

RISK FACTORS FOR ASYMPTOMATIC MALARIA INFECTIONS IN THE PERUVIAN AMAZON

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The Peruvian Amazon reflects a challenging scenario of malaria elimination. The high proportion of asymptomatic infections, only identified by active case detection, obscures the actual number of cases. Infected individuals who do not experience any symptoms are ignored, and if left untreated, could transmit the disease. In response to the burden of asymptomatic infections, it is necessary to know the epidemiology and the risk factors intrinsic to the hosts and the environment. From 2018 to 2020, population screenings were done in 11 communities around the Loreto region. The individuals answered a survey related to socio-demographics, malaria antecedents, and household conditions. Participants also provided blood samples for malaria diagnosis. Asymptomatic malaria infection was defined as an infection with the presence of fever, headache, or shaking chill. A univariate logistic regression analysis was performed to test the association of the potential risk factors with asymptomatic infection. For the multivariate logistic regression analysis, factors with p-values <0.2 for the likelihood ratio test in the univariate analysis were considered for further analysis. Of 3,839 surveyed individuals, 13.57% (N=521) were infected by *Plasmodium spp*. Among the malaria cases, 70% were asymptomatic infections. Seasonality, age, previous malaria infection, fumigation, and wooden households, were associated with asymptomatic malaria in the univariate analysis. The multivariate analysis showed a strong association between experience of previous malaria infection and an asymptomatic infection (AOR=0.06, 95%CI 0.01-0.18). During the wet season, individuals were 43.4% less likely to develop an asymptomatic infection (AOR = 0.57, 95%CI 0.37-0.88). Interestingly, patients over 46 years old have decreased odds of developing an asymptomatic infection (AOR=0.42, 95%CI 0.19-0.86). Our study contributes to the knowledge on transmission dynamics of asymptomatic malaria in the Peruvian Amazon by listing the risk factors that need to be considered by the National Malaria Control Program during the formulation of elimination strategies.

0235

CLOSING THE KNOWLEDGE GAP IN HUMAN MOBILITY AND CONNECTEDNESS BETWEEN RURAL VILLAGES ENDEMIC TO MALARIA IN THE PERUVIAN AMAZON

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Malaria exacts a massive toll on human health worldwide, with more than 229 million cases and 400 thousand deaths reported in 2019. In Peru, the disease mainly affects vulnerable populations from the Amazon. These are rural riverine villages that lack access to primary health care services. In recent years, human mobility has been associated with malaria transmission; yet, national malaria control plans do not include it in their control measures. Furthermore, population genetics analyses in these regions signal human mobility as a relevant factor in transmitting and maintaining the disease. However, few studies address precisely the travel profile of the inhabitants and the connectivity between these villages. Here, we characterized the travel profile of inhabitants from an Amazonian malaria endemic district in Peru and assessed the connectivity of their villages through social network analysis. We surveyed 2,378 persons from the Mazan district, an Amazonian region divided in the Napo and Mazan basin, during the 2018 dry season (July and October). Participants answered a sociodemographic survey, including questions about recent travels. Through non-parametrical statistical testing, we found that the Mazan basin inhabitants showed significantly higher travel mobility than those from the Napo basin, both in travel record proportion and total travel time. Additionally, we estimated the travellers' profile through multivariate logistic regression. Our results showed that people older than 16 years, currently studying, earning a salary in the last month and belonging to the Mazan basin are prone to show a travel record in the previous 30 days. Finally, our social network analysis proved that communities from the Mazan basin were strongly connected compared to villages from the Napo basin. Still, connectedness between basins was recorded. All in all, we described the villages' connectivity profile in Mazan and Napo basins, both inside the malaria-endemic district of Mazan. Our results provide more detailed connectivity than previous works done in the region.

0236

SPATIAL DISTRIBUTION OF PLASMODIUM FALCIPARUM AND P. VIVAX IN NORTHERN ETHIOPIA THROUGH MICROSCOPY, RAPID DIAGNOSTIC TEST, ANTIBODY AND ANTIGEN DETECTION DATA

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There are challenges to identifying areas of active malaria transmission within regions of low, heterogenous prevalence. Different tests provide different information including acute diagnosis (rapid diagnostic tests

(RDTs) and microscopy) or prior infection (IgG antibody data). This study describes the concordance of multiple field and laboratory-based malaria tests using data from the 2015 Ethiopian Malaria Indicator Survey. Blood samples were collected for microscopy and RDTs, and dried blood spot samples (n=2,279) from three regions in northern Ethiopia (Afar, Amhara and Tigray) were also analyzed by multiplex bead assay for antigen detection and IgG detection against 10 *Plasmodium* antigens (seven *P. falciparum* and three *P. vivax*). Geospatial analysis was conducted using spatial scan statistics and kernel density estimation to identify hotspots (spatial clusters) of malaria by the different tests. Prevalence of current or recent malaria infection was low (1.4% by RDT, 1.0% by microscopy, and 1.8% by sensitive bead antigen assay, which can detect lingering HRP2 antigens even after parasite clearance). Evidence for prior malaria infection found 38.1% seroprevalence for long-lived IgG against *P. falciparum* antigens and 39.9% seroprevalence for *P. vivax*. For *P. falciparum*, overlapping spatial clusters were identified for all four tests. Five *P. falciparum* antibody clusters were also found that had no overlap with clusters found by any other test, possibly indicating very low transmission areas. For *P. vivax*, three clusters identified based on bead antigen assay, microscopy and IgG antibodies partially overlapped. There were an additional two *P. vivax* antibody clusters identified with no overlap with other clusters. The abundance of antibody clusters detected indicate that in areas of low-transmission, IgG antibody data are a sensitive marker to assess malaria transmission in a population. As countries strengthen their routine surveillance system, assessing the geographic distribution of malaria infection and exposure using multiple metrics can help improve the understanding of malaria transmission dynamics in a region.

0237

STRONG CLUSTERING OF ASYMPTOMATIC PLASMODIUM FALCIPARUM AND P. VIVAX INFECTIONS AMONG ETHNIC GROUPS BUT ABSENCE OF POPULATION STRUCTURE IN CHITTAGONG HILL TRACTS, BANGLADESH

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Malaria remains endemic in eastern Bangladesh, with the most cases in the forested, mountainous region in the southeast called the Chittagong Hill Tracts (CHT). This area is home to Bengali settlers and diverse groups of indigenous people residing largely in mono-ethnic villages. We purposively selected the 10 most prominent ethnicities of the CHT and then randomly selected similar numbers of afebrile individuals from each ethnicity. All 1002 enrolled participants were screened for malaria by RDT and qPCR. The prevalence of *Plasmodium falciparum* and *P. vivax* infection was 0.7% by RDT (*Pf*: 6/1002; *Pv*: 0/1002, mixed: 1/1002) and 4% by qPCR (*Pf*: 21/1002; *Pv*: 16/1002, mixed: 5/1002). Infections were highly clustered, with 60% (30/50) occurring in only two ethnic groups, the Khumi and Mro. To investigate whether transmission occurs primarily within ethnic groups, parasites were genotyped. Twenty-one *P. falciparum* isolates were typed by *msp2* and deep sequencing of 5 amplicons (*ama1-D3, cpmp, cpp, csp*, and *msp7*), and 21 *P. vivax* infections by microsatellite (MS) typing of ten loci and amplicon sequencing of *msp1*. Diversity was high with 5-15 alleles per marker. Expected heterozygosity was 0.93 for *P. falciparum* and 0.81 for *P. vivax*. In total 85.7% (18/21) of *P. vivax* and 23.8% (5/21) of *P. falciparum* infections were polyclonal. No population structure was evident for either species, suggesting high transmission and gene flow among ethnic groups. However, MS pairwise relatedness of *P. vivax* among the Mro was higher than in other ethnicities. Identity-by-state (IBS) for *Plasmodium spp*. among samples from the Mro ethnicity was greater (*Pf*: 0.50; *Pv*: 0.25) than for all samples (*Pf*: 0.11; *Pv*: 0.22). This suggests that the Mro may be isolated from transmission from other ethnicities, perhaps due to geographic distance or some Mro-specific behavior. High asymptomatic and subpatent infection prevalence and high genetic diversity suggest sustained ongoing transmission in CHT. Control activities should be specifically directed to ethnicities at high risk.

0238

DIFFERENTIAL PATTERNS OF NATURALLY ACQUIRED IMMUNE RESPONSES TO 28 PLASMODIUM FALCIPARUM VACCINE ANTIGENS WITH VARIOUS LEVELS OF GENETIC DIVERSITY

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The study of antigenic targets of naturally acquired immunity (NAI) is essential to identify and prioritize antigens for effective vaccine development. NAI to *Plasmodium falciparum* malaria is mainly mediated by IgG antibodies but acquisition of protective antibody responses in relation to genetic diversity and signatures of immune selection of each antigen is largely unexplored. Using partial or full-length expressed recombinant proteins (3D7 allele), we measured total IgG responses to 28 *Plasmodium falciparum* antigens from more than 700 Papua New Guinean school-aged children (5 - 14 years old) at the first and last timepoints of two longitudinal cohorts from high and moderate transmission settings (n = 1500). We then measured correlations between antibodies, genetic diversity and immune selection (defined using MalariaGEN Pf3K sequences), and protective efficacy against malaria in the two transmission settings. Acquisition of total IgG was associated with repeated exposure to infection, genetic diversity, balancing selection, and antigen function. In the high transmission setting, high antibody levels were associated with protection from malaria; in contrast, in the moderate transmission setting they were typically associated with an increased risk of malaria. Among antigens with the highest total IgG at high transmission were rhoptry (e.g., RALP1), microneme (e.g., AMA1) or conserved antigens (e.g., CyRPA, TRAMP, Pfs25). Whilst, antigens with expected balancing selection hotspots such as AMA1, MSP3, MSP9, DBLMSP1, and RH5 were most frequently identified as the highest total IgG acquisition at moderate transmission setting, indicating allele-specific immune responses which can increase the chance of breakthrough infection. This study highlights different mechanisms of immunity at different transmission levels which enhances further understanding of naturally acquired antibody acquisition to different vaccine candidate antigens.

0239

POST-DISCHARGE BURDEN OF MORBIDITY AND MORTALITY IN CHILDREN ADMITTED WITH SEVERE ANEMIA OR OTHER SYNDROMES IN MALARIA-ENDEMIC SETTINGS IN AFRICA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Severe anemia (SA), hemoglobin <5.0 g/dL, is associated with high in-hospital mortality among young children. In malaria-endemic areas surviving children remain at increased risk of mortality or readmission for

at least six months post-discharge. We conducted a systematic review and performed random-effects meta-analyses to determine the risks of morbidity and mortality in the post-discharge period among children aged <15 years admitted with SA versus other syndromes in malaria-endemic Africa. Mantel-Haenszel odds ratios (MHOR) were used for paired binary outcomes. Pooled effect estimates were generated using DerSimonian and Laird random-effects model to obtain relative risks (RR) or hazard ratios (HR). Heterogeneity was expressed using the I^2 statistic. The primary outcomes were all-cause death and readmission within six months post-discharge. Twenty-four studies (N=24) including prospective and retrospective cohort studies and randomised-controlled trials were included. For children previously admitted with SA, mortality by six months post-discharge was higher than during the in-hospital period (studies N=5, MHOR=1·72, 95% CI 1·22-2·44, p=0·002, I^2=51·5%) and almost three-times higher compared to children previously admitted without SA (N=4, RR=2·80, 95% CI 1·614·86, p<0·0001, I^2=73·1%). Readmissions were also more common in children admitted with SA (N=1, RR=3·05, 1·12-8·35, p<0·0001, I^2=0·0%). Children admitted with severe acute malnutrition also had a higher 6-month post-discharge mortality than those admitted for other reasons (N=2, RR=3·45, 1·89-6·31, p<0·0001, I^2=81·3%). In malaria-endemic Africa, children hospitalised with SA and severe acute malnutrition are at increased risk of mortality in the first six months post-discharge. Improved strategies are needed for the post-discharge management in these high-risk groups.

0240

EPIDEMIOLOGICAL IMPACT OF PIPERONYL BUTOXIDE (PBO) LONG-LASTING INSECTICIDAL NETS AFTER A NATIONAL NET DISTRIBUTION CAMPAIGN IN RWANDA, 2020

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Rwanda is making progress in malaria control with the incidence of malaria reducing from 321 per 1,000 person per year in 2018-2019 to 198 per 1,000 in 2019/2020. The use of long-lasting insecticidal nets (LLINs) is a key malaria prevention tool used in Rwanda. However, pyrethroid resistance threatens gains made in malaria control. Rwanda has adapted a multi-pronged vector control strategy including the use of Indoor Residual Spraying (IRS) in high malaria burden districts and the distributions of LLINs in other districts. In 2018, the National Malaria Control Program strategic approach changed to include the use of newer types of LLINs such as Piperonyl butoxide (PBO) nets and Interceptor G2 to counter pyrethroid resistance. PBO nets have a synergist that partially restores pyrethroid susceptibility in mosquito vectors and were first distributed in Rwanda in 2019. In 2019-2020 Rwanda distributed 5,566,006 rectangular LLINs through a mass campaign in 24 districts. These included 1,399,528 PBO nets distributed between February to March 2020, in five medium malaria burden districts: Rulindo, Gicumbi, Kicukiro, Gasabo and Nyarugenge. Data on malaria cases were collected monthly through the Rwanda Health Information Management System (R-HMIS). We compared malaria cases reported during a period of 12 months before and after the mass distribution of the PBO nets. Data analysis showed a reduction in malaria cases in all five districts. In Gasabo district, malaria cases decreased by 71% from 292,431 to 85,454. In addition, malaria incidence in the same district reduced from 469 to 134 cases per 1,000. In Gicumbi, Kicukiro and Nyarugege districts, malaria cases reduced by 72%, 61% and 62% respectively. The highest reduction of 83% in malaria cases was observed in Rulindo district. Notwithstanding that other additional malaria control interventions such as larviciding and the weather during the study period may affect the incidence malaria, initial results from our evaluation indicate that PBO nets can reduce malaria incidence dramatically, making them a promising new tool to combat pyrethroid resistance on malaria control in Rwanda

0241

THE FIGHT AGAINST MALARIA: THE CASE OF THE US PRESIDENT'S MALARIA INITIATIVE (PMI) MALARIA ACTION PROGRAM FOR DISTRICTS

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The PMI Malaria Action Program for Districts (MAPD) project was implemented from 2016 to 2021 to reduce malaria related morbidity and mortality in Uganda, in collaboration with the National Malaria Control Division (NMCD). All 52 project districts signed a memorandum of understanding to commit to support the implementation of effective malaria interventions in line with the national malaria strategy, and to build the capacity of the NMCD and districts to effectively manage malaria programs. Key activities included the development of annual district malaria plans; the routine distribution of long lasting insecticidal treated nets (LLINs) and intermittent preventive treatment in pregnancy (IPTp); technical assistance for malaria case management with regular health facility support supervision; surveillance, monitoring, and evaluation including performance reviews; social behavior change; and gender and youth interventions. Comparing first to fourth project year, health facility data in project areas showed great progress in malaria health service delivery, mortality, and morbidity indicators. The proportion of women receiving three or more doses of IPTp increased from 1.9% (95% CI 1.8% - 1.9%) to 72.9% (95% CI 72.8% - 73.1%), the proportion of pregnant women receiving LLINs increased from 48.7% (CI 48.5% - 48.8%) to 78.8% (95% CI, 78.6 % - 78.9%), the proportion of suspected malaria cases tested increased from 76.0% (95% CI 75.9% - 76.0%) to 98% (95% CI 97.9% - 98.1%), and the proportion of patients with a negative malaria test who were treated with an antimalarial decreased from 46.8% (95% CI 46.7% - 46.8%) to 1.4% (95% CI; 1.47% - 1.51%). Moreover, a 49% reduction in annual reported malaria deaths (from 2142 to 1098) and a 15% reduction in annual parasite incidence (API) (from 109 to 92 per 1000 population) was seen. Analysis of non-MAPD district data showed a 7% reduction in malaria deaths (from 3178 to 2943), but API data lacks reliability for analysis. These results suggest that PMI support and collaboration with the Uganda NMCD, through MAPD and districts, were successful in considerably reducing malaria morbidity and mortality in Uganda.

0242

ASSESSING THE DIRECT EFFECT OF INDOOR RESIDUAL SPRAYING WITH FLUDORA® FUSION IN NCHELENGE DISTRICT, ZAMBIA

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In 2019, the National Malaria Elimination Program of Zambia transitioned to a novel insecticide, Fludora® Fusion, for its annual targeted indoor residual spraying campaign in Nchelenge District, an area with high year-round transmission. Monthly cross-sectional active surveillance data from 2014-2020 were used to determine the impact of the transition from Actellic to Fludora® Fusion in indoor residual spraying on parasite prevalence using Poisson regression models with robust variance estimation. Annual and rainy season models were adjusted for annual population coverage with indoor residual spraying in the sprayed area, weeks since annual spraying began, participant age, net use, population density, and distance to the nearest health facility, stream, and

lake. Fludora® Fusion use was not associated with decreased parasite prevalence in sprayed homes compared to unsprayed homes relative to Actellic use (relative risk ratio = 1.11, 95% confidence interval = 0.94, 1.30). However, household indoor residual spraying receipt in the past six months was only borderline statistically significantly associated with parasite prevalence under Actellic (relative risk = 0.91, 95% confidence interval = 0.82, 1.01). Further, increasing year-over-year indoor residual spraying coverage was not associated with malaria risk (relative risk = 1.00, 95% confidence interval = 1.00, 1.00), suggesting that, in addition to household benefit, community benefit was limited or absent. Self-reported bed net use was associated with a 19% decrease in risk (95% confidence interval = 0.75, 0.87). These findings indicate indoor residual spraying with neither Actellic nor Fludora® Fusion have sufficiently controlled malaria in Nchelenge District and should be supplemented with alternative strategies, such as housing improvements or universal long-lasting insecticide-treated net coverage.

0243

UNDERSTANDING CROSS BORDER MALARIA IN SAUDI ARABIA

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Border malaria is a major obstacle to malaria elimination in Saudi Arabia. Today malaria on the southern border of Saudi Arabia, a region where malaria cases are resurging, and elimination efforts are stalled mainly due to humanitarian crisis and the Conflict in Yemen. This study analyzes the current border malaria epidemiology in the southern border of Saudi Arabia from 2015 to 2018. All reported cases maintained by the malaria elimination centers in Aledabi and Baish, Jazan Province, Saudi Arabia, from 2015 to 2018 were analyzed to examine the epidemiological changes over time. Pearson's Chi-Square test of differences was utilized to assess differences between characteristics of imported and local causes and between border cases. A logistic regression model was used to predict imported status was related to living in the border area. A total of 3,210 malaria cases were reported in Baish, and Aledabi malaria centers between 2015 and 2018, of which 170 were classified as local cases and 3,040 were classified as imported cases. The majority of cases are male and residents of the border areas. Given the complexity of cross-border malaria, creating a malaria buffer zone that covers a certain margin from both sides of the border would allow for a joint force, cross-border malaria control operation. To conduct malaria elimination activity and cases reported as belonging to this zone, rather than being pushed from one country to the other, would allow malaria elimination staff to work collaboratively with local borderland residents and other stakeholders to come up with innovative solutions to combat malaria and reach malaria-free borders.

0244

CHARACTERIZING MOBILITY PATTERNS OF FOREST GOERS IN SOUTHERN LAO PDR USING GPS LOGGERS

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In the Greater Mekong Sub-region (GMS), engaging in forest activities is a major risk factor for malaria. As countries focus their malaria control and elimination efforts on forest-going populations, a better understanding of their mobility patterns and risk associated with specific types of forest-going trips, is essential. In 2018, we conducted a focal test and treat intervention (FTAT) in Champasak Province, southern Lao PDR, and recruited 2904 forest-goers in our study. A subset of them were offered to carry a "i-Got-U" GPS logger for roughly two months, collecting GPS coordinates every 15 to 30 minutes. The utilization distribution (UD) surface around each GPS trajectory was used to extract trips to the forest or forest-fringes. A hierarchical clustering algorithm identified trips with shared mobility patterns characteristics in terms of duration, timing of the trip and forest penetration further enabling classification of high-risk trips because of an increased exposure to dominant malaria vectors in the region. Finally, we used gradient boosting trees to assess which of the forest-goers' socio-demographic and behavioral characteristics predicted the best their likelihood to engage in trips at higher-risk for malaria. A total of 122 forest-goers accepted to carry a GPS logger resulting in the collection of 803 trips to the forest or forest-fringes. Six clusters of trips emerged, helping to identify 240 trips at higher-risk for malaria based on high forest penetration and whether the trip happened overnight. Age, size of traveling group, outdoor sleeping structures and number of children were the best predictors of forest-goers' probability to engage in high-risk trips. This study characterized the heterogeneity within the mobility patterns of forest-goers and further segmented their role in malaria transmission in the GMS. These results are key for national control programs across the region to assess and meet their 2030 malaria elimination goals.

0245

VEUPATHDB: COMPREHENSIVE INFORMATICS SUPPORT FOR YOUR RESEARCH NEEDS

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VEuPathDB is a free, online, NIAID-funded bioinformatics resource center focused on eukaryotic pathogens, vectors, fungal and host informatics. It represents the merger of VectorBase and EuPathDB and supports over 400 organisms. VEuPathDB empowers users to leverage omics data without the need for computational programming. VEuPathDB analyzes a wide variety of omics data and couples the analysis results to advanced search capabilities, data visualizations and custom tools to facilitate the discovery of meaningful relationships from large volumes of data. Available data range from basic sequence and annotation to transcript and protein expression, variation, domains, orthology, phenotypes, pathways, epigenomics and host-pathogen interactions. Data mining strategies include: records that compile all data for a single feature like a gene, a genome browser, a search strategy system, a Galaxy interface, and a geovisualization tool to observe population biology data. Users might begin at a gene page to view tables and graphs of that gene's behaviour in an RNA-Seq experiment, then transition to the genome browser for a dynamic view of the RNA-Seq data aligned to the genome, as well as the opportunity to view nearby gene models or other data types. The search strategy system allows users to query from a genome-wide perspective, easily merge evidence from diverse data and across species, and ask questions such as 'Find genes in these 4 species that are expressed in the first half of the organism's life cycle'. Users with their own omics data can use Galaxy to privately analyze and port their results to VEuPathDB for comparison. The MapVEu geovisualization tool facilitates mining of population data for traits such as insecticide resistance and infection status. Our active user support offers an email help desk, social media, tutorials, webinars, and workshops. Email us at help@veupathdb.org with questions or suggestions.

0246

EXPANDING THE UTILITY OF PLASMODIUM GENETIC CROSSES USING NANOPORE LONG-READ GENOME ASSEMBLIES FOR PARENTAL PARASITES

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Illumina short-read sequencing of parents and progeny from genetic crosses provides a useful approach to characterize malaria genetic crosses, but is limited to the core genome regions, and cannot resolve rearrangements and complex structural variants. *Plasmodium falciparum* has an AT-rich, haploid genome, with highly variable subtelomeric regions that contain polymorphic gene families involved in immune evasion and pathogenesis. These regions cannot be efficiently sequenced with short read Illumina methods, because it is not possible to map reads to the 3D7 reference sequence. To help resolve this issue, we used long-read technology (Oxford Nanopore Technologies, ONT) to sequence 6 parental parasites from three genetic crosses. We performed *de novo* assembly to generate highly contiguous, cross-specific reference sequences. By comparing the assembly of these parental parasites with the 3D7 reference genome, we uncovered a number of structural variants and rearrangements, as well as genes that are not present in the 3D7 reference. Furthermore, by mapping Illumina short read sequencing from the progeny to parental reference sequences, we were able to expand the percentage of genome sequenced and improve resolution of QTL peaks. This approach allowed us to identify a novel candidate gene (merozoite-associated tryptophan-rich antigen 2) that is not present in the 3D7 reference or NF54 nanopore assembly but is segregating (either present or absent) in the progeny of a cross (NF54×NHP4026) and falls under a prominent QTL for differential growth in serum or AlbuMAX based culture. These results demonstrate the value of high-quality cross-specific reference genomes for malaria linkage analysis.

0247

PIGGYBAC MUTAGENESIS APPLIED TO PLASMODIUM KNOWLESI

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Plasmodium vivax is the second most prevalent type of malaria worldwide and the most common cause outside of Africa. Unfortunately, an inability to maintain blood-stage parasites in the lab has severely limited experimental studies on this parasite. The adaptation of *Plasmodium knowlesi* (PkA1-H.1) to continuous *in vitro* culture in human RBCs now provides an important breakthrough to support a laboratory model for analysis of vivax-like malaria parasites. Using this culture system, we have successfully adapted the *piggyBac* transposon mutagenesis method for pursuing forward genetic screens and gene functional analysis at genome scale in *P. knowlesi.* To establish the *piggyBac* system for *P. knowlesi,* the 5′ UTR of *pkef1a* was amplified from genomic DNA and inserted as the 5′ regulatory element in the *piggyBac* transposase helper plasmid PfDCTH, replacing the original promoter. This helper plasmid PkETH was designed to drive transposase expression to mobilize and integrate the *piggyBac* transposon in *P. knowlesi*. In parallel, the 5′UTR of *pkhsp70* was inserted in the drug selection cassette carried by the transposon to drive expression of hDHFR-GFP in *P. knowlesi*. The two plasmids were co-transfected into cultured *P. knowlesi* schizont-infected human RBCs by direct electroporation using an Amaxa 4D-Nucleofector. Anti-folate resistant clones of transfected PkA1-H.1 were isolated by limiting dilution and *piggyBac* genome integration by sequence analysis. Consistent with results for random integration at TTAA-target insertion sites observed in other *Plasmodium* spp. insertions were widely distributed in different chromosomes throughout the genome in UTRs and CDS of genes. These preliminary results indicate that *piggyBac* transposable system is feasible for functional genomic analyses of *P. knowlesi*as it has achieved in *P. falciparum.*

0248

UNDERSTANDING RESIDUAL PLASMODIUM FALCIPARUM TRANSMISSION IN ZANZIBAR THROUGH MULTIPLEXED AMPLICON DEEP SEQUENCING

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Over the past 15 years, Zanzibar has achieved substantial reductions in the burden of malaria, but case numbers have recently increased again. Reactive case detection (RCD) using RDTs is routinely conducted by the Zanzibar Malaria Elimination Program (ZAMEP). To better understand residual *Plasmodium falciparum* transmission dynamics in Zanzibar, we characterized the genetic diversity of parasites circulating within 5 districts on both islands. We have developed a novel droplet digital PCR (ddPCR) based high-throughput method for highly multiplexed amplicon deep sequencing from dried blood spots (DBS). 518 samples were typed by a panel of 28 highly diverse microhaplotypes (median expected heterozygosity = 0.74) and 7 drug-resistance loci. The method was highly sensitive, genotyping data was obtained for >80% of markers in 80% of samples at densities of ≥5 parasites/µL. The parasite population in Zanzibar was highly diverse (mean heterozygosity = 0.71; mean allelic richness = 9.6). The majority of infections (75%) were polyclonal, with a mean multiplicity of infection (MOI) of 2.2. We found that index cases had significantly lower MOI than secondary cases (1.98 *vs*. 2.33, p=0.007), but no differences in MOI between travelers and non-travelers were observed. Low spatial and temporal genetic differentiation was observed, indicating frequent gene-flow between the two islands. Fine-scale population structure was detectable, with frequent clustering of highly related infections (IBS ≥0.5) within households. No *kelch13* mutations were observed, but mutations were found in drug-resistance genes *Pfdhfr*, *Pfdhps*, *Pfmdr1*, and *Pfmdr2*. No *hrp2/hrp3* deletions were observed. In conclusion, a highly diverse parasite population indicates sustained transmission. Closely related parasites within households point to clinical cases as important sources of onward transmission.

0249

SINGLE CELL ANALYSIS REVEALS TRANSCRIPTIONAL HETEROGENEITY AND CHANGES ACCOMPANYING PLASMODIUM SPOROZOITE DEVELOPMENT

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Sporozoites, the infective stage of the malaria parasite, develop in oocysts on the mosquito midgut and make their way to the mosquito salivary glands where they wait to be inoculated into the mammalian host. Despite their critical role in malaria transmission, little is known about the mechanisms underlying the maturation of *Plasmodium* sporozoites in mosquitoes. Here, we use single-cell RNA sequencing to characterize the gene expression profiles of 16,038 *P. berghei* sporozoites derived from 12 independent collections and isolated throughout their development: 614 sporozoites collected from punctured late-stage oocysts, 2,147 isolated

from mosquito hemolymph, 5,979 isolated from the salivary glands and 7,298 obtained from forced salivation experiments. Our single cell data reveal extensive transcriptional heterogeneity among parasites isolated from the same anatomical site, suggesting that *Plasmodium* development in mosquitoes is asynchronous and might be regulated by intrinsic as well as environmental factors. In addition, we describe a succession of tightly regulated changes in gene expression occurring during the maturation of sporozoites and highlight novel candidate genes that could play important roles in oocyst egress, sporozoite motility, and the mechanisms underlying the invasion of mosquito salivary glands and mammalian hepatocytes. Lastly, our analyses show a general decrease in transcriptional activity preceding the translational repression observed in mature sporozoites and associated with their quiescent state in salivary glands, followed by a rapid reactivation of the entire transcriptional machinery immediately upon salivation. This study provides a valuable resource and lays the foundation to characterize the role of novel genes required for sporozoite development and transmission, thus identifying new targets for the development of malaria control strategies.

0250

PFEMP1 EXPRESSION SPECIFIC TO SEVERE MALARIAL ANEMIA AND CEREBRAL MALARIA IN A CASE-CONTROL STUDY IN MALI

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The majority of severe and fatal malaria infections affect young children in sub-Saharan Africa. Most severe malaria cases are due to infection with *Plasmodium falciparum*. Variant surface antigens mediate cytoadhesion and rosetting, thereby enabling sequestration in the microvasculature, preventing clearance of infected cells by the spleen, and contributing to endothelial activation that increases vascular permeability and an inflammatory response that can be detrimental to the host if unregulated. The most well-known variant surface antigens, *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP1s), are encoded by *var* genes and mediate adhesion to host cell receptors such as endothelial protein C receptor (EPCR) and intercellular adhesion molecule 1 (ICAM1). Expression of particular PfEMP1s binding to EPCR and ICAM1 is associated with severe malaria syndromes, including cerebral malaria and severe malarial anemia. To identify PfEMP1 variants associated with cerebral malaria and severe malarial anemia, we conducted a case-control study of severe malaria cases matched to uncomplicated cases of malaria in Mali, West Africa from 2014-2018 that included children aged six months to five years. We report results from assembly of full-length PfEMP1 transcripts from specific severe malaria syndromes in comparison to uncomplicated malaria controls. We sequenced RNA extracted from whole blood, after enrichment for parasite RNA (and *var*s in particular) using a custom capture-based approach. Transcripts from malaria cases were *de novo* assembled, and those corresponding to PfEMP1 domain sub-classifications were determined to identify potential host cell receptor targets. We determined PfEMP1 transcripts in 14 cases of cerebral malaria, 11 cases of severe malarial anemia, and seven cases of cerebral malaria and severe malarial anemia compared to uncomplicated malaria matched controls. Our results have the potential to provide insight into the pathogenesis of specific syndromes of severe malaria and may inform drug and vaccine development efforts to prevent severe malarial disease.

0251

AN IN VITRO MODEL OF SEVERE MALARIA REVEALS ALTERED TEMPORAL EXPRESSION OF UBIQUITYLATION PROCESS GENES

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Plasmodium falciparum malaria infection continues to be among the principal causes of childhood morbidity and mortality worldwide. Ubiquitination is essential for regulating physiological processes, including innate and adaptive immunity. Our recent studies in children from a *P. falciparum* holoendemic region of Kenya with either uncomplicated (Hb≥9.0 g/dL) or complicated (Hb<6.0 g/dL) malaria (any density parasitemia) showed differential expression of genes involved in the human ubiquitination process. We and others have shown that treatment of peripheral blood mononuclear cells (PBMCs) from healthy, malaria-naïve donors with a physiological concentration (10 µg/mL) of *P. falciparum* hemozoin (*Pf*Hz) can serve as an *in vitro* model for severe malaria. As such, PBMCs from such donors (n=3) were cultured in the absence or presence of *Pf*Hz (10 µg/mL) for 3, 9, and 24 hrs. RNA from the samples were isolated (Qiagen RNeasy Mini Kit) and cDNA was synthesized (Qiagen RT^2 First Stand Kit). Gene expression profiles for a panel of 84 key ubiquitination genes were quantified (Qiagen Human Ubiquitylation Pathway RT^2 Profiler PCR Array). Although PBMCs treated with *Pf*Hz for 3 hrs showed no significant alterations in mRNA levels, stimulation for 9 hrs elicited significant transcriptional changes for 6 genes: 4 were down-regulated (*FBXO4*, *NEDD8*, *UBE2E3,* and *UBE2W*), whereas 2 were up-regulated (*HERC5* and *UBE2J1*). Moreover, *Pf*Hz treatment for 24hrs significantly altered mRNA levels of 14 genes: 12 being down-regulated (*ANAPC11*, *BRCC3*, *CUL4B*, *FBXO4*, *MIB1*, *SKP2*, *TP53*, *UBA2*, *UBA3*, *UBE2G1*, *UBE2G2*, and *WWP1*) and 2 being up-regulated (*UBE2J1* and *UBE2Z*). Collectively, these studies demonstrate temporal differential regulation of ubiquitylation genes and suggest a potential role for this process in the pathogenesis of severe malaria.

0252

COMPLEMENT COMPONENT 5 (C5) MISSENSE MUTATIONS ALTER THE LONGITUDINAL RISK OF MALARIA AND SEVERE MALARIAL ANEMIA IN CHILDREN RESIDING IN A HOLENDEMIC REGION OF PLASMODIUM FALCIPARUM TRANSMISSION

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Severe malarial anemia (SMA) is a leading cause of childhood morbidity and mortality in holoendemic *Plasmodium falciparum* transmission regions. To gain an enhanced understanding of predisposing factors for SMA (Hb<5.0g/dL), we explored the relationship between complement component 5 (C5) missense mutations [rs17216529 (433C>T, Val>Ile^{145}) and rs17610 (3929C>T, Ser>Asn^{1310})], malaria, and SMA in a cohort of children (n=1,487) over 36-months of follow-up in western Kenya. C5 variants were selected based on their ability to impart amino acid substitutions that can alter the structure and function of C5. We explored the relationship between C5 genotypes/haplotypes and the number of malaria and SMA episodes over the 3-year follow-up period by fitting a Poisson regression (R glm function, family=Poisson; model selection: forward-backward search based on AIC). Potential confounding risk factors, such as age at enrollment, sex, HIV, bacteremia, sickle cell trait status, G6PD deficiency, and α-thalassemia, were included as independent variables in the model selection. After adjusting for multiple comparisons, longitudinal analyses revealed that inheritance of the homozygous mutant (TT) at locus 433 enhanced the risk of malaria (RR=1.144, 95%CI: 1.059-1.236, *P*=0.001). The CT haplotype also enhanced the risk of malaria (RR=1.068, 95%CI: 1.017-1.122, *P*=0.009). Consistently, the TT genotype at locus 433 that increased the risk of malaria also increased the risk of SMA (RR=1.627, 95%CI: 1.201-2.204, *P*=0.002). The haplotype containing both wild-type alleles (CC) decreased the longitudinal risk of SMA (RR=0.679, 95%CI: 0.542-0.850, *P*=0.001). Collectively, inheritance of the investigated C5 missense mutations influence the longitudinal risk of malaria and SMA in immune-naïve children exposed to intense *P. falciparum* transmission.

0253

OPTIMIZATION OF PLASMODIUM FALCIPARUM WHOLE GENOME CAPTURE FOR MULTIPLEX SEQUENCING OF LOW PARASITEMIA DRIED BLOOD SPOTS

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Generation of high-quality *Plasmodium falciparum* whole genome sequencing data from low parasitemia dried blood spots (DBS) will increase our capacity to conduct population genomic studies and genomic surveillance. Generation of sequencing data with high breadth of coverage requires isolation or enrichment of parasite DNA from the predominating host DNA. Previous methods of parasite DNA enrichment have included leukocyte depletion, selective whole genome amplification, enzymatic digestion of host DNA, and to a more limited extent, hybrid capture. In this study, we designed a whole genome capture approach, using Roche's SeqCap EZ platform, for generation of whole genome sequencing data from *P. falciparum,* and evaluated the extent to which capture reactions can be multiplexed to increase throughput, avoid non-specific hybridization, and reduce costs. Laboratory-created DBS from NF54 cultured parasites diluted in whole blood resulting in final parasitemias of 500, 1200 and 10000 parasites/μL were generated. DNA extracted from DBS underwent library preparation and barcode labeling. Individually labeled samples were combined into multiplex mixtures, captured, and sequenced using an Illumina NovaSeq S1. Genome coverage statistics, including the percent of parasite reads and proportion of the genome with at least 5x depth of coverage, were compared between multiplexed and non-multiplexed samples and across parasitemias. The average percentage of the *P. falciparum* genome covered by ≥5x reads in 8-plexed samples was 88.2%, 74.1%, and 59.7% for 10000, 1200, and 500 parasites/μL, respectively, which is equivalent or better coverage than in 4-plex samples (Wilcoxon RS, 10000 p/μL p=0.030, 1200 p/μL p=0.059, 500 p/μL p=0.478). In addition, we are evaluating the distribution of genome coverage and the potential for differential capture of varied parasite strains. Our results indicate that multiplexing did not decrease genome coverage, and in some instances, improved coverage at different parasitemias, suggesting increased multiplexing may be possible.

0254

GENETIC EPIDEMIOLOGY OF SÉNÉGAL MALARIA INFECTIONS REVEALS PARASITE CONNECTIVITY AND CORRELATION WITH MALARIA INCIDENCE

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Genetic epidemiology of *Plasmodium falciparum* in Sénégal, utilizing identical-by-descent analysis of sequencing data, detects patterns of outcrossing and inbreeding that inform transmission dynamics and parasite connectivity across time and space. Parasite relatedness reveals transmission dynamics in Thiès, Sénégal from 2006 to 2020, including persisting clonal lineages across multiple transmission seasons and R_0 declines and rebounds. Working with the Sénégal National Malaria Control

Program, we identified distinct patterns of relatedness to guide decision-making about intervention use. Genetic relatedness among parasites collected at 26 health-posts with incidence from < 1/1000 to > 450/1000 reveals distinct within- and between-site patterns that provide actionable data toward intervention use. Parasite relatedness patterns suggest human mobility contributes to malaria infections, including sites with little local transmission but many shared infections (Koutal), and distant that share genetically related parasites sites (Kafountine to Dakar). Distinct parasite genetic relatedness patterns are observed in sites with similar incidence, including Diourbel, with large residential schools characterized by a highly clonal parasite population structure consistent with local transmission that may be amenable to vector-based interventions; and Touba, a pilgrimage site characterized by partially-related infections shared within Touba and across Senegal. Epidemiological modeling of population genetic parasite data correlate with transmission (i.e., R_0) and with epidemiological indicators (i.e., incidence). Key modeling parameters include complexity of infection or proportion polyclonal and measures of clonality or genetic identity among the parasite populations. Integration of genetic epidemiology into ongoing surveillance is being used to understand how parasite infections in a population are derived, shared, and maintained and to reveal otherwise undetected patterns of relatedness among infections that can help gauge transmission and guide appropriate intervention targeting and selection.

0255

APICAL MEMBRANE ANTIGEN-1 (AMA1) HAPLOTYPES SPATIOTEMPORAL DISTRIBUTION AND ENCODING GENES VARIANTS RELATION TO MALARIA SYMPTOMS IN BANDIAGARA, MALI

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Although the overall malaria incidence has decreased sharply over the past decade, there has been a plateau in the past three years. Vaccination is a control strategy that could limit malaria burden. While multiple malaria vaccines have been tested in phase 2/3 trials, and one undergoing a phase 4 trial, none has induced an overall clinical efficacy greater than 50%. Understanding the parasite genetic factors playing a role in malaria pathogenesis could help identify essential proteins and design an effective malaria vaccine. We hypothesize that clinical phenotypes are a function of parasite density, parasite and human genetic makeup and potentially parasite and host epigenetics factors. To identify a relationship between parasite genetics and clinical phenotypes, here we have focused on the relation between the AMA1, an antigen involved in parasite invasion into the red blood cells and the clinical phenotype of resulting malaria clinical disease. Using *ama1* gene sequences generated from samples collected from 425 children in an incidence study conducted in Bandiagara, Mali over three consecutive years, we assessed the relationship between individual haplotypes and malaria clinical symptoms and disease. The dynamic of AMA1 haplotypes showed a gene under balancing selection during the three years of follow-up. After adjusting for study participants' sample collection location, we found a significantly greater proportion of most frequent c1L haplotypes in male participants compared to female. (p=0.01). Also, the distributions of AMA1 haplotypes indicates a clustering of all major variants in study participants with splenomegaly and diarrhea together (p=0.01). Finally, none of the most frequent haplotypes were linked to malaria defined as any temperature greater than 37.5^0C and a parasitemia greater or equal to 2500 parasites/mcl. Our findings suggest a lack of association between AMA1 haplotypes and all malaria symptoms highlighting the role of multiple factors in malaria symptomatology.

0256

GENETIC DIVERSITY AND DEMOGRAPHICS OF PLASMODIUM SPECIES ON BIOKO ISLAND, EQUATORIAL GUINEA

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Since 2004, the Bioko Island Malaria Elimination Project (BIMEP) has been conducting annual malaria indicator surveys (MIS). Starting in 2014, the Equatorial Guinea Malaria Vaccine Initiative (EGMVI) conducted a series of clinical trials examining safety and protective efficacy of the attenuated whole-sporozoite malaria vaccine candidate, PfSPZ Vaccine of Sanaria Inc. A large-scale phase III study is planned for 2022-2023 to demonstrate the protective efficacy against *P. falciparum* field exposure. Despite continuous monitoring, the diversity and genotypes of *Plasmodium* spp. infections on Bioko Island are unknown. Determining the baseline population of *P. falciparum* will help to understand its evolution under sustained control interventions and illuminate potential targets of vaccine-induced immunity. During the 2019 MIS, ~8,000 dried blood samples collected by finger pricking were stored on Whatman filter papers. DNA was extracted using a high-throughput method developed at the Malaria Research Program, at the University of Maryland Baltimore. A multiplex PCR assay confirmed the presence and species identify of *Plasmodium* spp. A subset of samples based on geographic location and age group on Bioko, will undergo selective whole genome amplification (sWGA) and Illumina sequencing. Travel history, pregnancy status, and gender will be examined. Genetic diversity will be measured by genome-wide nucleotide diversity (average pairwise sequence divergence) based on single nucleotide polymorphisms (SNPs). Major allele frequency per site, in each sample, will be assigned when read support is greater than 70%. Results from our investigation will describe characteristics of circulating infections, including *Plasmodium* species, complexity of infection and parasite population structure. Travel history will enable potential differentiation between local strains on Bioko Island and imported malaria cases from mainland Equatorial Guinea. By establishing baseline characteristics, the impact of future interventions can more effectively be measured by following their consequence on *P. falciparum* population over time.

0257

SEASONAL RISK ASSOCIATED WITH FIXATION OF ANTIMALARIAL RESISTANCE THROUGH IMPORTATION

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The introduction of artemisinin combination therapies (ACTs) has been instrumental in the global effort to reduce the public health burden due to *P. falciparum* malaria. However, the use of ACTs is also associated with an increased risk of the emergence and evolution of antimalarial resistance. While *de novo* acquisition through mutation is one mechanism by which antimalarial resistance can arise, another is through the importation of a resistant genotype though mechanisms such as human movement and migration. Despite awareness of the role that importation may play in the regional emergence of antimalarial resistance, knowledge of how the risk of antimalarial genotype fixation fluctuates with the seasonal patterns in malarial prevalence remains limited. To address this gap, we utilized a national-scale, individually-based stochastic model of malaria; previously calibrated for the prevalence and seasonality patterns in Burkina Faso; and

introduced individuals carrying resistant genotypes at defined intervals in order to observe possible fixation patterns. We show under what conditions importation events may lead to regional anti-malarial resistance, independent of any *de novo* mutation events.

0258

CAN SELECTION ON HISTIDINE-RICH PROTEIN 2/3 GENE DELETIONS BE DETECTED BY TRACES OF SELECTION?

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Efficient malaria control requires access to antimalarials and appropriate diagnostics, especially in remote areas. Rapid diagnostic tests (RDTs), being as sensitive as light microscopy, are most appropriate for these settings. Confirming infections by RDTs is also recommended by the WHO before treatment with artemisinin in an effort to contain resistance. The antigens targeted by widely used *P. falciparum* RDTs are histidine-rich proteins 2 and 3 (pfhrp2 and pfhrp3). So far hrp-based tests showed the best performance. However, *P. falciparum* variants having deletions at pfhrp2 and/or pfhrp3 are increasingly being observed, leading to false-negative RDT results and hence jeopardize reliable malaria diagnostics. We introduce a population genetic framework to investigate potential mechanisms driving the spread of pfhrp2/3 deletions. Particularly, delays in chemotherapy due to false-negative RDT results are a potential mechanism driving the pfhrp2/3 deletions. We show how this mechanism - unlike the mechanism driving antimalarial drug resistance - is linked to transmission intensities as reflected by multiplicity of infection (MOI). Namely, selection in a low-transmission setting is more efficient. Moreover, we show how traces of selection as reflected by genetic hitchhiking manifest for selection for pfhrp2/3 deletions vs. drug resistance. The population genetic models sustaining our framework can be employed to study the combined effect of drug resistance evolution and the spread of hrp2/3 deletions and be readily adapted to estimate frequencies and prevalences of pfhrp2/3.

0259

ESTIMATING THE RETROSPECTIVE IMPACT OF MALARIA INTERVENTIONS USING MODELLING: AN EVALUATION OF IRS IN SOUTHERN MOZAMBIQUE

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As malaria strategies are scaling up, there is an increasing need to assess their retrospective epidemiological impact in order to optimize scarce resources in future. Interventions aimed at reducing malaria transmission are chosen based on the impact shown in trials, however, often lack of context-specific pre-implementation assessment due to funding and/or operational constraints. Evaluations of interventions therefore often need to be conducted in the absence of a control group or baseline. The use of transmission models can facilitate these evaluations by reconstructing a possible counterfactual to what would have happened in absence of intervention. In this work, we present the steps taken, from exploratory data analysis to the calibration and simulation of a malaria transmission model, for the ongoing impact evaluation of indoor residual spraying (IRS) in districts in South Mozambique for the period 2017-2019. We collated routine surveillance and operational data to understand the malaria situation and the implementation of IRS during the evaluation time and before. Additional data was also collated to investigate other possible drivers of malaria transmission, including data on climate, other malaria interventions and insecticide resistance. Intermittent time series analyses were performed to understand malaria trends following IRS implementation. We found a statistically significant decrease in incidence after the intervention for children under the age of five, but not for adults. OpenMalaria, a malaria transmission model, was calibrated and used to disentangle the impact of IRS from any other potential drivers and represent the situation in each district. The impact of IRS was quantified by estimating the number of cases averted when compared to the counterfactual. These analyses were complemented by detailed district-level costing of interventions, to explore return on investment and support further economic evaluations. The framework presented here is a novel method for impact evaluations and highlights the additional value modelling can bring for estimating past impact of malaria programs.

0260

AN ENSEMBLE MODEL FOR URBAN MALARIA INTERVENTION MICROSTRATIFICATION IN NIGERIA

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Malaria is a leading cause of childhood mortality in Nigeria, accounting for an estimated 30% of all deaths between the ages of 1-59 months from 2008 - 2013. Intervention strategies that account for spatial variation in malaria transmission intensity both within and across urban areas, to target areas with the greatest burden will lead to cost-savings and potentially improve intervention effectiveness. We developed an ensemble model for predicting areas of high malaria transmission in urban Nigeria using data from Malaria Indicator Surveys (2010, 2015), Demographic and Health Surveys (2018), and geospatial covariates. Covariates and individual-level malaria microscopy results were aggregated to an area level by computing cluster-level proportions, where clusters are defined by a geographic boundary and consist of sampled households with children under the age of five tested for malaria. Cluster-level malaria prevalence was stratified into areas of low (<10%) and high (≥10%) transmission to create a balanced binary target variable. Twenty-three features were used in the prediction. Eighty percent of the data was used to train a random forest model to classify clusters by transmission status. Model performance was evaluated with a test set comprising 20% of the overall dataset. Model hyperparameters were tuned to optimize performance using both a random search and grid search algorithm. Classification accuracy, the number of correct predictions divided by the total predictions, was 74% for the training set and 71% for the test set. The strongest prediction feature was the proportion of individuals in the highest wealth quintiles followed by the proportion of women with secondary or higher education suggesting the strong influence of socio-economic drivers on malaria transmission in urban areas. Our model outputs can be used by regional malaria control programs to inform decisions on where to concentrate malaria interventions in urban areas.

0261

CHALLENGES IN DEVELOPING STATISTICAL MODELS TO UNDERSTAND FACTORS DRIVING URBAN MALARIA TRANSMISSION IN NIGERIA.

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Nigeria is the largest contributor to the global malaria burden, accounting for 27% of global malaria cases and 23% of global malaria deaths. Malaria transmission in urban Nigeria is highly spatially variable. Understanding drivers of transmission in urban settings can provide insight into how interventions can be allocated most efficiently. We describe data quality issues and their impact on our ability to identify and describe factors that drive malaria transmission in urban Nigeria. We used cluster-level data from the Demographic Health Survey in Nigeria (2010, 2015, 2018) and publicly available socioeconomic and climate data. Hierarchical clustering was performed with population density, housing quality, and building density data to assess cluster-sampling heterogeneity. Cluster-level

estimates of malaria parasite prevalence in children under the age of five were categorized into low (<10%) and high (≥10%) prevalence groups. A Bayesian multilevel model was used to evaluate each explanatory variable's effect on the log-odds of a cluster being in the high prevalence group, and state-level variation was modeled with a random intercept. The clustering analysis suggested inadequate heterogeneity: very high population and building density areas were underrepresented in the analysis dataset. Density plots of individual covariates showed a skewness towards zeros and ones, suggesting that most were improper. Educational attainment and wealth index were the only statistically significant variables, and each showed a negative correlation with parasite prevalence. We interpret the lack of a statistically significant relationship between potential confounders and mediators of the relationship between socioeconomic variables and parasite prevalence as due to their improper data distribution rather than the absence of a link. Data quality issues and low-resolution data hinder models from identifying malaria transmission drivers. Acceleration of malaria control and elimination efforts in urban areas require more resources to be invested in urban subnational malaria data collection.

0262

MODELING THE POTENTIAL IMPACT OF ANTIMALARIAL INTERVENTIONS IN HIGH-BURDEN AREAS OF ETHIOPIA

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Over the last decade, Ethiopia launched a major scale-up of malaria control interventions following a renewed interest to further reduce morbidity and mortality and eventually eliminate the disease. High coverage of these interventions coupled with the extensive deployment of health extension workers at the lowest administrative unit, have led to a large reduction in malaria morbidity and mortality across the country. Motivated by the gains made, the country embarked on a sub-national malaria elimination in 2017. However, there remain pockets of relatively high disease burden, where large population movements and seasonal workforces have complicated efforts to reduce transmission in these areas. By focusing on the high-burden areas of the country and using a previously developed dynamic malaria transmission model, this study, conducted in partnership with the FMOH, quantified the potential impact of new malaria control interventions including seasonal malaria chemoprevention (SMC), mass drug administration (MDA), and use of Piperonyl Butoxide (PBO) nets in 74 high-risk districts that represent 50% of the malaria burden nationally. A combination of PBO nets and MDA was estimated to yield the highest reduction in annual clinical incidence (28% all ages and 24% for children under five [U5s]), followed by a scenario that combines PBO nets and SMC (25% for all ages and 46% for U5s). Our results also demonstrated an 18% greater incidence reduction for PBO nets compared to standard long-lasting insecticidal nets (LLINs), highlighting the importance of pyrethroid resistance profile in Ethiopia. With effective mix of interventions targeting the highest incidence areas, Ethiopia can further reduce malaria burden not only in those regions, but also nationally, bringing elimination closer. A national malaria strategy incorporating modelling based on local data could help to guide sub-national tailoring of malaria intervention mixes for optimal burden reduction and accelerate progress towards elimination.

0263

SAME BUDGET, MORE IMPACT: USING MATHEMATICAL MODELLING TO OPTIMIZE CAMEROON'S MALARIA PROGRAM

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National intervention plans are designed in the context of heterogeneous risks, multiple available tools, and non-linear disease dynamics. Coupling mathematical modelling of malaria dynamics with economic tools is a powerful way to explore trade-offs between strategies. We aim to identify two stratification plans that minimize all-age malaria cases and deaths within the program budget. Firstly, we developed a list of interventions considered under the national strategy; these reflect global guidelines. Second, we calibrated an established malaria microsimulation platform to historical prevalence. We used the model to predict the district-level impact (number of cases or deaths averted compared to the current national plan) and the cost of each possible combination of interventions. Using a linear optimization algorithm, we selected stratifications that maximise i. cases averted and ii. deaths averted, at a cost within the available budget. Piperonyl butoxide bednets were selected over standard long-lasting insecticidal nets for near blanket coverage both when optimizing for cases and deaths (in >90% of 189 districts). Indoor-residual spraying was rarely selected (<10% districts). Intermittent preventive treatment for infants was selected in fewer districts when optimizing to avert cases (~50% districts), than to avert deaths (~70% districts), with seasonal malaria chemoprevention selection following the same pattern (in ~9% and ~20% of 45 eligible districts, when averting cases and deaths, respectively). Enhanced case management was selected for blanket coverage for both targets. Both optimized strategies were projected to reduce the number of cases and deaths by 16-18% over the current plan. Our analysis combines information on malaria ecology, burden and intervention costs within a unified framework. Such analyses provide a valuable additional source of information for decision-makers when designing national strategies. Faced with competing health priorities and stagnating malaria funding, we must find ways to make money go further: optimized intervention stratification offers an exploration of possible paths.

0264

ESTIMATING THE IMPACT OF SINGLE LOW DOSE PRIMAQUINE ON REDUCING MALARIA TRANSMISSION IN NKOMAZI SUBDISTRICT, SOUTH AFRICA

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Single Low-Dose (SLD) Primaquine may be used successfully to decrease transmission of the *Plasmodium falciparum* parasite. In South Africa, a modelling study was conducted to estimate the community benefit of widescale distribution of SLD Primaquine in combination with Artemether / Lumefantrine in the Nkomazi sub-district in Mpumalanga Province. A nonlinear ordinary differential equation compartmental model was developed to simulate malaria transmission and estimate the intervention's impact on reported malaria incidence. The model was developed to capture the *in vitro* gametocyte stage of the parasite life cycle by accounting for sequestration and maturity of gametocytes. In order to quantify the contribution of SLD Primaquine in reducing transmission, it was also necessary to estimate the impact of supporting interventions like indoor residual spraying activities, active case detection, importation of malaria and the seasonality of transmission. The model was validated with local and imported case and intervention data from the Mpumalanga Malaria Control Programme (MMCP) in order to estimate the cases averted

due to the SLD Primaquine intervention. The impact of intervention at various coverage levels within the malaria season was also explored. The modelling study is currently being extended to other endemic subdistricts in South Africa where SLD Primaquine was introduced.

0265

ESTIMATING THE VARIABILITY OF ANOPHELES BIONOMICS AND ITS IMPACT ON TRANSMISSION WITH A HIERARCHICAL BAYESIAN MODEL

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Locality-specific information is generally unavailable on how the bionomics of *Anopheles* mosquitoes affect the vectorial capacity for malaria, or on the vulnerability of local vectors to different vector control methods. To address this, we estimated geography-, time- and species-specific survival and behavioral parameters of *Anopheles* mosquitoes using an augmented version of the Malaria Atlas Project's global database of bionomics data. A sensitivity analysis using an existing mathematical model of the impact of vector control was used to determine which bionomics parameters are most influential. We then applied inclusion and exclusion criteria to select subsets of studies with relevant experimental designs that minimize bias from collection methods for parous, endophagy, and endophily rates. For informing the human blood index, we separated data from indoor and outdoor collections. We performed a meta-analysis by fitting a Bayesian hierarchical model with levels based on *Anopheles* taxonomy. The resulting algorithm can be applied automatically to select relevant data for a specific country and/or time span. Using 26 studies, we found a mean endophagy rate of 40 ± 4% with high inter-species variability: on average, 23 ± 2% of *Anopheles albimanus* and 66 ± 0.5% of *Anopheles arabiensis* mosquitoes bite indoors. Location is also highly influential: 67 ± 0.5% of *Anopheles arabiensis* bite indoors in East Africa, but only 39 ± 3% in West Africa. We selected 61 studies for estimating the parous rate and observed it is highly species-variable, with averages ranging from 29 ± 2% for the *Nuneztovari* complex to 76± 0.8% for *Anopheles funestus* sensu stricto. There is little intra-continent variation, with the parous rate of *Anopheles gambiae* sensu stricto varying between 61 ± 0.4% in West Africa and 65 ± 2% in East Africa. The model allows extrapolation of knowledge on *Anopheles* bionomics and quantification of variability between species and geographies. The dataset can be readily augmented with additional data, but there is still a need for more frequent and standardized entomological data collections to understand local behaviors of malaria vectors.

0266

MODELING THE DIFFERENTIAL IMPACT OF IMPROVED EFFECTIVE COVERAGE FOR MALARIA CASE MANAGEMENT ACROSS TRANSMISSION SETTINGS

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Effective treatment of symptomatic malaria is a critical component of control efforts due to its role in reducing not only morbidity and mortality but also the transmission potential of detected cases. Directly measuring the impact of routine case management is challenging due to underreporting and the concurrent deployment of other interventions. Mathematical models can be applied to capture transmission events under pre-specified scenarios while accounting for underlying immunological profiles. Using an agent-based simulation model of *Plasmodium falciparum* malaria dynamics, we aimed to evaluate the impact of case management services at programmatically attainable effective coverage levels and in varying transmission contexts. Specifically, we estimated the impact on incidence, prevalence, and mortality of increasing realistic levels of baseline effective coverage (15%, 25%, or 50% cure rates of symptomatic malaria) by 10%, 30%, or 50% points in three hypothetical transmission settings: low (15% under 5 prevalence at baseline), moderate (25%), and high (50%). Across transmission settings, operationally feasible increases in effective coverage resulted in reductions in incidence, prevalence, and most notably mortality. For instance, increasing coverage by 30% pts—as might be achieved by preventing stockouts—was associated with reductions of ~0-23% in incidence, ~7-29% in prevalence, and ~16-38% in deaths. Impact was greatest among children and in low transmission settings. For example, given a low baseline coverage of 15%, increasing coverage by 30% pts was associated with 51% and 22% reductions in prevalence among children under five for low and high transmission settings, respectively. The same scenario was associated with 29% and 7% reductions in population-level prevalence. By isolating the impact of case management on malaria outcomes, we identified that, regardless of baseline coverage, even modest coverage increases have transmission and mortality burden impact, not only in low endemic areas but also in high endemic settings characterized by greater immunity and thus lower proportions of cases with symptoms.

0267

ESTIMATION OF MULTIPLICITY OF INFECTION IN MALARIA ASSUMING OVER-DISPERSED MOSQUITO BITING RATES

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In areas of high transmission, individuals are typically infected with multiple genetically distinct malaria lineages (haplotypes) due to multiple infective contacts (super-infections) during one disease episode. The number of (super-)infections is referred to as multiplicity of infection (MOI), which is considered an essential metric in malaria control. MOI correlates with transmission intensity, mediates recombination and hence affects the parasite's genetic diversity. MOI also impacts the spread of multidrug resistance characterized by point mutations in different genes. MOI estimates from heuristic methods are inferior to those from formal statistical frameworks. Maximum-likelihood (ML) frameworks to estimate MOI typically have desirable properties but are based on the assumptions made by the underlying model. A standard assumption is that of rare and independent infective events, leading to a Poisson distribution. ML methods to estimate MOI have been criticized because of the Poisson assumption. Namely, it implicitly reflects that the number of mosquito bites within a given time interval is also Poisson distributed - which is not supported by empirical evidence. The distribution of mosquito bites is typically overdispersed and accurately modelled by a negative binomial distribution. Here, we introduce a maximum likelihood framework under the assumption that MOI follows a negative binomial distribution. We show that this model is degenerate and always yields the same estimate as the corresponding Poisson model. This shows that the Poisson model is more generally applicable than previously thought and implies that the effect of overdispersion can only be accounted for if sustained by the data, e.g., by providing additional information on the risk of exposure of certain patients.

0268

PREDICTING THE EPIDEMIOLOGICAL IMPACT OF LARGE-SCALE IMPLEMENTATION OF INTERMITTENT PREVENTIVE THERAPY IN INFANTS IN SOUTHERN NIGERIA

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Intermittent preventive therapy in infants (IPTi) aims to reduce clinical malaria episodes and deaths by administering sulfadoxine-pyrimethamine (SP) at three time-points during infancy. The World Health Organization (WHO) recommends IPTi in moderate to high, non-seasonal malaria transmission areas. Although recommended since 2010, only one country has adopted the strategy. Delaying IPTi may miss the opportunity to reduce adverse malaria outcomes in infants and young children and to accelerate policy adoption, but stronger evidence on IPTi effectiveness as well as a re-assessment of its deployment schedule are urgently needed. This study estimates the likely impact of large-scale implementation of IPTi when administered as recommended at 2, 3, and 9 months of age or with additional doses during infancy and extended into the second year of life. We adapt an existing malaria transmission model (EMOD) to model the impact of IPTi in 365 eligible local government areas (LGAs) in Nigeria. The model was calibrated to capture the transmission intensity and seasonality in each LGA and parameterized using Demographic Health Survey (DHS) and Malaria Indicator Survey data for insecticide treated bednet coverage and case management. IPTi coverage was informed using pentavalent 2, 3, and measles vaccine coverage from the DHS in 2019. We use IPTi-SP efficacy estimates from a systematic review of IPTi trials inform the model. We estimate the reduction in malaria infections and clinical cases in infants at 21.5% (range across LGAs 6.8-34%) and 15% (4.2-21.0%) respectively. Results further suggest that an additional dose during the second year of live would be more impactful than an additional dose during infancy. Although relative reductions are small, absolute cases averted may be high and the results favor an extension of IPTi doses into the second year. Our modeling study provides IPTi impact estimates for different deployment schemes across various transmission settings in Southern Nigeria and contributes to strengthening evidence for informing national IPTi policy in high malaria burden countries.

0269

TRANSMISSION-BLOCKING INTERVENTIONS: FROM LABORATORY TO THE FIELD

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Transmission-blocking interventions (TBIs), designed to block transmission of malaria parasites from an infected human to a feeding mosquito, have been proposed as a potential tool in the fight against malaria. Interest in TBIs is increasing, as candidates are now being evaluated in malaria-endemic settings, rather than purely in the laboratory. It is important to understand how effective these vaccines are in the field, compared to in the laboratory, and how best to deploy them as a public health tool. Building on recent work, an established model of malaria transmission is used to provide insight into how potential candidate TBIs could be combined with existing control interventions to support malaria control and elimination. The work is illustrated using monoclonal antibodies with transmission-blocking properties. It is particularly important to identify which age groups within a population should be targeted with a TBI, and how these novel tools could be utilised alongside existing interventions. The composition of the infectious reservoir in a malaria-endemic setting will be influenced by the current intensity of malaria transmission but will also depend on how transmission intensity has changed in recent years. Model predictions are compared to evidence on the reservoir of infection from direct membrane feeding assays. The framework is used to predict the public health impact of different monoclonal antibody use cases in areas with different malaria endemicity and seasonality of transmission and investigate how these novel interventions could be evaluated in large scale clinical trials.

0270

EFFECT OF SOCIAL AND BEHAVIOUR CHANGE INTERVENTIONS ON MALARIA HEALTH BEHAVIOUR - LLIN USE AND TREATMENT SEEKING IN ETHIOPIA

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The Johns Hopkins Centre for Communication Programs (CCP) aims to improve malaria behaviours in Ethiopia. The project's baseline research included 2770 women in rural Ethiopia in 2016 and evaluated knowledge on malaria prevention and women's gender equitable norms as predictors for LLIN use; and self-efficacy a predictor for woman's treatment seeking behaviour for fever. Consequently, the program designed and implemented gender sensitive multi-channel social and behaviour change (SBC) interventions including roadshow community events, a mobile app, maternal and child health videos and weekly radio programs. Endline data were collected from 1773 randomly sampled rural women from intervention areas in 2019. Regression analysis suggests that program exposure significantly increased women's knowledge of malaria prevention methods (aOR: 3.21, 95% CI: 1.34-7.67), positive attitude towards gender equitable norms (aOR: 1.33; 95% CI: 1.07-1.670), and self-efficacy on treatment seeking for fever (aOR: 3.50; 95% CI: 1.70-7.46). These behavioural antecedents also were significantly associated with LLIN use and treatment seeking for fever. Treatment seeking for children with fever under five improved from 66.9% to 70.5% between baseline and endline, respectively ($P<0.05$). Women exposed to the interventions were 2.5 times (95% CI: 1.15-5.42) more likely to seek treatment than those unexposed. Significant dose-response relationships were also seen between the women's LLIN use by level of exposure (none, some, high) to the SBC interventions: LLIN use was 55.9%, 62.1% and 75.8%. The results support that well-designed "community and gender sensitive" SBC interventions can improve malaria prevention and treatment seeking behaviour.

0271

RE-INTRODUCED MALARIA IN QUEENSLAND, AUSTRALIA DURING THE SECOND WORLD WAR

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Once malaria has been eliminated from an area, it is difficult re-introduce it despite the continued presence of competent *Anophelines*. One of the greatest tests of this principle occurred during the Second World War when thousands of soldiers with malaria infections from New Guinea were stationed in Queensland. A *P vivax* epidemic arose in 1942 in Cairns which was the evacuation point for many military and civilians from the Imperial Japanese Army's offensive in New Guinea. Malaria in Cairns was noted from May 1942 especially in outer areas (Bungalow) where brackish water creeks promoted mosquito breeding. The epidemic of nearly 700 total cases peaked at mid-year which are the cooler months (average 17-26° C) and then fell rapidly in September. Sporozoite positive mosquitoes (1.5% positive out of 1891 dissected) were first documented in Australia during the Cairns epidemic from *Anopheles farauti* (known then as *An punctulatus moluccensis*). Thus warned, public health authorities made

great efforts to suppress parasites in transiting soldiers and to position them south of 19° S latitude away from most vectors. Only two distinct outbreaks other than Cairns were recorded in Queensland during the war. Pamaquine, an old 8-aminoquinoline, was extensively used in combined treatment regimens killing most gametocytes. Queensland experienced some scattered locally transmitted epidemics in 1943-44 but by 1945 malaria transmission had largely been eliminated. Improved 8-aminoquinoline regimens such as tafenoquine could assist blocking future re-introductions of malaria into eliminated areas.

0272

IMPACT OF COMMUNITY DELIVERY ON COVERAGE OF INTERMITTENT PREVENTIVE TREATMENT FOR MALARIA IN PREGNANCY IN MALAWI

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Increasing coverage of at least three doses of sulfadoxine-pyrimenthamine (SP) for intermittent preventive treatment in pregnancy (IPTp3) is a priority to prevent malaria in moderate to high transmission areas. In 2020, only 30% of pregnant women across sub-Saharan Africa received IPTp3. Coverage of IPTp3 in Malawi was higher (41%, 2017 MIS), but still low. From February 2019-August 2020, we implemented a cluster randomized controlled trial in 20 health facilities (HF) in two districts in Malawi. The purpose of the trial was to assess whether community IPTp delivery (cIPTp) by Health Surveillance Assistants (HSAs) can increase IPTp3 coverage, compared with IPTp delivery at antenatal clinics (ANC) alone. Potential adverse effects of cIPTp on ANC attendance were also evaluated. HFs were randomly assigned 1:1 to control or intervention. All HSAs affiliated with a HF were assigned to the same group. Household surveys were conducted at baseline (Dec 2017) and endline (Aug 2020) among women who had completed a pregnancy in preceding 12 months. Effects were estimated using a difference-in-difference regression analysis, accounting for clustering by HF. Surveys were conducted with 370 women at baseline, and 687 at endline. Half of those surveyed at both timepoints were from intervention areas. Median age was 24 years; 35% of women were primigravid. Socio-demographic features were similar between intervention and control areas. Compared with controls, IPTp1, IPTp2 and IPTp3 in intervention areas changed by 13.5 (95% CI: 4.7, 22.3), -2.5 (95% CI:-16.3, 11.4) and 6.9 (95% CI:-5.9, 19.6) percentage points, respectively, while ANC2, ANC3 and ANC4 in intervention areas compared with controls changed by -0.3 (95% CI:-8.4, 7.8), 17.7 (95% CI: 0.9, 34.4) and 25.3 (95% CI: 1.3, 49.3) percentage points, respectively. Women initiated ANC on average 2.5 weeks earlier in intervention versus control areas (95% CI: 1.4, 3.7). In a setting with above average baseline coverage of IPTp3, cIPTp failed to significantly increase coverage of IPTp. cIPTp was associated with earlier initiation of ANC and may have prevented a decrease in IPTp coverage during COVID lockdowns.

0273

THE CONTRIBUTION OF COMMUNITY DELIVERY TO THE UPTAKE OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY WITH SULFADOXINE-PYRIMETHAMINE IN THREE DISTRICTS OF THE DEMOCRATIC REPUBLIC OF THE CONGO

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According to the 2017-2018 Multiple Indicator Cluster Survey (MICS), 56.0% of pregnant women in the Democratic Republic of Congo initiated intermittent preventive treatment of malaria in pregnancy (IPTp) with quality-assured Sulfadoxine-Pyrimethamine (QA SP). However, only 13.4% of pregnant women received the minimum three doses recommended by the WHO. To help close this gap, community-based distribution of QA SP (C-IPTp) by community health workers (CHWs) was introduced as a pilot in 88 health facilities across three districts. C-IPTp was launched in Kenge district in August 2018 followed by Bulungu and Kunda districts in November 2019. Community selected CHWs were trained and deployed to identify pregnant women and refer them to ANC, as well as to screen them for eligibility to receive QA SP and administer QA SP to those eligible. Another key part of this intervention is focused social and behavior communication change activities and ANC outreach promotion. Health facility service delivery data from the National Health Management Information System, coupled with supplementary data from CHW activities were analyzed to examine trends in ANC and IPTp uptake. We used estimated number of pregnant women as a denominator in our analysis. Across the three districts, the IPTp uptake trend showed clear progress. In Kenge, 37% of pregnant women received IPTp3 in 2017 prior to introduction of C-IPTp which increased to 90% in 2020 with 30% of doses given by CHWs. In Bulungu and Kunda, 48% of pregnant women received IPTp3 in 2017, which increased to 72% in 2020 with 12% of doses given by CHWs. In addition, pilot districts experienced improvements in ANC attendance. The percentage of pregnant women attending at least 4 ANC visits increased from 41% in 2017 to 64% in 2020. In Kenge district, early ANC initiation (before 16 weeks) increased from 36% to 67% over the same time period. These data suggest that complementing ANC distribution of QA SP for IPTp with CHW distribution can contribute to overall increases in IPTp uptake as well as improvements ANC attendance.

0274

IDENTIFICATION OF NATIVE SECRETED PROTEINS WITH ASAIA BOGORENSIS FOR PARATRANSGENIC MODIFICATION

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Malaria is a major global health concern, responsible for 229 million cases and over 400,000 deaths in 2020. This disease is caused by parasites of the genus *Plasmodium* and spread through the bite of infected female *Anopheles* mosquitoes. While preventative measures such as bednet usage, and indoor spraying of insecticides have reduced the global incidence, it is obvious that new measures are desperately needed to combat the spread of this disease. Paratransgenesis is one such technique, and refers to engineering a native symbiont of mosquitoes to produce antiplasmodial molecules and ultimately prevent parasite transmission. *Asaia bogorensis* is a native symbiont of *Anopheles* mosquitoes, and an ideal candidate for paratransgenic modification. However, little is known about the native secretion systems present in *A. bogorensis* and how to harness them for use. Through the use of an online prediction tool called BastionHub, we have identified over 300 predicted Type I and Type II secreted protein. We have narrowed these down to the top 30 most likely candidates and used qPCR analysis to determine which, if any, of these candidates were upregulated in the presence of bloodmeal-like conditions. Upregulation in bloodmeal-like conditions may improve a

paratransgenic system by narrowing the production of antiplasmodials to when *Plasmodium* is present. The candidates identified through qPCR were then tested for secretion, by directly inserting an epitope tag into the chromosome of *A. bogorensis.* These strains were analyzed for secretion in minimal and bloodmeal-like conditions. This identified the first confirmed secreted proteins within the *A. bogorensis* proteome and can be targeted for paratransgenic modification with antiplasmodial effectors.

0275

IMPACT OF THREE MONTHS OF MALARIA CHEMOPROPHYLAXIS AFTER SEVERE FLOODING IN WESTERN UGANDA: A PRAGMATIC TRIAL IN A HUMANITARIAN CONTEXT

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Nearly half of global disasters over the past two decades were attributable to extreme precipitation and flooding, including 64% of events in the Africa region, where malaria is endemic. Unlike the immediate impacts of flooding, however, epidemics of malaria emerge months after flooding and cause substantial excess morbidity and mortality. We conducted a pragmatic, quasi-experimental trial of malaria chemoprophylaxis in response to severe flooding that occurred in the Kasese District of western Uganda in May 2020. All children 12 years of age and younger in one village were eligible to receive monthly rounds of dihydroartemisinin-piperaquine (DP) in the three months following flooding. During each round, field staff geolocated households with a handheld GPS, administered a brief questionnaire. And performed a rapid diagnostic test. Tablets of DP were provided according to weight-based dosing guidelines. To assess the impact of the program, we selected two neighboring villages where no malaria-specific interventions were undertaken to serve as controls. We abstracted routine clinical information from registers at the five nearest health facilities to measure the incidence of clinical malaria. A total of 554 children completed at least one round with 413 (74.6%) completing at least two rounds. Compared to control villages, we estimated a 30.1% (p = .03) and a 55.0% reduction (p<.001) in test positivity and incidence, respectively. Using differences in the observed incidence between the intervention and control villages, we estimate that the chemoprophylaxis intervention resulted in 318 cases averted (95% CI 294 - 342) among a population of 1,188 residents. While eligible represent the largest share of averted cases (217, 68.2%), the model also predicted a substantial decrease in cases among older individuals who were not eligible for the intervention. Total program costs were $3.89 per course of DP delivered or $15.87 (95% CI $14.75 - $17.16) per case averted. Our study adds to evidence base of opportunities where targeted, relatively short periods of malaria chemoprophylaxis may be used to reduce excess disease burden.

0276

MALARIA CARE-SEEKING AND TREATMENT IDEATION AMONG GOLD MINERS IN GUYANA

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Guyana hinterland regions 1, 7, 8, and 9 have the country's highest malaria transmission rates, particularly among remote mobile gold miners. The USAID-funded Breakthrough ACTION project, in collaboration with the Guyana National Malaria Program (NMP), sought to understand how gold miners' malaria-related ideation - knowledge, beliefs, attitudes, and feelings - influence the adoption and maintenance of malaria care-seeking/treatment behaviors. 1685 miners from 233 mining camps in project focal areas in Regions 1, 7 and 8 were surveyed. Malaria care-seeking and treatment ideation was defined as a composite additive score consisting of general malaria knowledge, perceived susceptibility, perceived severity, and interpersonal communication in addition to specific care-seeking/treatment beliefs, perceived self-efficacy, norms, and response efficacy. Higher scores indicate more positive ideation. Logistic regressions assessed the relationship between ideation score and care-seeking/treatment behavior. Overall, miners' care-seeking/treatment ideation score ranged from 0 to 8 with a mean (SD) of 3.8 (1.7), and scores were higher among miners who were in Region 7, Christian, older than 35 years, more educated, or had prior episodes of malaria. Higher levels of ideation were associated with higher rates of any care-seeking (aOR: 1.19, 95%: 1.04 - 1.36), but not prompt care-seeking; with higher rates of malaria testing (aOR: 1.22; 95% CI:1.07 - 1.38); and with lower rates of self-medication (aOR: 0.80, 95%CI: 0.77 - 0.99). Miners' care-seeking/treatment ideation was linked with improved malaria behavior outcomes. The NMP is implementing a community case management (CCM) initiative using trained volunteers to test and treat uncomplicated malaria in mining camps. Breakthrough ACTION Guyana is implementing a mass media campaign to address miners' ideation related to care-seeking and treatment; distributing counseling materials to ensure high quality of testing and treatment services; and branding of malaria testing and treatment locations and testers with flags and certificates to increase the visibility of the CCM program.

0277

ASSESSING SEASONAL MALARIA CHEMOPREVENTION ADHERENCE IN NIGER THROUGH AN INDEPENDENT MONITORING SURVEY USING A RANDOMIZED CLUSTER DESIGN

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In Niger, community distributors give Seasonal Malaria Chemoprevention (SMC) medication through home visits to children aged 3 -59 months at monthly intervals for four months during the rainy season. Despite the scale-up of SMC since 2016 to all eligible districts and high administrative coverage (>85%), the reduction in child morbidity due to malaria remained below the expectations of the Niger National Malaria Control Program (NMCP). To assess adherence to the full SMC treatment regimen, the NMCP instituted monitoring surveys after each cycle of SMC campaign. In 2020, an independent monitoring survey using randomized cluster sampling was conducted to collect quantitative data from caregivers using an electronic tool (KoBoCollect) in the 17 health districts (HD) (eight in the Dosso region and nine in the Tahoua region) supported by President's Malaria Initiative Impact Malaria (IM) project. For each of the four cycles, two to three HDs per region were surveyed, covering each district once. In each HD, one urban and two rural health areas were selected; in each health area, three villages were selected and 20 households per village. All the sampling was done through systematic sampling. A total of 153 villages in 17 districts were sampled and 3,059 households were visited with caregivers of 5,130 children assessed on the medication's adherence. According to parent declaration, 99.6% of children (5,107/5,130) received at least one dose and among them, 99.2% (5,064/5,107) could show proof (filled SMC card or empty blister pack); 90.6% of children (4,180/4,616) received the second dose and 84% (2,008/2,392) the third dose according to parent report; parents could show proof of administering medicines to 80% (3,306/4,180) of children on the second day and 77.6% (1,558/2,008) on the third day. Loss or damage to cards were cited as the primary reason for not being able to show proof. Independent monitoring surveys (using the randomized

cluster sampling approach) implemented over each SMC cycle suggest that the less than expected reductions in morbidity are unlikely to be the result only of a lack of adherence to the full treatment regimen.

0278

INCREASED INTERMITTENT PREVENTIVE TREATMENT (IPTP) WITH SULFADOXINE PYRIMETHAMINE IN PREGNANT WOMEN THROUGH THE USE OF A MONITORING REGISTER: EXPERIENCE FROM THE STOPPALU+ PROJECT IN 19 DISTRICTS IN GUINEA

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Malaria infection in pregnancy is associated with anemia, low birth weight and neonatal mortality. The WHO recommends using intermittent preventive treatment in pregnancy (IPTp) to reduce the risk of malaria, giving at least three doses of sulfadoxine-pyrimethamine (SP) at scheduled antenatal care (ANC) visits. IPTp in pregnant women remains low in Guinea. According to national health information system (HIS) data, in 2018, 58% of pregnant women received three doses of SP and only 53% received four doses. While IPTp can be measured using survey data, coverage estimates from surveys represent a retrospective time period of up to 2 years. Routine data from ANC can be used to provide a more current estimate of IPTp coverage; however, there are challenges such as nonstandard ANC registers across health facilities and calculation methods that are aggregate instead of individually-based and are therefore less accurate. It is within this framework that the StopPalu+ project funded by the President's Malaria Initiative (PMI) supported the ministry of health to develop ANC monitoring registers. The objective was to document the actual level of IPTp coverage and design methods to improve coverage in areas that were lagging in the 19 districts supported by the project. Pregnant women who came for their first ANC visit in HCs from April 1, 2019 to March 31, 2020 were entered in the cohort register and followed until delivery. IPTp coverage with three doses increased over the monitoring period from 62% in the Apr-June 2019 cohort to 78% for Jan-March 2020 cohort. Documenting and following pregnant women using the cohort registers allowed for closer

0279

MALARIA IN PREGNANCY AND ANTENATAL CARE KNOWLEDGE, ATTITUDES AND INTERVENTION COVERAGE IN ATLANTIQUE DEPARTMENT, BENIN

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PMI Impact Malaria, in collaboration with the Benin National Malaria Control Program, is conducting a cluster randomized controlled trial in 40 health facilities (HF) comparing the impact of group antenatal care (gANC) versus standard (individual) ANC on uptake of malaria in pregnancy (MiP) interventions and quality of antenatal care. From November to December of 2020, we conducted a baseline cross-sectional household survey to assess coverage of MiP interventions, birth outcomes, knowledge and attitudes about ANC, role of gender in decision-making around ANC attendance, and behavior differences between those attending and not attending ANC among women who had completed a pregnancy in the last 12 months. One enumeration area (EA) per HF catchment was selected population proportional to size, and 33 women were randomly selected per EA. Analyses were done in SAS V9.4 and accounted for clustering at the HF level. Of 1259 women included, 21% were primigravid, 23% were secundigravid; 94% attended ANC1 but only 58% attended ANC4+. Of those who did not attend any ANC, 29% stated the facility was too far and 36% stated care was too expensive. Women initiated ANC at a mean of 3.3 months. Half reported that their husband had accompanied them to ANC. While 88% of women received at least 1 dose of intermittent preventive treatment in pregnancy (IPTp), 69% received 2 doses, and 40% received 3 doses. A total of 97% of pregnant women had their blood pressure taken, 96% had urinalysis, and 82% were dewormed. Most women (89%) reported using long-lasting insecticide-treated nets (LLIN). The group ANC intervention began in March 2021 and will continue for 18 months. The baseline data, as well as findings from the trial, may be used to improve ANC quality and better tailor and target malaria in pregnancy interventions for pregnant women in Benin, and throughout the region. Results may also be used to refine messaging and communication to promote early and continued ANC attendance.

0280

INTRODUCING FIELD DIGITAL DATA COLLECTION SYSTEMS INTO SEASONAL MALARIA CHEMOPREVENTION CAMPAIGNS - LESSONS LEARNT AND OPPORTUNITIES FOR ROBUST EVIDENCE DEVELOPMENT

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Data management has been cited as one of the challenges hindering the development of evidence generation for health interventions in Africa. To address these challenges, field digital data collection tools were introduced to support data management for health interventions, including malaria. Investigating the impact of the use of digital data collection tools in Seasonal Malaria Chemoprevention, a multi-dose health intervention that requires eligibility verification and follow-up for compliance, could benefit other multi-step health interventions that experience compliance challenges. Recently, Benin, the Gambia, Ghana, and Nigeria introduced digital android mobile data collection systems into their SMC campaigns. Based on qualitative evidence provided, this presentation shows the benefits, challenges and lessons learnt from the incorporation of digital data collection tools in the implementation of SMC. It discusses the challenges, and opportunities of linking field digital data collection tools into routine healthcare data management. Digital data collection systems were instrumental in the enumeration, satellite mapping and registration of children for SMC as well as in tracking the coverage of SMC. They were also useful in tracking dose compliance and monitoring of adverse drug reactions. Countries, however, cited that internet connectivity issues as well as limited resources to scale up the use of digital data collection systems were limiting the effective use and scale up of digital data collection tools, respectively. While all countries recognized the benefits of linking the data from digital collection tools with routine healthcare data collection systems none had implemented this till date due to resource constraints. The authors encourage that field data collection tools be scaled up for use in SMC campaigns. They advocate those solutions that can effectively link field data collection tools to routine health management systems be pursued to support robust evidence generation for policy development and innovation.

0281

SOCIAL NORMS, SOCIAL SUPPORT AND AVAILABILITY ARE CRITICAL DETERMINANTS OF CONSISTENT ITN USE BY FOREST-GOERS IN CAMBODIA: IMPLICATIONS FOR SBC PROGRAMMING FROMA RESPONDENT-DRIVEN SAMPLING STUDY

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Malaria transmission in Cambodia is highly localized to forests and forest fringes. Forest-goers are a hard-to-reach population at heightened risk of malaria infection and rely on repellents, coils, clothing and mosquito nets (ITNs) and hammock nets for prevention. However, ITNs are an imperfect tool in this context and insights into determinants of use can improve targeted SBC activities. We used respondent-driven sampling to conduct a survey of 654 forest-goers in 2020 to understand determinants of ITN use. One seed from each of 16 villages in Kampong Chhnang and Pursat provinces was recruited and referred up to three forest-goer contacts. Eligible participants reported spending at least one night in the forest in the past month. Recruitment continued until the required sample was reached, yielding up to 9 waves for some seeds. Multivariate logistic regression analysis was conducted using sleeping under ITN as the dependent variable. Three-fourths (75%) of forest-goers reported sleeping under an ITN every night during their last forest visit ("users"). The most common reasons for not consistently using an ITN were "too hot to use net" (51%), "no net available for use in forest" (21%) and "net available but wasn't brought" (19%). ITN users were more likely to own one or more ITNs that could be brought to the forest compared to non-users (96% vs 66%, p<0.0001) and were more likely to report prevalent social norms on ITN use within their community when in the forest than non-users (95% vs 65%, p<0.0001). ITN users also reported stronger social support than non-users, agreeing that their family and friends support the use of ITNs (79% vs 21%, p<0.0001). Finally, ITN users were less likely than non-users to believe that ITN use carried adverse health effects than non-users (40% vs 50%, p=0.03). This study revealed that social norms and social support for forest ITN use and perceived adverse effects were strongest behavioral determinants of consistent ITN use by forest-goers in these sites. Future SBC activities should target these levers to support ITN use, while complementary activities will be required to ensure adequate ITN availability for forest use.

0282

LESSONS FROM DIGITIZINGA MASS DRUG ADMINISTRATION CAMPAIGN FOR MALARIA DURINGA COMPLEX EMERGENCY — CABO DELGADO, MOZAMBIQUE, 2021

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In February-May 2021, a malaria mass drug administration (MDA) campaign using dihydroartemisinin-piperaquine was conducted in two districts of Cabo Delgado province, Mozambique amidst a complex humanitarian emergency that could increase mortality due to limited access to care. Limited information on the impact of MDAs in these settings underscores the importance of coverage monitoring. Data was collected on size, geolocation, treatment eligibility, and acceptance from each household and institution visited. This data was monitored daily via Google Sheets and then Power BI Desktop. Dashboards summarized coverage, absences, ineligibility, refusals, and team performance. Daily data quality reviews were conducted, and data error reports were provided to field teams. Daily monitoring and clear feedback loops with field teams strengthened campaign activities. Supervisors used team-specific daily reports on coverage and data quality issues to provide tailored supervision to teams and adapt coverage strategies. Google Sheets was not suited to process the volume of data reaching capacity after one day. The dashboard built in Power BI was able to effectively process and display large data volumes in digestible visuals that were updated daily. Direct use of the Power BI dashboard by supervisors was initially impeded by lack of familiarity with Power BI and software access. In one district, lack of connectivity prevented daily data uploads, so teams could not benefit from dashboard use during Round 1. Changes based on lessons learned from Round 1 included offline data collection and monitoring capacity for areas with no connectivity via ODK Briefcase and Power BI Desktop; one-step monitoring for supervisors via online dashboards; and adapting data collection and workflow to improve coverage estimates. In areas with connectivity, daily monitoring effectively identified coverage issues, informed mop-up efforts, and corrected data quality concerns. Flexible and proactive monitoring allowed for strengthening of systems during MDA implementation and highlight good practices for dashboards in low connectivity settings.

0283

MODELLING MALARIA ELIMINATION STRATEGIES IN ZANZIBAR, TANZANIA

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Despite the implementation of vector control interventions, use of artemisinin-based combination therapies, and reactive case detection, malaria continues to persist at a low prevalence on the island archipelago of Zanzibar. Human movement between mainland Tanzania and Zanzibar is hypothesized to be a significant contributor to disease persistence, as prevalence is substantially higher in some areas of the mainland than on the islands. Studies have previously shown that repeated importation of infections can allow for disease persistence in areas where the disease would otherwise become extinct. In this study, we developed a metapopulation model of malaria transmission on the two main islands of Zanzibar and mainland Tanzania to provide insights into the local reproduction number in the absence of human movement. Then, we calibrated the transmission and human movement rates between the islands and the mainland to data on malaria prevalence and travel history collected during a cross-sectional survey, and data from the reactive case detection programme carried out in Zanzibar. We explored potential improvements to reactive case detection, with the aim of finding strategies that would substantially lower the infection prevalence on the islands. We found that human movement between the islands and the mainland played a pivotal role in disease persistence on Zanzibar, with the local reproductive number being below one on both islands in the absence of human movement. Tackling the issue of imported cases is crucial, as improvements to reactive case detection were projected to have only a small impact on prevalence. Shifting from reactive case detection to a reactive focal mass drug administration programme also showed substantial reductions, as patients with sub-patent infections, who would

typically be missed by screen-and-treat programmes, would also receive treatment. Additionally, we found that treating imported infections could lead to a large reduction in prevalence, but only if the majority of imported cases are successfully treated.

0284

ASSESSING THE CONTRIBUTION OF ACTIVE CASE DETECTION TO MALARIA ELIMINATION IN THAILAND

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Thailand's malaria surveillance system complements passive case detection in health facilities with several methods of active case detection (ACD). ACD comprises both proactive methods (PACD) that seek new cases in high-risk areas and reactive methods (RACD) that target community members near index cases. As Thailand approaches elimination, with just 4,474 cases in fiscal year 2020 (FY20), it is unclear if these resource-intensive surveillance strategies are yielding the same output as in the past. This study used routine data to examine achievements and trends in ACD among all 9,833,506 patients tested from FY15-20. ACD accounted for 42.3% of blood tests and 5.4% of confirmed cases, which was consistent over the study period (range = 4.3%-6.0%). As expected in an elimination setting, test positivity was low for both ACD (0.09%) and passive case detection (1.12%) and declined over time (from 0.14% to 0.05% for ACD). Whereas PACD and RACD contributed nearly equal proportions of confirmed cases at the start of the study period, by FY20, PACD accounted for just 29.7% of ACD cases, with 0.02% test positivity. Fixed-schedule malaria clinics (PACD) were the lowest-yielding ACD method, and case investigation surveys (RACD) were the highest ($p<0.05$). Both PACD and RACD contributions were significantly higher among active foci than other foci with a confirmed index case ($p<0.05$). ACD enhances surveillance by capturing cases that would not be discovered in health facilities but could spark indigenous transmission. The results of this study can be used to optimize ACD deployment as incidence continues to drop. For example, Thailand may consider reducing the frequency or radius of PACD screenings, and revised focus investigation protocols could restrict RACD screenings to only the highest-risk individuals or to active foci. Strategically targeting ACD could release resources for emerging priorities, such as prevention of reintroduction, and complementary modeling analyses could quantify potential risks of undetected cases. The results may also be useful to other countries aiming to strengthen surveillance for elimination.

0285

PROGRESS TOWARD PLASMODIUM FALCIPARUM ELIMINATION IN THAILAND

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The evolution of multidrug-resistant parasites has made the Greater Mekong Subregion's goal of eliminating *Plasmodium falciparum* (*Pf*) by 2023 increasingly urgent. Thailand's program includes the 1-3-7 strategy: notification within 1 day, case investigation within 3 days, and response within 7 days. This study used national surveillance data and logistic regressions to assess the program's progress and identify characteristics of remaining *Pf* cases and foci. A panel vector autoregression with a Granger causality Wald test assessed the effects and causality of historical values of variables (time lags) on current values. From fiscal year 2013 through 2020 (FY13-20), *Pf* infections dropped from 11,991 cases among 1,943 foci to 250 among 125 foci; mean cases per focus declined from 6.2 to 2.0. Although incidence of other malaria species also declined, the proportion of *Pf* among all cases dropped from 43.4% to 5.8%, and the proportion of active foci with *Pf* infections dropped from 53.3% to 11.3%. A spatial-temporal analysis showed that *Pf* foci are increasingly clustered in high-burden provinces at the Myanmar border. Among 23,113 cases from FY17-20, after new elimination strategies were launched, increased odds of *Pf* were significantly associated ($p<0.05$) with Thai citizenship, male sex, and historical values in the proportion of *Pf* cases, the proportion of imported cases, and foci classification. Granger test results suggest that historical values of model variables have a causal relationship with the presence of *Pf* in a given year. Among active foci for all species, presence of *Pf* infections was associated with adherence to 1-3-7 protocols ($p<0.05$). These results highlight Thailand's progress and reveal unique factors of *Pf* infections. Although malaria epidemiology is heavily influenced by the matrix of Thai and migrant workers in border areas, *Pf* cases are concentrated among Thai men, suggesting that elimination may require more targeted approaches. Foci with *Pf* infections may be better implementing surveillance policies; further analyses could clarify whether this association is explained by programmatic or other health system factors.

0286

ROLE OF RURAL PRIVATE SECTOR PROVIDERS IN MALARIA SURVEILLANCE AND CASE MANAGEMENT DURING COVID-19 PANDEMIC IN MYANMAR

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During COVID-19 pandemic in Myanmar, essential health services were disrupted including malaria case management. Population Services International Myanmar (PSI/Myanmar) provided malaria testing with rapid diagnostic tests (RDT) and case management through its networks such as private medical doctors (Sun Quality Health clinics,SQH) in urban areas, community volunteers (Community Health Services Providers,CHSP) and private non-formal providers (Private Outlets,PO) in rural areas. We explored their performance and service quality prior and during the pandemic. As the first COVID-19 case was found in March 2020, we compared 9-month data of 2020 (Apr-Dec) with that from 2019 using program data. The data for comparison was confined to the providers present in both periods. Service quality assessments were done via a standard electronic checklist where scores above 80 points were considered satisfactory. Statistical significance was determined as $p<0.05$. The data came from 783 SQH (30.3%), 1163 CHSP (45%), and 637 PO (24.7%) providers. A total of 384971 and 346725 patients were tested with RDT and 3413 and 3543 were found to be positive in 2019 and 2020 (positivity rates 0.9% and 1.0%). The mean number of fever patients tested per provider per month were CHSP (16.3 vs. 15.7, p=0.11), PO (10.3 vs. 12.3, p=0.02), SQH (6.8 vs. 4.1, p<0.001). The mean number of RDT positive patients per provider per month were CHSP (0.1 vs. 0.1, p=0.51), PO (0.1 vs. 0.5, p=0.005), SQH (0.6 vs. 0.5, p=0.44). Positivity rates were CHSP (0.8% vs. 0.7%), PO (1.2% vs. 1.0%) and SQH (0.9% vs. 2.7%). The mean quality scores were CHSP (76.4 vs. 83.4, p<0.001), PO (87.9 vs. 86.6, p=0.01) and SQH (94.5 vs. 96.7, p<0.001). During the COVID-19 pandemic, malaria testing dropped among private medical doctors in urban clinics but rural private sector providers continued malaria services as before, at similar levels to traditional volunteers, maintaining adequate service quality. Thus, their role in malaria surveillance and case management was important and they could be one of the promising channels for providing such services during crisis situations in Myanmar and similar settings.

0287

CROSS-BORDER MOVEMENT AS A KEY CHALLENGE IN MALARIA ELIMINATION EFFORTS IN THE GUIANA SHIELD: ASSESSMENT OF MOBILE MIGRANT POPULATION CHARACTERISTICS

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Migration along the porous borders of Suriname with French Guiana, a European Territory, and Brazil, represents a continuous challenge to the national elimination goal in Suriname. A key population that engages in this migration are Artisanal Small-Scale Gold miners (ASM) that work throughout the Guiana Shield. This study focused on understanding the size, characteristics, migration patterns and health-related perceptions on risk of disease, health needs and access to care among mobile migrant ASM communities in Suriname. Mobile Migrants' population characteristics were examined using a mixed methods approach combining document review, a quantitative survey and qualitative in-depth interviews with the target population and experts. The total ASM population was estimated at around 31,000 individuals, of which 20,000 active in Suriname and 11,000 in French Guiana. Turnover was estimated to be 10,5% of which 95%, or some 2,000 persons annually are newcomers to the sector. International migration patterns were mapped from Brazil, but also from Venezuela, Dominican Republic, Cuba and China. In terms of perceived health risks, we find that most ASM workers are somewhat concerned with malaria, leishmaniasis and COVID-19. The improved understanding of the size, characteristics, migration patterns and health needs of ASM communities carries important implications for targeting the health needs of these vulnerable and hard-to-reach mobile communities in the Guiana shield. Recommendations include 1) using data to target most vulnerable subgroups 2) Increasing efforts to communicate risks, preventive measures and treatment options 3) Scaling up support by increasing service delivery, specifically for most at-risk subgroups to implement prevention and treatment options for malaria elimination and 4) Prioritization by the Surinamese government to work with the French government and regional institutions to guarantee access to diagnosis and treatment for ASM workers in remote areas in French Guiana

0288

IDENTIFYING AND ADDRESSING GAPS IN THE MALARIA ELIMINATION STRATEGY: ACTIVE CASE DETECTION IN MADAGASCAR, 2020

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Progressive malaria elimination is a goal of Madagascar's National Malaria Control Program (NMCP). In 2020, 13 (11.4%) of 114 districts met the NMCP's malaria elimination criterion (incidence <1 case per 1,000 inhabitants). To support these 13 districts, the U.S. President's Malaria Initiative (PMI) Measure Malaria and Impact Malaria projects implemented elimination strategies in Madagascar, including training health workers at the community level in surveillance, active case detection, investigation, and electronic case reporting. From December 1, 2020 to February 28, 2021, the NCMP conducted three rounds of active case detection, investigating, and screening of all household members where an index case (a passively identified confirmed malaria case) was reported, as well as the 10 surrounding households, in 18 rural communities in the health district of Antsirabe II. A malaria rapid diagnostic test was done on every individual, and real-time polymerase chain reaction was performed. Data from the screened individuals were recorded in a digitalized tool running on the District Health Information Software 2 (DHIS2) platform. The team screened 913 individuals based on 40 index cases reported from 18 communities in 20 health centers. Of the 913 individuals screened, 31 (3.4%) had a positive malaria rapid diagnostic test and were locally transmitted infection. Real-time polymerase chain reaction was performed on 507 of the 913 individuals due to limited stock on reagent. During the February round of foci investigation, a stock-out of primaquine, the recommended national treatment protocol (single-dose primaquine plus artemisinin-based combination therapy to treat a positive case), was reported in 11 health centers (55%). Active case detection and integrated vector management will only be as strong as the treatment supply chain that supports it (procurement system for primaquine and other malaria commodities) is well functioning to ensure adequate stock availability.

0289

ACCELERATE EFFORTS TOWARDS MALARIA ELIMINATION TO ACHIEVE ACTIVE FOCI FREE STATUS IN RAMREE TOWNSHIP, RAKHINE STATE OF MYANMAR

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Ramree, the largest island in Rakhine State, has two more islands within the township territory. About 98,097 people reside in the 6 wards and 222 villages which contribute a total of 228 malaria reporting units as a whole township. Pilot malaria elimination activities were started in 2018 by the National Malaria Control Programme in collaboration with Defeat Malaria project of the University Research Co. with the support of the U.S. President's Malaria Initiative. After three years of implementing activities, 198 out of 228 villages (87% of total) achieved Annual Blood Examination Rate (ABER) of at least 10%. Out of 198 villages with adequate ABER, the villages were classified as 197 residual non-active foci and one active focus. Htauk Kyant Taw village, surrounded by mountainous forest and has 42 households with a population of 202, was classified as an active focus at the end of 2020 due to the occurrence of 1 indigenous, 1 imported, and 3 introduced cases. A village Malaria Worker (VMW) provides community-based malaria elimination activities. An entomological survey conducted in 2019 found primary vectors *An. dirus* and *An. minimus*. The index case (Pv) was detected on 4th November 2021 and immediately notified by the VMW and was classified as imported after conducting a case investigation in the next day by township response team. Foci investigation and response activities were initiated by the township response team within 7 days of case detection. An additional 3 *P.v* cases were detected on 19th, 22nd, and 23rd of November 2020 during reactive case detection (RACD). The last indigenous case was found in on 16th December 2020. Based on foci investigation, the accelerated efforts to interrupt the local transmission in the village comprised of RACD, filling the gaps after bed-nets coverage and utilization assessment, larval source management, mobility monitoring and provision of mosquito repellents to night-time forest goers. Building on this, it should be explored how *P.v* transmission dynamics would potentially impact the existing surveillance and response strategy for effective intervention of onward malaria transmission.

0290

ADAPTATION OF RAPID MULTI-OBJECTIVE LOT QUALITY ASSURANCE SAMPLING (LQAS) SURVEYS AS PART OF SEASONAL MALARIA CHEMOPREVENTION PROGRAMS IN BURKINA FASO, CHAD, NIGERIA AND MOZAMBIQUE FOR IDENTIFYING ISSUES AND DRIVING IMPROVEMENTS IN SMC DELIVERY

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Malaria Consortium supports delivery of seasonal malaria chemoprevention (SMC) to children aged 3-59 months as monthly doses of sulfadoxine-pyrimethamine plus amodiaquine against *Plasmodium falciparum* malaria during the rainy season in several countries where transmission is highly seasonal, typically in four monthly cycles. In 2020, Malaria Consortium adapted the lot quality assurance sampling (LQAS) method for its end-of-cycle SMC monitoring surveys in Burkina Faso, Chad, Nigeria and Mozambique to measure quality of delivery across 16 indicators pertaining to SMC coverage, use of SMC Record Cards, dissemination of information on SMC to caregivers, and safe SMC implementation during the COVID-19 pandemic. LQAS data facilitated local assessment of program performance at the supervision area (SA) level using hypothesis tests to determine whether standards for one or more indicators were met. Targets and decision values were defined for each indicator. Districts were divided into SAs based on groups of health facility catchments, from which lots of 25 households were sampled; when numbers of households fell below the decision value for an indicator an issue was identified at the SA level. In all countries LQAS surveys were completed within two weeks of each of the first three cycles, giving time to process data, identify and prioritize issues, communicate issues to local stakeholders involved in SMC delivery, and engage them to implement improvements in distributor training and SMC delivery in the subsequent month's cycle. While overall programme coverage was high, coverage in some SAs was below the 80% target (e.g., in Chad cycle 3, coverage was 95% but the target was not met in 21/98 SAs). We compiled results for each indicator and attainment of targets; engaged with national, regional and local stakeholders; and noted changes to SMC delivery in some SAs. Results contributed to continuous improvement of SMC delivery. There were challenges in interpreting results and attributing causes of issues identified, identifying stakeholders in each country, and optimizing messaging to motivate further improvements to SMC delivery.

0291

COMMUNITY HEALTH COUNCILS FOR MALARIA ELIMINATION IN HAITI: LESSONS FROM A PILOT PROJECT IN GRAND ANSE DEPARTMENT

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Community engagement (CE) in vector-borne disease control and elimination, including malaria, is often more experimental, messy and adaptive than we anticipate. This paper examines the social construction and micro-politics of program design and implementation, focused on a volunteer-based CE model developed in Haiti as part of the Malaria Zero Initiative. The study draws on quantitative and qualitative data collected from 23 Community Health Councils (CHCs) from 2018-2021 in Grand Anse, a malaria hotspot region in Haiti. Project plans were disrupted by political crisis, a severe weather event, administrative hurdles and COVID-19; this conspired to create a "natural experiment" where greater emphasis was placed on self-organization than intensive facilitation. Despite this, none of the 23 CHCs ceased functioning over a two-year period, and an average of 0.86 monthly meetings were held with a 78% attendance rate. A high degree of transparency and diversity in membership helped create strong micro-planning and involvement. CHCs conducted an average of 1.8 community-based activities per month, with high levels of fluctuation indicative of local ownership. This included school and church sensitization, environmental sanitation campaigns, mass education, support for case referrals and community mobilization during Mass Drug Administration and Indoor Residual Spraying. The nature of volunteerism and incentives were a constant topic of negotiation; members drew on the tradition of *konbit* (mutual self-help), local histories of health and development campaigns and a lexicon of "solidarity" in difficult times. Small incentives played both symbolic and supportive roles and some level of politicization was viewed as inevitable, even beneficial. Rumors about financial and political profiteering of CHC volunteers took time to dispel while the tendency towards vertical planning in malaria control created conditions to exclude CHCs from some activities. With the end of Malaria Zero in 2020, there is now an opportunity to monitor the continued self-sufficiency of CHCs in promoting anti-malaria activities within their communities.

0292

DEVELOPMENT OF A SECOND-GENERATION GENETICALLY ATTENUATED MALARIA VACCINE: EN ROUTE TO A FIRST CLINICAL TRIAL

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Among different approaches to developing an effective malaria vaccine, immunisation with live, attenuated *Plasmodium falciparum* (*Pf*) sporozoites has so far induced the highest protective efficacy (>90%) in clinical trials. There are several approaches to immunisation with whole sporozoites (Wsp); the currently most advanced Wsp vaccine consists of sporozoites that have been attenuated by radiation (RAS). As an alternative to radiation attenuation, Wsp vaccine approaches are being developed based on the use of genetically attenuated sporozoites (GAP). The first generation of GAP, so-called early-arresting GAP (EA-GAP), abort their development within 2-3 days after invading liver cells and as such have a similar attenuation phenotype to RAS. We recently completed the world's first clinical safety and efficacy assessment of an EA-GAP malaria vaccine (GA1 vaccine). The GA1 was immunogenic and showed protective efficacy comparable to RAS immunization, although protective efficacy of both RAS and the EA-GAP in malaria-naïve Dutch individuals was lower than desired[1]. Thus, despite theoretical advantages, Wsp vaccines still require substantial improvement in order to further enhance their efficacy. One way to increase the immunogenicity and thus efficacy of Wsp vaccines is to broaden the array of antigens displayed to the immune system by extending the duration of parasite exposure to the immune system. In rodent malaria models it has been shown that GAP that arrest late during liver stage development (LA-GAP) induce significantly higher protective immune responses as compared to both RAS and EA-GAP, most likely resulting from increased antigen breadth and biomass of LA-GAP. We have therefore created an equivalent *Pf*LA-GAP that arrests late during liver stage development. We will report on the generation and pre-clinical testing of this *Pf*LA-GAP parasite and plans for the first clinical

trial to evaluate the safety and protective efficacy of a second-generation LA-GAP vaccine (GA2 vaccine) that arrest growth late during liver stage development. [1]Roestenberg, M. *et al. Science translational medicine* (2020)

0293

SAFETY AND EFFICACY AGAINST NATURALLY TRANSMITTED PLASMODIUM FALCIPARUM MALARIA OF PFSPZ VACCINE IN 2- TO 50-YEAR-OLDS LIVING ON BIOKO ISLAND, EQUATORIAL GUINEA

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PfSPZ Vaccine is comprised of radiation-attenuated, aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ). Clinical trials of PfSPZ Vaccine in >1700 5-month to 61-year-olds in the USA, Europe, and six African countries, including three trials in Equatorial Guinea (EG), have shown PfSPZ Vaccine to be safe and well-tolerated including in infants, children, adolescent, and HIV- and HIV+ adults. The vaccine has shown sustained (6-18 month) protection against naturally transmitted Pf in 4 separate field trials We are now planning to demonstrate safety and vaccine efficacy (VE) in a Phase III randomized, double-blind, placebo-controlled trial of 2100, 2- to 50-year-olds living on Bioko Island, EG. Preparatory work for implementation is ongoing and has included a recently completed trial for down selection of an optimal regimen to be used for Phase III testing and licensure. The selected regimen, 3 doses of $9.0x10^5$ PfSPZ administered on days 1, 8 and 29, has given the best VE against homologous controlled human malaria infection (CHMI) and is practical for deployment as compared to longer 16-20 week regimens tested in earlier trials. Two epidemiological studies have been conducted to assess local (Bioko Island) malaria incidence and risk factors (EGMALEP) and to pilot recruitment and screening procedures while creating a registry of potential participants for the upcoming trial (EGRESPAR). The clinical protocol for this trial - EGSPZV4, has gone through ethical and regulatory reviews in the USA, Europe, and two African countries with positive opinion and approvals. EGSPZV4 is expected to begin during first or second quarter of 2022. Details of the design, follow-up schedule for safety and efficacy endpoints as well as implementation plan in the post COVID-19 era and milestones will be presented. In addition, critical issues related to development pathway, study endpoints in malaria pre-exposed volunteers, ethical and regulatory review will be discussed.

0294

PHASE 2 SAFETY AND EFFICACY EVALUATION OF RADIATION ATTENUATED PLASMODIUM FALCIPARUM SPOROZOITE (PFSPZ) VACCINE IN HEALTHY AFRICAN ADULT WOMEN OF CHILDBEARING POTENTIAL IN OUÉLESSÉBOUGOU, MALI

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Sanaria® PfSPZ Vaccine, composed of radiation-attenuated, aseptic, purified, cryopreserved, *Plasmodium falciparum* (Pf) sporozoites (SPZ), is an advanced malaria vaccine candidate being developed for use in Africa, including in pregnant women. Previous studies of PfSPZ Vaccine in African infants, children and adults demonstrated an excellent safety profile with no differences in frequency or severity of adverse events (AEs) compared to normal saline (NS) controls. Vaccine efficacy (VE) over 6 months against naturally transmitted Pf infection calculated as 1 minus the hazard ratio in 3 studies was 48%, 51% and 52% in fully vaccinated adults. Accelerated regimens offer an advantage to pregnant women as they could confer protection earlier in pregnancy. This double blind NS placebo-controlled trial assessed safety and VE of $9.0x10^5$ or $1.8x10^6$ PfSPZ administered on accelerated 1, 8 and 29 day schedule. Women of childbearing potential were immunized before pregnancy, discontinued birth control following immunization and were followed for two years including during any subsequent pregnancies. 407 volunteers were screened June-July 2019, 324 enrolled and 300 were equally randomized to $9 x10^5$ PfSPZ, $1.8x10^6$ PfSPZ or NS injected via direct venous inoculation (DVI). All were administered artemether/lumefantrine 2 weeks prior to 1st and 3rd vaccinations to clear any existing parasitemias. Vaccinations were well tolerated, with the majority of AEs classified as mild (Grade 1). There were no statistically significant differences been vaccines and controls for total AEs, related AEs, solicited AEs (local, systemic, individual), laboratory abnormalities, or vital sign changes. VE against Pf infection of fully vaccinated individuals per protocol was, was 41% (95% CI 14%- 59% p=0.005,) in $9x10^5$ PfSPZ arm and 57% (95% CI 37%- 71%, p< 0.001) in $1.8x10^6$ PfSPZ arm. Durability of VE was assessed during the 2020-2021 malaria transmission season, without further study product administered, and will be presented. These promising results set the stage for immunization during pregnancy to reduce the risk and deleterious consequences of pregnancy malaria.

0295

PRODUCTION OF PFSPZ FOR MALARIA VACCINES IN A BIOREACTOR WITHOUT MOSQUITOES

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Sanaria has developed methods to produce *Plasmodium falciparum* (Pf) sporozoites (SPZ) *in vitro* (iPfSPZ) without mosquitoes to eventually replace mosquito-produced PfSPZ (mPfSPZ) in our PfSPZ-based vaccines. We produced hundreds of millions of iPfSPZ without mosquitoes in 12-well culture plates. iPfSPZ invade and develop *in vitro* to late liver stage parasites expressing Pf merozoite surface protein 1 (PfMSP1) in the 6-day hepatocyte potency assay using HC-04 cells and human primary hepatocytes as well, if not better than mPfSPZ, and to late liver stage parasites expressing PfMSP1 when injected into FRG mice with humanized livers at ~50% the efficiency of mPfSPZ. We have now progressed to a bioreactor system for producing iPfSPZ. Using a mini-bioreactor, we produced up to 2.3×10^8 iPfSPZ per culture, and in a medium bioreactor that was 6.25 times larger, we produced up to 1.5×10^9 iPfSPZ per culture. iPfSPZ expressed Pf circumsporozoite protein (PfCSP). Bioreactor-produced iPfSPZ were equal or better in potency compared to mPfSPZ in the 6-day *in vitro* hepatocyte potency assay. Importantly, for the first time, ~20% of the iPfSPZ were free in the supernatant of the culture harvest and not contaminated with the *Drosophila* S2 feeder cells used in the cultures.

We harvested up to 1.58x10^8 free iPfSPZ from a mini-bioreactor in three independent experiments. These free iPfSPZ were PfCSP positive. This is an extremely important step forward which will facilitate the use of larger capacity bioreactors for mass production of free iPfSPZ and subsequent optimization of purification and cryopreservation of iPfSPZ for use in PfSPZ vaccines. By eliminating the need for mosquitoes and dissectors from manufacturing, the transition from mPfSPZ to iPfSPZ will dramatically increase the efficiency and consistency of production of PfSPZ vaccines, and reduce cost of goods by more than 90%.

0296

PARASITE TARGETS OF PROTECTION INDUCED BY PFSPZ VACCINE: A WHOLE ORGANISM-BASED VACCINE AGAINST MALARIA

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A placebo-controlled efficacy study of Sanaria® PfSPZ Vaccine, composed of radiation attenuated, *Plasmodium falciparum* (Pf) sporozoites (SPZ), was conducted in 5-12 month olds in Western Kenya. This pre-erythrocytic vaccine did not induce detectable T cell responses and while there was 41% vaccine efficacy (VE) at 3 months in infants receiving the highest PfSPZ dose, no VE remained at 6 months. We were interested in determining if the genotype distribution of infecting Pf strains was identical in controls and vaccinees or if, instead, Pf genotypes observed differed between study arms. The former would be consistent with the absent T cell responses and lack of durable VE in these infants, while the latter would suggest genotype-specific vaccine-induced protection in a setting with high parasite genetic diversity and attack rate, enabling vaccine evasion. Isolates analyzed correspond to first infections in each infant, starting at 3 weeks post last immunization. Illumina short read data was generated from DNA extracted from leukocyte-depleted blood, and SNPs called according to best practices. Allele frequency distribution differences between Pf isolated from vaccinees in the high dose group (n=44) and controls (n=56) were estimated using F_{ST}. Of 21,738 bi-allelic, non-synonymous SNPs, 442 differed significantly between study arms (p<0.05, permutation test, 5,000 replicates). They mapped to 338 genes, which were significantly enriched for membrane-associated cellular localization, as expected of antigens, and consistent with a sieve effect imparted by vaccine-induced protection. Unexpectedly, among these genes are several blood stage antigens, including apical membrane antigen-1 and rhoptry-associated membrane antigen. Although the results are partly consistent with genotype-specific PfSPZ Vaccine-induced protection, they could be confounded by factors such as variable previous exposure or the presence of maternal antibodies. To determine the main driver(s) of the patterns observed, bioinformatics analyses and immunological experiments are ongoing to characterize host responses to antigens/variants of interest.

0297

PFSPZ VACCINE EFFICACY WITH AND WITHOUT ANTIMALARIAL PRE-TREATMENT-COMPARISON BETWEEN STUDIES

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Sanaria® PfSPZ Vaccine, composed of aseptic, purified, radiation-attenuated *Plasmodium falciparum* (Pf) sporozoite (SPZ) of the NF54 strain, is an advanced malaria vaccine candidate used in multiple studies conducted in Mali. The vaccine has shown an excellent safety profile and significant vaccine efficacy (VE) at different dosages and schedules administered by direct venous inoculation (DVI). We previously reported 100% VE against controlled human infection with homologous NF54 parasites in individuals who did or did not receive antimalarial treatment with artesunate/amodiaquine (ASAQ) before PfSPZ Vaccine (1.8x10^6 PfSPZ at 0, 8 and 16 weeks) in Mali. A phase 1 study ("Study 1") in healthy adults investigated two PfSPZ Vaccine regimens (0.9x10^6 PfSPZ at 0, 8, 16 weeks versus 0.9x10^6 PfSPZ at 0, 1, 4 weeks, with 2nd year booster doses); subjects received antimalarial treatment with artemether/lumefantrine (AL) pre-treatment before 3rd but not 1st or 2nd doses in Year 1 (2018) and before 4th dose in Year 2 (2019). A phase 2 study ("Study 2") in healthy adult women of childbearing potential investigated two PfSPZ Vaccine regimens (0.9x10^6 PfSPZ versus 1.8x10^6 PfSPZ at 0, 1, 4 weeks) and subjects received AL pre-treatment before 1st and 3rd doses in Year 1 (2019). Follow-up for Pf infection in both studies for 24 weeks started immediately after 3rd dose. PfSPZ Vaccine was well-tolerated in all study arms, with no significant differences in AE profiles compared to placebo study arms. In Study 1, VE was not significant for either regimen versus normal saline (NS) placebo control group. In Study 2, VE was significant for both regimens versus NS group: VE 42% (95%CI 16-59, p=0.003) after 0.9x10^6 PfSPZ Vaccine; VE 56% (95%CI 36-70, p< 0.001) after 1.8x10^6 PfSPZ Vaccine. Notably, the regimen of 0.9x10^6 PfSPZ Vaccine given at 0, 1, 4 weeks achieved significant VE in Study 2 but not Study 1. Taken together, these studies indicate that antimalarial pretreatment before initial doses of PfSPZ Vaccine is required to maximize protective immunity.

0298

GIVING MALARIA VACCINATIONS THE ALL CLEAR: THE IMPORTANCE OF CLEARING ASEXUAL ERYTHROCYTIC STAGE PARASITEMIA BEFORE ADMINISTRATION OF PFSPZ VACCINES

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Symptomatic and asymptomatic malaria have been associated with decreased antibody responses to multiple vaccines; asexual erythrocytic stages of *Plasmodium falciparum* (Pf) are immunosuppressive. Results of controlled human malaria infection (CHMI) assessment of PfSPZ-CVac vaccine efficacy (VE) and field trial assessment of PfSPZ Vaccine and PfSPZ-CVac VE indicate that the presence of blood stage Pf at the time of immunization can eliminate vaccine-induced protection. When doses of 5x10^4 PfSPZ of PfSPZ-CVac were administered at 5-day intervals, the 1st and 2nd doses were administered before development of blood stage parasitemia, and the 3rd dose was administered at the nadir of blood stage parasitemia from the 1st dose and before blood stage parasitemia from the 2nd dose; VE against CHMI 10 weeks later was 63%. When the same dosage was administered at 7-day intervals, the 2nd and 3rd doses were administered at the peak of blood stage parasitemia from the preceding dose and VE against CHMI 10 weeks later was 0. The mean peak parasitemias recorded by qPCR in this group with no protection were 1.7 and 1.6 parasites/µL after doses 1 and 2. In Africa, we have results of 8 clinical trials of PfSPZ Vaccine or PfSPZ-CVac in infants to adults. In 5 of these trials in Mali and Burkina Faso, blood stage parasitemia was cleared presumptively before the first immunization; in all 5 of these clinical trials there was statistically significant VE against field transmission of Pf during the next 6 months (42% to 56% VE). In 3 trials in Mali and Kenya, blood stage parasitemia was not cleared presumptively and VE against Pf infection during the next 6 months was not statistically significant. Results from a clinical trial in Gabonese children who did not receive presumptive treatment before dose 1 are still blinded. These data indicate immunosuppression caused by asexual erythrocytic stage infection, even at submicroscopic levels, significantly reduces the VE of PfSPZ vaccines, and provide a compelling imperative for the clearing of blood stage Pf parasitemia in study subjects prior to the start of clinical trials of any PfSPZ vaccine, and potentially any malaria vaccine.

0299

SAFETY AND PROTECTIVE EFFICACY OF PFSPZ-CVAC (PYR) VACCINATION AGAINST PLASMODIUM FALCIPARUM INFECTION IN HEALTHY ADULTS IN BANCOUMANA, MALI

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We assessed safety and vaccine efficacy (VE) of Chemoprophylaxis Vaccination (CVac) against naturally transmitted *Plasmodium falciparum* (Pf) infection in a Phase 2 randomized double blind placebo-controlled study in Mali. PfSPZ-CVac comprised 3 doses of $4.0x10^5$ PfSPZ Challenge [NF54] (aseptic, purified, cryopreserved, infectious Pf sporozoïtes [SPZ]) administered by direct venous inoculation (DVI) at 0, 4, and 8 weeks combined with oral pyrimethamine (PYR) and, pending an interim analysis of Year 1 efficacy, of a 4th (booster) dose given in Year 2. In 2019, we randomized 240 adults to 4 arms to receive artemether/lumefantrine pre-treatment and then PfSPZ Challenge (V) or normal saline placebo (P) DVI: Arms V_0 (n=90) and P_0 (n=54) received 75mg of oral PYR on day of DVI (day 0) while Arms V_{2+3} (n=60) and P_{2+3} (n=36) received 75mg of oral PYR on days 2 and 3 post-DVI. After 3rd vaccination, participants were monitored for *Pf* by thick blood smear (TBS) every two weeks for 24 weeks. As previously reported, PfSPZ-CVac was safe and well tolerated, with two SAEs unrelated to study procedures (intestinal volvulus and death by rifle injury). Of 1651 adverse events (AEs), 1.2% (19/1651) were grade 3, and 3 of these (all neutropenia) were considered possibly related. Grade 1 and 2 AEs frequencies were respectively 35.01% (578/1651) and 63.8% (1053/1651), mostly lab abnormalities or episodes of rhinitis that were unrelated. In an interim analysis, significant VE was observed in at least one of interventional main phase arms, which allowed the booster phase in 2020. After 4th vaccination, participants were again monitored for Pf by thick blood smear every two weeks for 24 weeks. The booster phase is now complete and final VE analyses are underway. We will present safety and VE results as well as immunology data. Sanaria PfSPZ CVac (PYR) dosed at $4.0x10^5$ was safe and well-tolerated and at least one dosing regimen of PYR or both showed protective efficacy against Pf infection in adults in Bancoumana, Mali. This is the first demonstration of VE in a field trial of PfSPZ-CVac.

0300

IMPACT OF COVID-19 ON PLANNED AND ONGOING PFSPZ VACCINE CLINICAL RESEARCH AND SITE MITIGATION STRATEGIES IN BIOKO ISLAND, EQUATORIAL GUINEA IN 2020

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The world has been impacted by the 2019 novel coronavirus SARS-CoV-2 and the resulting disease, "COVID-19". The current containment efforts in Equatorial Guinea and the world at large have disrupted the implementation of ongoing and planned clinical research of the Bioko Island Malaria Elimination Program (BIMEP). BIMEP executes the study entitled "Pilot Study to Optimize Recruitment and Screening Procedures for Future Clinical Trials and to Create a Registry of Potential Research Participants on Bioko Island, Equatorial Guinea" (EGRESPAR) and plans to conduct a Phase 3 clinical trial of Sanaria® PfSPZ Vaccine on Bioko Island, initially planned to start in 2020 with enrolment of 2100 research subjects, aged 2 to 50 years. Clinical trial execution during the pandemic could significantly increase risk to study participants and study staff by creating opportunities for SARS-CoV-2 transmission, thereby seeding communities with infected individuals. Therefore, we continue to document how the COVID-19 pandemic has impacted upon BIMEP and the mitigation strategies to minimize this effect. We will use qualitative techniques to analyse study documents and reports to gain a broad understanding of the situation. The impact of the COVID-19 pandemic will be described in terms of the financial impact on the program, monitoring of participant safety and willingness to participate, potential loss of experienced staff, and the delays to the clinical trial schedule.

0301

PFSPZ-LARC1 VACCINE, A PLASMODIUM FALCIPARUM SPOROZOITE VACCINE GENETICALLY ATTENUATED TO ARREST AT THE LATE LIVER STAGE

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There has been little to no change in the numbers of cases and deaths caused by malaria in the past 5 years. A vaccine is needed. An ideal vaccine would prevent infection, thereby preventing all clinical manifestations and transmission of malaria. 100% vaccine efficacy (VE) against heterologous controlled human malaria infection (CHMI) 12 weeks after last dose has been achieved by immunizing subjects taking chloroquine (CQ) chemoprophylaxis with 3 doses of $2x10^5$ aseptic, purified, cryopreserved Pf sporozoites (SPZ) (Sanaria® PfSPZ Challenge). This is the best VE ever achieved against heterologous CHMI. This vaccine, known as PfSPZ-CVac, is limited because there is 1) full development of the liver stage and transient parasitemia after the 1st dose which can cause malaria-like symptoms and 2) risk of uncontrolled infection if CQ is not properly taken or absorbed. To produce a vaccine with the strengths, but not the drawbacks of PfSPZ-CVac, we have genetically engineered PfNF54 parasites so that PfSPZ invade hepatocytes and develop to the late liver stage normally, and then arrest in development. This was done by deletion of the gene encoding Plasmei2, an essential protein exclusively expressed in the late liver stage. Challenge with these late arresting replication competent (LARC) PfSPZ in FRG mice containing humanized livers showed complete attenuation. We now report that we have robustly produced this PfSPZ-LARC1 vaccine in aseptic mosquitoes. The fresh PfSPZ-LARC1 had a viability of 98%, and in a 6-day *in vitro* human hepatocyte assay, aseptic, purified, cryopreserved PfSPZ-LARC1 invaded and developed as expected to late liver stage parasites expressing PfMSP1 comparably to wild-type PfSPZ. All GMP manufacturing process steps previously used are suitable for manufacture of PfSPZ-LARC1. We will report on our IND-directed activities, production of a GMP clinical lot, quality control release of the lot, and design of and plans for clinical trials in the US and Germany to assess safety, immunogenicity and VE of PfSPZ-LARC1 Vaccine.

0302

TOWARDS HUMAN USE OF A GLYCOLIPID ADJUVANT FOR PLASMODIUM FALCIPARUM SPOROZOITE (SPZ)-BASED VACCINES AGAINST MALARIA

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We are working to further enhance *Plasmodium falciparum* (Pf) sporozoite (SPZ)-based vaccines against malaria. In malaria naïve individuals, PfSPZ Vaccine demonstrated 100% vaccine efficacy (VE) at 12 weeks after last vaccine dose against controlled human malaria infection (CHMI) with heterologous Pf and 46% to 56% VE lasting 6 to 18 months in adults in Africa against intense transmission of heterogeneous Pf. Using a unique glycolipid adjuvant 7DW8-5, our goal has been to prolong the duration of VE and to increase efficacy in endemic settings. In a mouse model using *P. yoelii* (Py) SPZ, we had achieved greater than 80% protection at 16 weeks with 2 dose and 4 dose accelerated regimens of irradiated (irr) PySPZ plus 7DW8-5 adjuvant administered by direct venous inoculation (DVI) representing a 2-fold enhancement over irr PySPZ without adjuvant. The adjuvant can be mixed with irr PySPZ. High level (>80%) protection of mice persisted at 16 weeks with irr PySPZ administered by DVI, in the presence of 7DW8-5, but not by non-DVI routes. Manufacturing of 7DW8-5 under cGMPs was completed and in a pilot study with *P. knowlesi* (Pk) SPZ, irr PkSPZ we achieved 50% VE, but no improvement with the adjuvant, likely attributable to sub-optimal comparative dose or dosing regimens, or the short-term infectious challenge design. However, we demonstrated an excellent safety profile of the combined PkSPZ-adjuvant vaccine in NHPs. Body weights remained constant throughout the study with no changes in physical, or behavioral patterns indicating pain or distress in animals injected with the adjuvant. In addition, a 58-parameter chemistry panel was assessed at baseline and after the 2nd and 3rd vaccination, and no abnormalities were noted. With regard to functionality, in a co-culture system with human iNKT cells *in vitro,* the GMP adjuvant exhibited comparable bioactivity with research grade product, paving the way for further confirmatory studies in animal models. Our efforts represent steps toward the first development of an adjuvant for a live eukaryotic parasite vaccine.

0303

ASSESSING THE BEHAVIOURAL AND PHYSIOLOGICAL EFFECTS OF PIPERONYL BUTOXIDE (PBO) EXPOSURE ON ANOPHELES GAMBIAE

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Long lasting insecticidal nets (LLINs) remain one of the frontline tools in malaria vector control, however due to insecticide resistance, Piperonyl butoxide (PBO) has been incorporated into LLINs. These LLINs have been deployed around Africa and have proven to be efficient in areas that have resistant vectors. However, a knowledge gap exists when it comes to the interactions between the vectors and PBO LLINS. This project assessed the impacts of exposure to PBO-LLINs and PBO-alone on *Anopheles gambiae* s.l. with focus on longevity, host-seeking behaviour, and reproductive capacity in a laboratory environment. A baited-box video assay was used to assess the behavioural responses of susceptible lab reared *Anopheles gambiae* to three different net types: untreated, deltamethrin only and PBO- deltamethrin. The mosquitoes were then observed post assay for longevity. The sublethal effects of PBO alone on both male and female mosquitoes was observed using the WHO tube assay. The effects of PBO on blood-feeding and longevity were characterized. An Olfactometer was then used to quantify host-seeking behavior. There were significant differences between the time spent blood-feeding on both the strains. However, no differences were seen for the time taken before the mosquito appears in the box. For both the Kisumu and Banfora strains, longevity significantly differed between the net types (Kisumu, X^2=128.0, df=2, P <0.0001; Banfora X^2=114.3, df=2, P <0.0001). WHO tube assay results showed that where blood-feeding was allowed immediately after exposure there was a significant reduction in feeding (X^2=429.0, df=7, P <0.0001). Male mosquitoes lived nearly three times as long if exposed to control papers rather than those treated with PBO ($\chi 2$ = 66.1, df = 1, p = 0.0001). The Olfactometer assay confirmed that mosquitoes that are exposed to PBO are less likely to seek a host successfully. In conclusion, the PBO LLINs show efficacy against both resistant and susceptible mosquitoes in lab-based assays. This study highlights that PBO remains an important compound in the fight against resistance in malaria vectors

0304

EFFECT OF MASS-DISTRIBUTION CAMPAIGN OF INSECTICIDE-TREATED NETS AND INDOOR RESIDUAL SPRAYING ON ENTOMOLOGICAL MEASURE OF MALARIA TRANSMISSION IN BURUNDI

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Malaria is the main public health challenge in Burundi with a high incidence of the disease. Insecticide treated nets (ITNs) and indoor residual spraying (IRS) are the main vector control interventions in the country. *Anopheles gambiae* s.l. and *An. funestus* s.l. represented the major malaria vectors in the country. In December 2019, a mass-distribution campaign of ITNs was conducted in all the eighteen provinces of the country. All provinces received a standard deltamethrin-based ITN (Yorkool), except Kirundo province where piperonyl butoxyde (PBO) ITNs (Permanent 3.0) were distributed. IRS was only conducted in Ngozi and Muyinga provinces in October 2019. Monthly entomological surveys were conducted before (October 2018-September 2019) and after the implementation of vector control interventions (October 2019-September 2020) in eight sentinel sites (including one which received both IRS and ITNs) using human landing catches (HLC). The highest Entomological Inoculation Rate (EIR) based on HLC was observed in December 2020 just before the distribution of ITNs with 11.32 infective bites/person/month, while the highest EIR after the ITN mass campaign was 2.81 infective bites/person/month and observed in March 2020. In general, in most of the sentinel sites EIR was reduced after the implementation of vector control interventions with an average EIR of 8.88 infective bites/person/month before the ITN mass campaign versus 1.09 infective bites/person/month after the campaign (p<0.0001). The average parous rates were 79.34% (95% CI: 77.47-81.10) for *An. gambiae* s.l. populations and 76.75% (95% CI: 72.53-80.60) for *An. funestus* s.l. Parity was significantly lower as compared to the rate in 2019 before the ITN mass campaign with 84.31% for *An. gambiae* s.l. (p<0.0001) and 83.27 % (p=0.013) for *An. funestus* s.l., suggesting impact on the older population of the malaria vectors that would potentially transmit malaria in the community. The results demonstrated a reduction in malaria transmission risk after the implementation of the two vector control interventions.

0305

TARGETING STRONGLY INSECTICIDE RESISTANT ANOPHELES FUNESTUS BY USING IVERMECTIN BASED ATTRACTIVE TOXIC SUGAR BAITS IN THE SEMIFIELD SYSTEM

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Vector control strategies are crucial for malaria elimination. Each current vector control tool has its own set of limitations and additional new tools with novel active ingredients (AI) or modes of action to work synergistically with current tools are required. Attractive Toxic Sugar Baits (ATSBs) which exploit mosquito sugar feeding behavior to lure individuals into a bait station treated with a killing agent have proven to be effective in controlling malaria vectors. But little is known if ATSB can control *Anopheles funestus*. Here we evaluated the efficacy of a cost-effective ivermectin-based ATSB as an intervention for controlling *An. funestus*in the semi-field. A 3×3×2 m cage was constructed with 4 separate chambers. Four ATSB containers with only sugar meals were put at each corner on the ground floor, inside the chambers. 100 female *An. funestus* were released in each chamber and left there for 7 days, while only given sugar meal, scoring fed and dead mosquitoes daily. After 7 days, two chambers were used as treatment and the other two chambers remained as control with the addition of 100 female *An. funestus* to each chamber. The number of mosquitoes sugar-fed or dead was recorded daily. The survival rate of *An. funestus* were similar in all chambers before the introduction of 0.01% ATSB container on day 7 and lower in the treatment chamber shortly after the introduction of ATSB container, suggesting an impact of ATSB on the survival rate of *An. funestus*. A significant difference in *An. funestus* survival between the control and treatment chambers was observed ($P = 0.0025$). The feeding success was 61% in both chambers within 24 hrs. *An. funestus* were susceptible to pirimiphos-methyl, but resistant to all pyrethroids commonly used on LLINs. In conclusion, *An. funestus* can feed on ivermectin-based ATSB, and subsequently affects their survival rate in the semi-field. Therefore, targeting this strongly insecticide-resistant and endophilic *An. funestus* using ivermectin-based ATSB indoor is very crucial towards malaria control and elimination, and further field trials are necessary to assess its efficacy in a real environment.

0306

THE TRIPARTITE RELATIONSHIP BETWEEN ANOPHELES ARABIENSIS' GUT MICROBIOME, 20-HYDROXYECDYSONE (20E) SIGNALING, AND VECTORIAL CAPACITY

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20-hydroxyecdysone (20E) agonists have emerged as promising insecticides to target malaria vectors. Indeed, they can reduce *Anopheles* mosquitoes› numbers even in areas with high levels of pyrethroid resistance. These agonists bind to *EcR*, mosquitoes' 20E receptor, and activate 20E signaling. Whether 20E agonists can target mosquitoes that have fed on antibiotics-containing blood is still unknown. Addressing this question is essential since antibiotic-fed *An. gambiae* females are more fertile. Therefore, the over-prescription of antibiotics observed in African countries may contribute to their high number of mosquitoes and malaria transmission. Here, we explored the relationship between *An. arabiensis*' 20E signaling, gut bacteria, and reproductivity. First, we tested if *EcR* levels and the gut bacterial load are linked. For this purpose, we (i) measured *EcR* expression post-blood meal, a time where the gut bacteria proliferates, (ii) quantified the gut bacteria after depleting *EcR* with RNA interference, and (iii) assessed if antibiotic-fed mosquitoes have fewer *EcR* transcripts. We found that *EcR* expression is significantly induced post-blood meal, and *EcR* transcript levels correlate with gut bacterial load, notably bacteria of the Enterobacteriaceae and Acetobacteriaceae family. Next, we investigated how 20E titers regulate gut bacteria. Mosquitoes were injected with 20E, and the expression of ten Imd pathway-related immune genes was measured at different time points post-injection. Strikingly, 20E injection initially decreased the expression of these genes but later increased their expression, probably to maintain bacteria homeostasis. Our last objective explored if the gut bacteria affect how 20E signaling regulates mosquitoes› reproductivity. We thus compared the reproductivity of *EcR*-depleted aseptic and septic mosquitoes. Both fecundity and fertility were equally affected by *EcR*-depletion, regardless of mosquitoes' gut bacteria load. Altogether, *EcR*'s ability to regulate *An. arabiensis'* reproductivity suggests that 20E agonists could effectively control malaria vectors in Africa.

0307

LONG-ACTING IVERMECTIN AGAINST MALARIA: A ONE-HEALTH APPROACH CONSIDERING ECOLOGICAL SIDE-EFFECTS OF TREATED PERIDOMESTIC ANIMALS

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In Burkina Faso, the ANIVERMATE project investigates the treatment of peridomestic animals (cattle) with ivermectin (IVM) as complementary malaria vector control. However, excreted veterinary drugs can enter the environment and burden ecosystems. Therefore, we consider ecological consequences of excreted residues. We monitored IVM concentrations in dung of Zebu cattle, treated with either an innovative injectable long-acting depot (MedinCell's Bepo® technology, see companion abstract by Pooda *et al.*) or monthly injections of a commercial IVM formulation (Ivomec®). IVM treatments were designed to last six months. In addition to residues in fresh dung, we studied the environmental fate of IVM in stored dung and conducted sorption studies with soil samples from local villages. The Bepo® depot resulted in significantly lower IVM peak concentrations in cattle dung. Excreted IVM showed a bimodal curve with moderate fluctuations over the trial period. Monthly injections of Ivomec® peaked within 7 days after each injection, followed by exponential decline. Ecotoxicological effects of excreted IVM on dung-dependent organisms are common. For adult species of Coleoptera and Diptera, IVM in dung appears to be of lower toxicity. Due to uniformly moderate IVM dung concentrations with the depot formulation compared to fluctuating concentrations with Ivomec® injections, the depot induces less toxicity for larvae of dung organisms that can tolerate concentrations in a medium range. However, for more sensitive larvae, the prolonged exposure to toxic IVM concentrations in fresh dung warrants further ecotoxicological investigation. More knowledge on local dung/soil fauna is desirable. After 3 months of dung storage, IVM displayed low degradation. Soil sorption studies revealed strong sorption, indicating high hydrophobicity of IVM. ANIVERMATE highlights the importance of integrating ecological risk assessment with regards to a One-Health approach. A long-acting depot administered in cattle may provide a complementary and reliable mosquito control with manageable ecological side-effects to reduce impacts on agricultural production.

0308

BIOEFFICACY OF CLOTHIANIDIN AND PIRIMIPHOS METHYL IN NORTH WESTERN LAKE ZONE REGIONS IN TANZANIA TO CONTROL PYRETHROID RESISTANT MALARIA VECTORS

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The aim of this study was to assess the residual efficacy of clothianidin and pirimiphos-methyl (p-methyl) used as indoor residual spray (IRS) on different wall surfaces inside houses and their effectiveness in controlling malaria vectors in Tanzania from October 2019 to September 2020. WHO wall cone and fumigant bioassays were conducted monthly for 12 months with laboratory susceptible *An. gambiae* s.s. Kisumu strain on different wall surface types sprayed with either clothianidin or p-methyl. The walls were mud, oil or water painted, lime washed, unplastered cement and burnt bricks. Claypots, CDC light traps, Prokopack aspirators and collection bottle rotators with CDC light traps were used during monthly entomological surveillance conducted in 10 districts (6 IRS and 4 without IRS as control). Collected mosquitoes were identified morphologically and sibling species identified by PCR. Mean mortality on all wall surface types was above the 80% WHO threshold for eleven months post-IRS with clothianidin. All p-methyl-sprayed wall surfaces had a mean mortality above 80% post-IRS for seven consecutive months. There was no significant variation among different wall surfaces ($p>0.05$). A total of 39,686 female *Anopheles* mosquitoes were collected and morphologically identified as *An. gambiae* s.l. (71.9%), *An. funestus* s.l. (21.7%), *An. coustani* (3.7%), *An. pharoensis* (1.8%) and *An. rufipes* (0.9%). Molecular identification conducted on 23,961 mosquitoes revealed the local malaria vector population was predominated by *An. funestus* s.s. (40.9%), *An. arabiensis* (37.6%), *An. gambiae* s.s. (15.7%) and *An. parensis* (1.1%). In sprayed sites, *An. funestus* were predominant before IRS and *An. arabiensis* after IRS. *An. funestus* s.s. was predominant in unsprayed control sites.The average pre-IRS sporozoite rate was 1.6 and reduced to 0.9 after spraying. The sporozoite rate remained higher (1.8%) in unsprayed sites ($p<0.01$). Clothianidin and p-methyl remained efficacious on all types of sprayed wall surfaces post-IRS during peak transmission season. IRS with these insecticides has significantly reduced sporozoite rates in *Anopheles* vectors.

0309

TWO-YEAR ENTOMOLOGICAL SURVEILLANCE RESULTS FROM A CLUSTER-RANDOMIZED CONTROLLED TRIAL ASSESSING THE EFFICACY OF DUAL ACTIVE INGREDIENTS LONG-LASTING INSECTICIDAL NETS (LLINS) IN MISSUNGWI DISTRICT, SOUTH-WESTERN TANZANIA

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The effectiveness of current malaria vector control tools mainly pyrethroids-based long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) is threatened by the widespread pyrethroid resistance in malaria vectors. Next-generation LLINs with two novel active ingredients of different mode of actions were evaluated against malaria transmitted by pyrethroid-resistant vector mosquitoes in Missungwi district, North-western Tanzania. A 2-year single-blind, four arm cluster-randomized controlled trial was conducted to evaluate the efficacy of dual-active LLINs compared to the standard pyrethroid-LLIN on density, and entomological inoculation rates of malaria vectors across 84 study clusters. Indoor light trap collections were carried out quarterly in eight houses in each cluster that received study interventions, between February 2019 and December 2020. Dual-active LLINs deployed included Royal Guard®, a mixture LLIN combining pyriproxyfen and the pyrethroid alpha-cypermethrin; Olyset® Plus, a mixture LLIN combining the synergist piperonyl butoxide and the pyrethroid permethrin; Interceptor® G2, a mixture LLIN contains chlorfenapyr and alpha-cypermethrin in comparison with a standard LLIN that contains alpha-cypermethrin alone. All Anopheles mosquitoes sampled were morphologically identified to species or species group, and a subset of malaria vectors were further tested for *Plasmodium falciparum* circumsporozoite protein and identified to sibling species using polymerase chain reaction. Analysis of mosquito's outcomes by interventions and dual active LLINs will be presented. Together with the epidemiological outcomes, the findings will provide the first body of evidence on the efficacy of Royal Guard and Interceptor G2 in a RCT against pyrethroid-resistant malaria vectors.

0310

ENTOMOLOGICAL IMPACT OF PIPERONYL BUTOXIDE (PBO) NET DEPLOYMENT ON PYRETHROID-RESISTANT ANOPHELES GAMBIAE S.L. MOSQUITOES IN EBONYI STATE, SOUTHEASTERN NIGERIA

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Pyrethroid insecticide resistance has been widely reported in 20 of the 36 states in Nigeria, where insecticide-treated nets (ITNs) are the main malaria prevention method. Insecticide susceptibility data helps inform which ITNs will be most effective for local mosquito populations. Susceptibility data generated in Ebonyi by the President's Malaria Initiative VectorLink project guided the deployment of deltamethrin+piperonyl butoxide (PBO) ITNs in November 2019. VectorLink assessed the entomological impact of

these nets in three Local Government Areas (LGAs): Ezza North, Izzi and Ohaukwu. *Anopheles* mosquitoes were collected monthly from human baited CDC light traps and pyrethrum spray collections and identified using morphological keys. Species specific polymerase chain reaction assays were conducted on all samples from the *An. gambiae* complex. The indoor resting density (IRD), human biting rate (HBR), entomological inoculation rate (EIR), and biting time of *Anopheles* mosquitoes pre-and post-distribution were compared using Chi square analysis. *An. gambiae* s.s., *An. coluzzii,* and *An. arabiensis* were the major vector species found. The number of *Anopheles* resting indoors/room/night reduced by 2.7-fold in Ezza North (9.78/3.60), 3.4-fold in Izzi (4.58/1.36), and 4.1-fold in Ohaukwu (5.46/1.32) post-intervention. Significant decreases in IRD were recorded post-intervention in all three LGAs (p≤0.03). The average HBR (number of bites/person/night) was significantly reduced indoors (p≤0.05) in Ezza North (14.28 vs 6.78), Izzi (8.22 vs 1.33) and Ohaukwu (11.10 vs 4.60). The baseline outdoor HBR did not differ significantly post-distribution in Ezza North (3.22 vs 1.10, p=0.48), Izzi (2.04 vs 0.44, p=0.32) and Ohaukwu (3.62 vs 1.34, p=0.21). The proportion of mosquitoes (73% vs 72%) biting at peak indoor and outdoor times (10 p.m.-5 a.m.) was similar (p=0.93). A significant reduction of mean EIRs (number of infective bites/person/year) was recorded indoors in Ezza North (29.50 to 0) and Ohaukwu (18.50 to 0). These results suggest that PBO ITNs improved protection for humans from mosquito bites and reduced malaria transmission.

0311

SMARTNET INITIATIVE: HARNESSING MOBILE TECHNOLOGY AS PART OF COMMUNITY ENGAGEMENT TO SUPPORT MALARIA CONTROL IN MALAWI

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Malawi reported over 5 million cases of malaria in 2019 and it remains the main cause of death for children under the age of 5. Successful malaria control efforts require: 1) timely and accurate data collection, analyses, and use; 2) optimization of proven interventions such as insecticide treated nets (ITN); 3) sufficient resources; and 4) community engagement. Mobile technology use in Malawi has increased to around 48 mobile phone subscriptions per 100 people in 2019. The Malawi SmartNet Initiative leverages 2/3/4G mobile technology to reach ITN users in real time. In March 2020, 300,000 ITN with a short code were distributed via antenatal clinics. The short code allowed recipients to complete a survey via their mobile phones. Questions included sociodemographic and geographic indicators, net usage, presence of malaria-like illnesses, and location. Among the 300,000 recipients, as of March 2021, 54,682 voluntarily responded and of those 18,555 (34%) completed a Unstructured Supplementary Service Data (USSD) mobile phone survey; 14,947 of the 18,555 (80%) also provided the unique ITN code. Respondents answered 12 questions including basic demographics, number of household members, ITN possession and use, malaria-like illnesses and location. Dialling the short code was free and of respondents who completed the survey, the average response time of 2 min 25 seconds and 42% completed it during first call. Respondents were from each of the 28 districts and represented an estimated 273,410 household members (54,682 households) or 51% of the estimated households expected to be reached by the 300,000 ITNs. Limitations include potential lack of representativity of this sentinel sampling approach; biases include increased response from those who are literate and have mobile phones. Although generalizability of these data remains to be determined, through future surveys, this sentinel engagement with 21,434 individuals is one of the largest conducted in African malaria control and provides opportunities to both disseminate and collect information in real time.

0312

HOUSEHOLD OUTDOOR NIGHTTIME ACTIVITY AND SLEEPING BEHAVIOR AND POTENTIAL IMPLICATIONS FOR MALARIA VECTOR CONTROL IN THE NORTHERN BENIN, WEST AFRICA

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Nighttime outdoor practices of household residents may limit the effectiveness of vector control interventions and sustain residual malaria transmission. To identify and characterize these practices, the location and nighttime activity of household members (HHMs) were observed in the departments of Alibori, Atacora, and Donga in northern Benin from 2018 to 2020. Monitoring of outdoor and nighttime resting and sleeping practices was done by direct overnight observation of the households. Three household visits were done per year, with one visit occurring during each rainy (wet period), dry (hot period), and harmattan season (cold period). A convenience sample of ninety-six households with at least 5-9 HHMs was enrolled each year in the study. One HHM was recruited as a household activity enumerator to monitor HHMs' nighttime activities and location every 30 minutes between 7 pm and 7 am for five nights per season per year. The activities monitored included resting or sleeping locations, ITN use, and other mosquito avoidance practices. More than 50% of the HHMs were resting or sleeping outdoors between 7 pm-10 pm throughout the study duration. During the hot and dry season, 80% of the HHMs were resting or sleeping outdoors. A high proportion of HHMs (44.4%) was observed staying all night outdoors. Most of them did not use mosquito nets or other mosquito avoidance paraphernalia such as mosquito coils, insecticide sprays, or repellents. Hot ambient temperature and limited lighting and space within the household were the main reasons given as to why HHMs rested or slept outdoors. Outdoor resting and sleeping are common behaviors for the majority of HHMs in northern Benin due to the heat during the dry season and limited lighting and space within their house; also, HHMs often forgo protecting themselves from mosquito bites, likely increasing the risk of HMMs receiving infectious bites. It may be necessary to enhance messaging on ITN use when resting or sleeping outdoors in Benin, specifically targeting the dry season. Also, hammock ITNs could be considered for use in Benin, or better outdoor tools, could be developed.

0313

CHARACTERIZING LATE NIGHT ANOPHELES MALARIA VECTORS IN NCHELENGE, ZAMBIA

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With effective drugs and public health interventions in place, malaria remains a major burden in sub-Saharan Africa and is among the leading causes of hospitalization, morbidity, and mortality in this region. Vectored by mosquitoes of the *Anopheles* genus, the transmission dynamics of the *Plasmodium* parasite to humans are complex, and elements including time of day, season, location, and parasite infection rate within the mosquito must be considered. In the Nchelenge district of northern Zambia, *Anopheles funestus* is the predominate malaria vector, while species such as *An. gambiae* and other understudied vectors are present and contributing to transmission. Variable feeding behaviors exist between species, emphasizing the importance of understanding species-specific preferences to interrupt transmission via implementation of appropriate vector control interventions. To further discern these behaviors, mosquitoes foraging within twenty Nchelenge households were captured overnight during two weeks in August of 2019 utilizing CDC light traps placed in indoor sitting rooms of households between 4-10pm and from 10pm-6am. Overnight mosquitoes will be morphologically identified, species confirmation and bloodmeal source will be analyzed via PCR assays, and presence of parasite within mosquitoes will be determined by ELISAs specific for *P. falciparum*. It is expected that *An. funestus* make up a vast majority of captured mosquitoes and roughly 1-2% of these harbor *Plasmodium* sporozoites. Results from these analyses regarding species abundance, foraging patterns, host preference, and parasite infection rates will be compared with early evening collections and will translate to a better understanding of malaria transmission to humans. These results coupled with data from household surveys on human behavior including bed net usage and IRS status will assist in identification of risk factors for increased densities of anophelines, allowing for implementation of more species-specific vector control strategies, ultimately contributing to the continued effort to decrease the burden of malaria in Zambia.

0314

EFFICACY OF CDC LIGHT TRAP AND HUMAN DECOY TRAP (HDT) COMPARED TO HUMAN LANDING CATCH FOR ESTIMATING MALARIA VECTOR BITING RATES IN RURAL TANZANIA

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The human landing catch (HLC) is considered the best trap for biting mosquitoes, yet there are concerns about its safety for extensive use. We compared the numbers of mosquitoes caught by the CDC light trap, the human decoy trap (HDT) and HLC in Tanzania in 2017 and 2019. We collected the mosquitoes as part of an exercise to evaluate the impact of indoor residual spraying (IRS) products in a rural malaria-endemic setting. We conducted CDC light trap surveys indoors, the HDT outdoors and the HLC both indoors and outdoors. We used negative binomial mixed-effects models to compare nightly catches of the CDC light trap to the indoor HLC and of the HDT to the outdoor HLC. Overall, we trapped 14,606 *Anopheles arabiensis*, 66,807 *An. funestus* and 75,248 *Culex* spp adult female mosquitoes. We observed consistently higher numbers of *Culex* spp than *An. funestus* and higher numbers of *An. funestus* than *An. arabiensis* across all traps. Compared to indoor HLC, we found that the CDC light trap caught about half as many *An. arabiensis*, (RR = 0.42(0.31-0.58), $p < 0.0001$), over two thirds of *An. funestus*, (RR = 0.64(0.50=0.83), $p = 0.0008$), and an approximately equal number of *Culex* spp, (RR = 0.93(0.74-1.18), $p = 0.57$). We found that HDT caught just about a tenth of both *An. arabiensis*, (RR = 0.07(0.01-0.31), $p = 0.0006$) and *An. funestus*, (RR = 0.10(0.06-0.18), $P < 0.0001$) and a third of *Culex* spp, (RR = 0.29(0.17-0.50), $P < 0.0001$) caught by the reference trap. Differences between the CDC light trap and the indoor HLC did not appear to vary greatly depending on mosquito density. The CDC light trap may be used for regular indoor malaria vector monitoring as long as a correction factor is applied to match mosquito catches of the HLC gold standard trap. Due to low mosquito numbers caught by HDT in this study, we recommend further investigations with a more robust study design to reassess its efficacy and potential use as an alternative to outdoor HLC.

0315

KEY FINDINGS FROM DIGITIZING SUPERVISION OF CONTINUOUS DISTRIBUTION CHANNELS FOR INSECTICIDE TREATED NETS IN TANZANIA, 2019-2020

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Continuous distributions of insecticide treated nets (ITNs) through health facilities, schools, and communities have been shown to maintain high population access to ITNs and have the potential to replace mass campaigns in some settings. Supervision helps optimize these distribution channels by enabling supervisors and decision-makers to respond to identified gaps in logistics management (e.g. ITN storage), data management (e.g. accuracy of control cards against physical stock), and provider behavior (e.g. adherence to issuing ITNs to eligible clients). However, ensuring quality distribution of ITNs through these channels is limited by the lack of consistent supervision and timely access to supervision data. We implemented a digital tool of the supervision checklist and compared it to the customary paper-based system. In 2019, 4939 schools on the Mainland and 374 health facilities across the Mainland and Zanzibar received supervision visits using paper-based forms. Between January 2020 and March 2021, 2043 schools were visited (but only 89 were visited with digital forms) and all 990 visits to facilities used the digital tool. Access to supervision data for reporting and analysis improved from a minimum two week wait to real-time. Staff were not able to quantify past performance due to the vast number of paper reports. Among visits to facilities using the digital tool, 94% observed an ITN inventory control card (to measure stock levels) available, but card contents were correct in only 63% of visits. Approximately half of visits (56%) observed acceptable (5%) variance between card values and a physical stock count. In total, 85% of visits confirmed facilities had sufficient physical stock, but ITNs were stored appropriately in only 65% of visits. Qualitative results revealed many facilities lacked pallets. The digital tool highlighted nuances within continuous distribution of ITNs previously unquantified, enabled District Medical Officers to act more rapidly (e.g., to review unreported commodities), and provided data which can be further used to adapt training curricula to improve future ITN distributions.

0316

PYRETHROID-PIPERONYL BUTOXIDE (PBO) NETS REDUCE THE EFFICACY OF INDOOR RESIDUAL SPRAYING WITH PIRIMIPHOS-METHYL: AN EXPERIMENTAL HUT EVALUATION IN SOUTHERN BENIN

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Most indoor residual spraying (IRS) campaigns are deployed against a background of high long-lasting insecticidal net (LLIN) coverage. Pyrethroid-piperonyl butoxide (PBO) LLINs have recently been recommended for malaria control and are replacing pyrethroid LLINs in endemic communities. PBO acts by inhibiting enzymes responsible for pyrethroid resistance, namely cytochrome P450 monooxygenases (CYPs). Pirimiphos-methyl is also widely applied for IRS campaigns however; it is a pro-insecticide that requires activation by CYPs in order to exert full toxicity. The inhibitory action of pyrethroid-PBO LLINs against CYPs may, therefore, reduce the toxicity of pirimiphos-methyl IRS when these interventions are combined. We performed a series of experimental hut trials to evaluate the impact of combining different pyrethroid-PBO LLINs with pirimiphos-methyl IRS against a pyrethroid-resistant vector population in Benin. Comparison was made to either method alone and combinations based on pyrethroid LLINs as a positive control. WHO susceptibility bioassays were performed to determine the resistance profile of the vector population. WHO susceptibility bioassays revealed the vector population was highly resistant to pyrethroids but susceptible to organophosphates. PBO pre-exposure only partially restored pyrethroid susceptibility. Vector mortality in huts containing pirimiphos-methyl IRS and pyrethroid-only LLINs (81%) was similar to IRS alone (85%, p=0.129). In contrast, mortality was significantly reduced with combinations of pirimiphos-methyl IRS and pyrethroid-PBO LLINs (55-59%) relative to IRS alone (77-78%, p<0.001). This study provides evidence for reduced efficacy of pirimiphos-methyl IRS in the presence of pyrethroid-PBO LLINs. Control programmes may consider withholding pyrethroid-PBO LLIN distribution in areas scheduled for IRS with pirimiphos-methyl as a precaution.

0317

USING TRANSFER LEARNING COUPLED WITH DIMENSIONALITY REDUCTION TO IMPROVE GENERALISABILITY OF MACHINE LEARNING PREDICTIONS OF MOSQUITO AGES FROM MID INFRARED SPECTRA

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Accurate prediction of mosquito population age-structure helps to monitor the efficacy of interventions which target adult mosquitoes. Mid-infrared (MIR) spectroscopy provides biochemical signals of the mosquito cuticle that correlate with age, which can be used to train machine learning (ML) models for age prediction. We assessed whether dimensionality reduction and transfer learning (adding few observations of the target population into training data to improve model-performance) could improve generalisability of ML predictions for mosquito ages from MIR spectra. We used *Anopheles arabiensis* mosquitoes reared in two insectaries maintained at different rearing conditions. Heads and thoraces of day 1-17 mosquitoes were scanned using a MIR spectrometer to obtain high resolution MIR spectra. Mosquito ages were grouped into three classes (i.e. 1–5, 6–10, 11–17 day-olds). The dimensionality of the spectra data was reduced using either principal component analysis (PCA) or t-distributed stochastic neighbor embedding when training a deep learning (DL) and standard ML classifiers. Transfer learning was later used to improve generalisability of the models in predicting mosquito ages. We found that when dimensionality reduction by PCA was used, DL achieved an overall accuracy of 38% in generalizing prediction for mosquito ages. On the other hand, when transfer learning (5% of the unseen data) and dimensionality reduction by PCA were both used, DL improved generalisability with an overall accuracy of 95% in predicting mosquito ages. Standard ML also achieved 94% accuracy in generalizing prediction of mosquito ages. Thus, dimensionality reduction itself does not improve generalisability of the models in predicting mosquito ages from another rearing condition. However, using both dimensionality reduction and transfer learning, generalisability of the deep learning in predicting mosquito ages improved, achieving >90% accuracy. Additionally, standard ML can reduce computational time and achieve high accuracy matching the DL performance, when dimensionality reduction and transfer learning are both used.

0318

IMPACT OF NEXT-GENERATION DUAL-ACTIVE INGREDIENT LONG-LASTING INSECTICIDAL NET DEPLOYMENT IN TANZANIA ON INSECTICIDE RESISTANCE IN ANOPHELES FUNESTUS S.L. AND ANOPHELES GAMBIAE S.L.: AMELIORATION OR INTENSIFICATION?

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Insecticide resistance among mosquito species is now a pervasive phenomenon, which threatens to jeopardize global malaria control efforts. Novel vector control tools, including long-lasting insecticidal nets (LLINs) incorporating new active ingredients with different modes of action are urgently needed to delay the evolution and spread of resistance. During a four-arm cluster-randomised trial in Misungwi district, Tanzania, evaluating the effectiveness of (1) Royal Guard, a LLIN combining pyriproxyfen and alpha-cypermethrin; (2) Interceptor G2, a LLIN combining chlorfenapyr and alpha-cypermethrin; (3) Olyset Plus, a LLIN combining piperonyl butoxide (PBO) and the pyrethroid permethrin; compared to (4) Interceptor, a standard alpha-cypermethrin LLIN, we measured longitudinal trends in insecticide resistance among >27,000 wild mosquitoes collected over 24 months. Prior to LLIN distribution, pyrethroid resistance was high, with 30-min knock-down ranging from 43.7-59.4% for *Anopheles funestus* s.l. and *An. gambiae* s.l. following exposure to alpha-cypermethrin and mean 24-hr mortality of 38.3-56.5% in permethrin bioassays. Following LLIN distribution, we detected a significant increase in permethrin resistance intensity in *An. funestus* s.l. across all trial arms. Levels of alpha-cypermethrin resistance also increased but only in clusters which received Interceptor® or one type of LLIN under evaluation. PBO pre-exposure restored permethrin susceptibility among vector populations and high levels of chlorfenapyr susceptibility were also observed. We assessed oviposition inhibition to pyriproxyfen using dissection 3-days after exposure with variable results between trial years. *An. gambiae* s.s. populations were characterised by high frequencies of L1014FS-*kdr* (98%) and overexpression of *CYP6M2, CYP6P3, CYP6P4* and *CYP9K1*. Study findings highlight increasing pyrethroid resistance intensity in *An. funestus* s.l. associated with the deployment of next-generation LLINs, with additional work required to thoroughly understand the operational implications and potential mechanisms driving cross-resistance.

0319

ASSESSMENT OF THE DURABILITY OF LONG-LASTING INSECTICIDAL NETS FROM THE 2017 ITN MASS CAMPAIGN IN BENIN, WEST AFRICA

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Long-lasting insecticidal nets (LLINs) are widely used for malaria prevention and control in Benin since 2007. Although LLINs have been used for nearly 15 years, there is limited information on LLIN durability in Benin. The survivorship, physical integrity, and bioefficacy of LLINs were assessed through longitudinal surveys conducted at 6, 12, 18, 24, 30, and 36 months following a July-October 2017 mass distribution campaign. Three brands of LLINs (DawaPlus®2.0, PermaNet®2.0, and Yorkool®) were distributed in 3 districts (Ketou, Dogbo, and Djougou). Five hundred LLINs per district (250 in rural areas and 250 in urban areas) were monitored for a total of 1500 LLINs. Bioefficacy tests were done on 50 LLINs per brand per site and assessed by WHO cone tests. After a 3-year follow-up period, only 16% (241/1500) of the original LLINs were found and assessed by the investigators in the sampled households. The associated net loss rate (84% overall) was similar across all three brands of LLINs (p=0.6868). This high net loss rate was largely due to the displacement or replacement of LLINs. Out of the 241 LLINs found, 48% (95% CI: 32 – 64) of the LLINs had physical damage, of which 13% (95% CI: 9 – 18) were severely torn. The median pHI of LLINs (all brands) was 578 (IQR = 219 – 843) at 6 months versus 197 (IQR = 49 – 666) at 36 months. This poster further describes the proportion of bednets in "good condition" (pHI ≤ 64) as classified by the WHO, the loss rate, and the median survival. The bioefficacy of the three LLINs after 2 years was greater than 80% in mosquito mortality. Although our evaluation showed that all three brands of bednets were in a relatively good physical and chemical condition, the high loss rate observed may limit the overall efficacy of LLINs in preventing malaria. Strengthening behavioral change communication to encourage communities to retain, use, and maintain LLINs may improve and sustain their effectiveness.

0320

LIMITED IMPACT OF INDOOR RESIDUAL SPRAYING FOR MALARIA CONTROL IN TANZANIA 2014 - 2020: A RETROSPECTIVE ANALYSIS

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Indoor residual spraying (IRS) has been a key malaria intervention in Tanzania since 2006. This retrospective analysis aims to evaluate the impact of IRS on malaria incidence in Tanzania's high burden Lake Zone (district level) and Zanzibar (shehia [sub-district] level). Routine data from 2014 to 2020 were collected (District Health Information System 2 for mainland Tanzania and Malaria Case Notification system for Zanzibar) and separately analyzed. Districts and shehias were grouped into 'No IRS' and multiple years of IRS in the same locations ('1-2'; '3-4'; '>4' years). The annual epidemiological trend was obtained by calculating the percentage difference between the incidence in the month pre-IRS (baseline month) and the incidence during the 9 months post-IRS (i.e., corresponding to the organophosphate insecticide's residual efficacy used for the corresponding IRS year). Spatial regression models, with random effect (to adjust for other factors), were developed to evaluate IRS's effect on incidence between and within years, and accounting for multiple IRS rounds. During the study period, 23 districts and 205 shehias received at least one round of IRS. For the entire period, model results showed a significant protective effect of IRS on malaria incidence in mainland Tanzania (-63.9%: 95% CI: -183.3%; 17.6%) and Zanzibar (-24.9%; 95% CI: -52.6%, -3.2%). However, on a yearly basis, the protective effect of IRS was temporally and spatially heterogeneous: in Mainland Tanzania, the highest number of sprayed districts where IRS showed a protective effect was in 2016 and 2017 (7/9, of which 4 were statistically significant); in Zanzibar, 19% of sprayed shehias in 2018 showed a significant protective effect versus 4% in 2019. In Zanzibar, incidence (OR 1.15; 95% CI: 1.10, 1.19) and cases in the prior year (OR 0.98; 95% CI: 0.96, 0.99) were positively and negatively associated with malaria incidence trends, respectively. Our analytical approach can be used to improve IRS monitoring as well as targeting, ensuring that the impact of IRS on malaria is maximized.

0321

SITE-SPECIFIC INCIDENCE RATE OF DIFFERENT GENOMIC STRAINS OF ENTEROAGGREGATIVE ESCHERICHIA COLI & ASSOCIATION WITH ENTERIC INFLAMMATION & GROWTH IN CHILDREN OF A MULTICOUNTY BIRTH COHORT STUDY

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There is a lack of information that highlights the possible association between different potential virulence genes among the Enteroaggregative *Escherichia coli* (EAEC) and environmental enteric dysfunction (EED), and their eventual combined effects on nutritional status and growth among children. Infections by different genomic strains of EAEC were detected by the use of a customized multiplex quantitative polymerase chain reaction array system known as the TaqMan Array Card (TAC) from stool samples collected from a total of 1705 children enrolled in the MAL-ED birth cohort. We measured site-specific incidence rate by using Poisson regression models, identified the risk factors, and estimated the associations of different genomic strains of EAEC with the composite EED score and child growth at 24 months of age. Overall highest incidence rate (49.1%) was found among children having infection with the concomitant presence of *aaiC* and *aatA* gene, which was the greatest in Tanzania (65%). Less maternal education, lack of improved floor, and ownership of cattle in the household were found to be risk factors for EAEC infection by different genomic strains. In the multivariate models, after adjusting for age, sex, WAMI index (Wealth, Asset, Maternal education, Income), enrolment length-for-age z score, maternal BMI, number of children in the family, poultry/cattle in house, seasonality, serum zinc, and alpha-1-acid glycoprotein, co-pathogens, and site for the overall estimate; and different genomic strains of EAEC infections showed strong positive associations with different biomarkers of EED in generalized estimating equations and with poor linear growth at the age of 24 months. Different genomic strains of EAEC were associated with enteric inflammation and linear growth faltering among children. Our analyses may aid an epidemiologic investigation to propel a potential vaccine development aimed at reducing the burden of EAEC infections and resulting phenotypic aberrations in the form of combating EED and its consequent childhood malnutrition.

0322

COMPLICATED COURSE OF A PRIMARY KLEBSIELLA PNEUMONIAE LIVER ABSCESS REQUIRING EXPLORATORY LAPAROTOMY AND PARTIAL HEPATIC LOBECTOMY

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Klebsiella p. is a well-known cause of community acquired pyogenic liver abscesses in Taiwan and East Asia. These abscesses can become invasive, and are even described as metastatic in some cases. Although previously uncommon in the US, there has been an increase in incidence of KLAs (Klebsiella Liver Abscess) in the past few decades. Case: 42 year old male with no known past medical history presented to the hospital for progressive abdominal pain, generalized weakness, and increased thirst. 3 weeks prior to presentation, he was seen at another facility for fever, chills, and body aches; he was prescribed amoxicillin with initial improvement of his symptoms. Later, his symptoms returned, along with the development of RUQ abdominal pain. He denied any significant past medical history, including any prior diagnosis of diabetes. He did not take any medications, denied any prior major illnesses, surgeries, or hospitalizations. He was born in the Philippines, but moved to the US in 1990. He had not travelled outside of the US since that time, and had not traveled outside of California in over 3 years. Vitals: temp 99.2, HR 112, RR 18, BP 121/71. Exam: RUQ tenderness and mild scleral icterus. labs: AST 213, ALT 136, Alk phos 459, t bili 3, WBC 11, crp 181, procal 7.8, glu 364, hba1c 11. Abdominal CT revealed an 11 cm liver abscess. A percutaneous drain was placed and he was started on IV abx. Cultures from the aspirate grew Klebsiella p. Initially he improved with IV abx and continued drainage, but worsened again on day 4 of the hospitalization with fever, chills, and worsening abdominal pain. Repeat CT showed improvement of the hepatic abscess, but interval development of perihepatic, perisplenic, and pelvic abscesses. General surgery was consulted. An exploratory laparotomy showed necrosis of hepatic lobes 6 & 7, gross inflammation of the gallbladder, paracolic gutter abscess involving the appendix, and multiple other abscesses in the peritoneal cavity. He underwent a partial hepatectomy, cholecystectomy, appendectomy, and drainage of multiple intraabdominal abscesses. He was treated with 4 weeks of abx therapy with resolution of his infection.

0323

IMMUNOGENICITY AND WANING OF IMMUNITY TO ORAL CHOLERA VACCINE (SHANCHOL™) IN ADULTS RESIDING IN LUKANGA SWAMPS OF ZAMBIA

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In cholera endemic areas, the periodicity of cholera outbreaks remains unpredictable, making it difficult to organize preventive efforts. Lack of data on duration of protection conferred by oral cholera vaccines further makes it difficult to determine when to deploy preemptive vaccination. We report on the immunogenicity and waning of immunity to an oral cholera vaccine (Shanchol™) in Lukanga Swamps – a known hot spot in Zambia. We enrolled 223 participants aged 18-65 years from whom serum samples were collected at baseline prior to vaccination with dose 1 and at day 28 before administration of dose 2; then consecutively, at 6, 12, 24, 30, 36, and 48 months. We assessed vibriocidal antibody titres against *Vibrio cholerae* O1 Inaba and Ogawa serotypes. The vibriocidal antibody titres were $\log_{10}$ transformed and expressed as geometric mean titres (GMTs) and vaccine seroconversion defined as four-fold increase in vibriocidal antibodies from baseline. From a baseline of 13.58, anti-Ogawa GMT increased to 21.95 after the first dose, but rapidly began to wane to 14.52, 13.13, and 12.78 at months 6, 12 and 24 respectively, and then increased to 13.21, 18.67 and 23.65 at months 30, 36 and 48 respectively. A similar trend was observed for anti-Inaba GMT across the same time points. Overall, seroconversion against Inaba and Ogawa after 1st dose was 35/134 (26%) and 34/134 (25%) respectively, and these were statistically significant when stratified by baseline titre; Inaba (p=0.02), and Ogawa (p<0.0001). We found Shanchol™ to be immunogenic and that participants with high baseline titres were less likely to seroconvert. Vaccine response was highest soon after the first dose. The second dose did not seem to add any significant immunogenic value, suggesting that 1st dose deployment to a larger population may have protective value over second dose to a fewer population with the same vaccine stockpile. The observed rise in GMT's at months 36 and 48 suggests natural exposure, making this period a critical time for natural transmission. Therefore, we recommend re-vaccination at 36 months in high risk areas.

0324

ASSESSMENT ON ENTEROPATHOGEN DIARRHEA-ASSOCIATED AMONG UNDER-FIVE CHILDREN IN BENGO ANGOLA

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Diarrhea is the most important event in children under five among the poorest people in Africa. Some studies in Angola show evidence of enterobacteria, viruses, and helminths in diarrhea diseases like *Giardia lamblia* strains. In Bengo province studies carried out between 2012-2013 before the introduction of *Rotavirus* vaccination, showed evidence of *Rotavirus* infection in acute gastroenteritis, and the most common *G1*-genotype (83.6%) was revealed. The Monitoring and the assessment of photogenic enterobacteria in Bengo, can provide data for outbreak prediction and forecast of several enteropathogens or emerging oldest agents in the same area. It allows epidemiologic studies which could be arising prophylactic measures and strength the decision make on health in remote locations in Bengo. This study aims to assess the most important pathogenic enterobacteria and *Rotavirus* among children aged 1-59 months in the main public Hospital of Bengo Province, in Angola, through fecal culture test, molecular approach, beside a questionnaire for the caregiver. Children were enrolled according to strict inclusion criteria. Fecal samples have been cultured in MacKonkey agar and XLD (18- 24h at 37°C) and tested (OD600nm) by Vitek2 Compact®. The rapid test (SD®) and PCR for Rotavirus detection performed. A preliminary result from n=112 samples analyzed, evidences infection by pathogenic *Echerichia coli* spp n=41 (36.6%), *Klebisiella* spp n=30 (26.78%), *Salmonella* spp n=3 (2.67%), and *Rotavirus* n=7 (6.48%). However, *Rotavirus* infection is common in children up to 3 years old and co-infection was observed n=6 (5.35%). Poverty in rural areas, low education, and the economic status of caregivers could be affecting the occurrence of diarrhea among children living in Bengo province.

0325

CAN A FOOD HYGIENE AND NUTRITION INTERVENTION REDUCE DIARRHEA AND ENTERIC DYSFUNCTION AMONG CHILDREN IN RURAL BANGLADESH?

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Key causes of undernutrition in children are insufficient intake of nutritious food as well as repeated infections and enteric dysfunction caused by poor sanitation and hygiene practices. We aimed to evaluate the impact of a combined home gardening, chicken rearing, nutrition education, and food hygiene intervention on diarrhea prevalence and markers of enteric dysfunction in young children in rural Bangladesh. We analyzed data on 614 children under 18 months of age participating in the Food and Agricultural Approaches to Reducing Malnutrition (FAARM) trial in Sylhet, Bangladesh. We analyzed diarrhea prevalence using 1-week recall and assessed fecal myeloperoxidase (MPO), neopterin (NEO), and alpha-1 antitrypsin (AAT) as biomarkers of enteric dysfunction and serum C-reactive protein (CRP) and alpha-1 acid glycoprotein (AGP) as biomarkers of systemic inflammation, measured by ELISA. We used multilevel regression to quantify the intervention's impact on diarrhea prevalence and enteric dysfunction biomarkers. Diarrhea prevalence was about 5% and did not differ between children from intervention and control arms. Biomarkers of enteric dysfunction were overall elevated, which was comparable to previous studies in similar settings and age groups. There was no difference in CRP, AGP, and AAT levels between arms. However, children from intervention households showed a 48% increase in MPO levels and a 25% increase in NEO levels compared to children from control households. Overall, we found high levels of biomarkers indicating widespread enteric dysfunction in children, while the prevalence of symptomatic diarrhea was rather low in both arms. Our data suggest that changes induced by the combined nutrition and food hygiene intervention were not sufficient to decrease intestinal dysfunction in young children in our study setting. We are currently investigating whether the increase seen in some biomarkers may be related to aspects of chicken rearing.

0326

PROTECTION AFFORDED BY PREVIOUS VIBRIO CHOLERAE INFECTION AGAINST SUBSEQUENT DISEASE AND INFECTION: A REVIEW

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Cholera is an acute, diarrheal disease caused by *Vibrio cholerae* O1 or 139, with an estimated 2.8 million cases and 95,000 deaths worldwide annually. Knowledge of the duration of protection following infection with *V. cholerae* is critical as we seek to optimize intervention strategies in the prevention and control of cholera. However, current estimates of the duration of protection following natural infection span widely from a few months to ten years. We analyzed the estimated duration of immunity following cholera infection from available published studies. We searched Pubmed and Web of Science for studies of the long-term immunity following cholera infection. We identified 22 eligible studies and categorized them as either (i) an observational study; (ii) a challenge study in which participants were subsequently challenged after an initial challenge with *V. cholerae*; or (iii) a serological study in which potential immunological markers of long-term protection of cholera were measured and assessed. We found strong evidence of protection at three years after infection in observational and challenge studies. However, serological studies show that elevated humoral markers of potential correlates of protection returned to baseline within one year. We also found that a subclinical cholera infection may confer lower protection than a clinical one, as suggested by three studies that found that, albeit with small sample sizes, most participants with a subclinical infection from an initial challenge with cholera experienced symptoms when rechallenged with a homologous biotype. Given that as much as 75% of *V. cholerae* infections are asymptomatic, care must be taken to extrapolate the findings from symptomatic cholera infections to all cholera infections. This review underscores the need to elucidate potential differences in the protection provided by clinical and subclinical cholera infections. Further, more studies are warranted to bridge the gap between the correlates of protection and cholera immunity. Understanding the duration of natural immunity to cholera can help guide control strategies and policy.

0327

ASSOCIATIONS BETWEEN BACTERIAL ENTEROPATHOGENS AND SYSTEMIC INFLAMMATION, IRON STATUS, AND ANEMIA IN PRESCHOOL-AGE CHILDREN IN GHANA

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Poor water, sanitation, and hygiene (WASH) are associated with anemia in young children, yet linkages between bacterial enteropathogen infections and child anemia remain largely unexplored. Enteropathogens may cause anemia via reduced micronutrient absorption and inflammation-mediated functional iron deficiency. The aim of this cross-sectional study was to determine the burden of bacterial enteropathogen infections among children 6-59 months old in Greater Accra, Ghana, and examine associations between infections and systemic inflammation, iron status, and anemia. Blood samples from 262 children were analyzed for hemoglobin (Hb), serum ferritin (SF), serum transferrin receptor (sTfR), C-reactive protein (CRP), and α-1-acid glycoprotein (AGP). Stool samples were analyzed for *Campylobacter jejuni/coli* (*C. jejuni/coli*), *Escherichia coli* (*E. coli*) pathotypes, *Shigella spp.*, and *Salmonella* using quantitative polymerase chain reaction (qPCR). Logistic regression models, adjusting for child age and sex, were used to estimate associations between detection of each enteropathogen and elevated systemic inflammation (CRP>5 mg/L or AGP>1 g/L), iron deficiency (SF<12 μg/L or sTfR>8.3 mg/L) and anemia (Hb<11.0 g/dL). Forty-six percent of children had anemia though only 18% of children had iron-deficiency anemia. Enteropathogens were detected in 87% of children's stool, and infections were predominantly subclinical. *C. jejuni/coli* infection was significantly associated with elevated CRP [Odds Ratio (95% CI): 3.49 (1.45, 8.41)] and AGP concentrations [4.27 (1.85, 9.84)], but not with iron deficiency or anemia. Enteroinvasive *E. coli/Shigella* infection was associated with higher odds of iron deficiency [SF: 2.55 (1.23, 5.29)] and anemia [2.34 (1.15, 4.76)], and with elevated CRP at higher relative detection of the *ipaH* gene [3.24 (1.18, 8.89)]. Subclinical enteropathogen infections, particularly involving enteroinvasive bacteria, appear to contribute to systemic inflammation and anemia in young children. These results suggest that improvements in WASH may help reduce the anemia burden among young Ghanaian children.

0328

UNDERSTANDING THE INCIDENCE AND RISK FACTORS ASSOCIATED WITH TRAVELERS' DIARRHEA IN INTERNATIONAL TRAVELERS DEPARTING FROM UTAH, USA

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Despite current knowledge on the causes and prevention strategies for travelers' diarrhea (TD), TD continues to be one of the most common medical concerns experienced by international travelers in the US, with 10%-40% of travelers reporting TD over a two-week trip depending on the travel destination and other characteristics. However, studies involving US travelers are limited, and a nuanced understanding of the risks associated with specific travel behaviors is lacking. In this study, we aim to explore the frequency of TD among international travelers who

sought pre-travel counseling in Utah, and the risk factors associated with TD. In this cross-sectional study, we've collected and analyzed data from anonymous post-travel questionnaires submitted by international travelers seen at the University of Utah and Salt Lake County travel clinics. Questionnaires administered from October 2016 to March 2020 collected information on demographics and health-related behaviors during travel. To analyze the 22 potential risk factors, we fit bivariate logistic regression models adjusted for a priori selected confounders, and present the odds ratio (OR) and 95% confidence intervals (95% CI) of travelers' diarrhea. Out of 570 completed post-travel surveys, 477 (83.7%) answered the TD question, and 110 (23.1%) had TD. Risk factors associated with increased odds of travelers' diarrhea included travel to Southeast Asia (2.08; 1.34-3.23), visiting an urban destination (2.92; 1.32-6.98), taking medications/supplements to prevent TD (2.73; 1.78-4.22), and inadequate hand hygiene behaviors (2.84; 1.04-7.64). Travelers' diarrhea continues to be common in international travelers from the US. Our findings provide insight into traveler's behaviors, and shows the need for additional research into prevention strategies for travelers' diarrhea.

0329

ETIOLOGY OF DIARRHEA AMONG PEOPLE LIVING WITH HUMAN IMMUNODEFICIENCY VIRUS AND HUMAN IMMUNODEFICIENCY VIRUS NEGATIVE PATIENTS IN IBADAN, NIGERIA

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Diarrhea is common in sub-Saharan Africa and is one of the leading causes of infantile deaths globally. Few etiologic studies in West Africa focus on individuals who are not young children or travellers. This study profiles the etiologies of diarrhea among adults living with human immunodeficiency virus (HIV) and HIV-negative patients in Ibadan Nigeria. Following ethical approval, we examined 329 stool samples from 127 patients with diarrhea who are living with HIV and 202 HIV-negative patients with diarrhea for occult blood, parasites and bacterial enteropathogens. Parasites were sought using iodine and normal saline wet mount and modified acid fast methods. Stool samples were cultured on MacConkey, eosin methylene blue and xylose lysine deoxycholate agars after enrichment in selenite F broth for the isolation of pathogenic enterobacteriales. Diarrhoeagenic *Escherichia coli* pathotypes and *Salmonella* were confirmed by polymerase chain reaction. The results were analyzed using Chi square and Fischer's Exact tests. Seven parasites, *Ascaris lumbricoides, Trichuris trichiura, Strongyloides stercoralis, Entamoeba histolytica, Cryptosporidium parvum, Cyclospora cayetanensis* and *Isospora belli* were detected in the stool samples. Enteroaggregative *E. coli* (EAEC) was the most commonly detected bacterial pathotype, recovered from 103 (31.3%) of all the patients and *Salmonella spp.* were detected in 6 (1.8%) of stool specimens. *C. cayetanensis* ($p = 0.0008$) and enterotoxigenic *E. coli* (ETEC) ($p = 0.00005$) were recovered significantly more often in HIV-negative patients with diarrhea but HIV positive status was not associated with any of the other parasites or bacteria sought ($p > 0.05$). Positive HIV status was strongly associated with detection of occult blood in patients' fecal samples ($p = 0.000004$). We conclude that bacteria- and parasite-associated diarrhea is common in Ibadan, with a wide range of pathogens implicated. With the exception of ETEC and *C. cayetanesis*, enteropathogen local epidemiology is not dependent on HIV status. Our data support the need for routine use of laboratory diagnostics in patient care.

0330

GUT MICROBIOME COMPOSITION PRIOR TO CAMPYLOBACTER GASTROENTERITIS AMONG NICARAGUAN INFANTS

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Campylobacter is increasingly recognized as a leading bacterial etiology of acute gastroenteritis (AGE) in young children in low- and middle-income countries. We have recently shown that *Campylobacter* species were detected in 22.4% (95% CI: 11.2-32.1) of AGE episodes in León, Nicaragua. Furthermore, experiencing a prior AGE episode of any cause conferred a threefold increase in the odds for *Campylobacter* AGE, which may modify the composition of the gut microbiome, altering susceptibility to *Campylobacter spp*. Thus, in the present study we assessed the gut microbiome composition among infants who went onto developing their first *Campylobacter* AGE episode as compared to those infants who did not. We conducted a pilot nested case-control analysis using a random subset of 20 Campylobacter gastroenteritis cases and their age-matched controls by comparing microbiome composition in the monthly routine stool specimen collected prior to the onset of AGE symptoms, and the corresponding routine stool specimen for the age-matched controls, from a population-based Nicaraguan birth cohort of 444 children. Characterization of gut microbiota was done by 16S rRNA amplicon sequencing and Quantitative Insights Into Microbial Ecology software. Relative abundance of bacterial taxa between cases and controls were compared using the Wilcoxon-Mann-Whitney test. We identified no significant differences in α-diversity between cases and controls. However, there was a trend toward a higher relative abundance of *Lachnospireacea* and *Veionellaceae,* in the control group when compared to cases, [should be explored in larger samples]. Contrary, in the group of cases, we found higher relative abundance of *Streptococcaceae* and *Enterobactereaceae* as compared to the control group. Furthermore, independent of the *Campylobacter species*, we found decreased bacteria genus (≥0.5-fold differences), *Holdemanella, Lachnospiraceae UCG 004, Pseudocitrobacter* and *Eubacterium*, as compared to the control group. Additional research is needed to improve our understanding if prior AGE episode on gut microbiome might affect susceptibility to Campylobacter AGE.

0331

MOST ANTIBIOTIC EXPOSURE TO ENTERIC PATHOGENS OCCURS DURING SUBCLINICAL INFECTIONS AMONG CHILDREN UNDER TWO YEARS OF AGE IN LOW-RESOURCE SETTINGS

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Because subclinical enteric infections are common among children in low-resource settings, enteric pathogens may be frequently exposed to antibiotics even when they are not causing diarrhea that prompts treatment. We quantified antibiotic exposures to subclinical infections in the MAL-ED birth cohort study. We included 15697 antibiotic courses in 1715 children. We identified subclinical infections exposed to each antibiotic course as all pathogens detected in the most recent stool sample up to 30 days prior to starting the course of antibiotics. Antibiotic courses were attributed to specific pathogens if they occurred during a diarrhea episode in which the episode-specific attributable fraction for that pathogen from mixed-effects models associating pathogen quantity

with diarrhea was >0.5. We estimated the total incidence of antibiotic exposure to each subclinical pathogen and estimated the proportion of that exposure that was attributed to treatment of diarrhea caused by other pathogens. The incidence of antibiotic exposure for subclinical bacterial and parasitic infections was 366.5 and 198.1 antibiotic courses per 100 child years, respectively. *Shigella* and *Campylobacter*, for which antimicrobial resistance is of particular concern, were exposed to 45.5 and 147.2 antibiotic courses per 100 child years, respectively. Almost all antibiotic exposure for *Campylobacter* (98.7%), *Cryptosporidium* (95.7%), ETEC (95.4%), and tEPEC (99.2%), and majority for *Shigella* (75.6%), occurred when they were carried subclinically. Compared to other causes of diarrhea, treatment of *Shigella* and rotavirus were the largest contributors to antibiotic exposure. However, only 3.6% and 2.1% of antibiotic exposure for subclinical bacterial infections was due to *Shigella* and rotavirus, respectively. The vast majority of antibiotic exposure to enteric pathogens occurred during subclinical infections, and treatment of etiology-specific diarrhea was responsible for a minority of this exposure. Interventions preventing subclinical carriage in addition to diarrhea are important to reduce antibiotic selection pressure to these pathogens.

0332

CLOSTRIDIOIDES DIFFICILE CARRIAGE IN THE FIRST TWO YEARS OF LIFE AMONG CHILDREN IN RESOURCE-LIMITED SETTINGS

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Clostridiodes difficile (*C. diff*) infection is an important cause of diarrhea associated with morbidity and mortality globally. In industrialized countries, colonization with *C. diff* is common among young children, but prevalence in low-resource settings is unknown. We included 34623 non-diarrheal monthly surveillance and 6731 diarrheal stools tested for *C. diff* toxin genes (tcdA and tcdB) from 1715 children in the 8-site MAL-ED cohort study. We estimated the prevalence, cumulative incidence, and seasonality of *C. diff* by site. Additionally, we investigated the associations of *C. diff* detection in the stool with risk factors of infection, markers of enteropathy, and growth. The prevalence of *C. diff* detection was lower in diarrheal stools (2.2%) compared to non-diarrheal stools (6.1%). *C. diff* detection was most prevalent in the second half of the first year of life and tapered off during the second year of life. Cumulative incidence of *C. diff* varied widely by site, ranging from 17% (Pakistan) to 76% (Peru) of children having at least one positive stool by 24 months of age. There were no seasonal differences in *C. diff* detection except in Bangladesh (peaked in rainy season, p=0.003) and Pakistan (peaked in dry season, p=0.01). After adjustment for site and age, female gender (RR:1.18, CI:1.02-1.35), cephalosporin use in the past 15 days (RR:1.73, CI:1.39-2.16), and treated water (RR:1.24, CI:1.02-1.50) were associated with *C. diff* positivity. Macrolide use in the past 15 days (RR:0.66, CI:0.51, 0.84) and diarrhea in the past 15 days (RR:0.71, CI:0.60, 0.83) were protective against *C. diff* detection. Across sites, *C. diff* carriage was significantly associated with elevated fecal myeloperoxidase, neopterin, and α-1-antitrypsin, but there were no associations between *C. diff* and child weight or length attainment at 24 months of age. *C. diff* colonization among young children in low-resource settings was highly variable by site. Although not associated with diarrhea and growth shortfalls, detection of *C. diff* in stools was associated with significant elevation of intestinal inflammation markers suggesting subclinical impact of carriage.

0333

REAL-TIME SURVEILLANCE FOR PEDIATRIC ENTERIC PATHOGENS OF PUBLIC HEALTH CONCERN IN BELIZE

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Globally, gastrointestinal (GI) illness is the fourth leading cause of death in children under 5, particularly in low to middle income countries (LMIC). The aim of this study was to determine the prevalence and causes of enteric infections in children ≤ 18 years old presenting with two or more GI symptoms in Belize. We conducted a prospective study of children aged >60 days to 18 years who presented with two or more GI symptoms to one of 11 participating healthcare facilities throughout all six Belize districts from January 16, 2020 to March 25, 2021. Stool specimens were tested for 22 pathogens using a film array panel. During this timeframe, 442 children were enrolled; 146 (33%) had two or more GI symptoms. Of 146 participants, 83 had stool specimens tested; 66 (79.5%) were positive for at least one pathogen, with 16 different pathogens identified. Positivity was higher among children ≤ 5 years (46/55; 83.6%) compared to those 6 to 18 years (20/28; 71.4%). The most common pathogens identified were enteropathogenic *E. coli* (EPEC; n=31), followed by enteroinvasive *E. coli* (EIEC; n=21), enteroaggregative *E. coli* (EAEC; n=21), norovirus (n=13), enterotoxigenic *E. coli* (ETEC; n=11), *Campylobacter* (n=11), *Giardia lamblia* (n=7), *Salmonella* (n=5), STEC (n=5), adenovirus F40/41 (n=4), sapovirus (n=3), *Plesiomonas shigelloides* (n=2), *E. Coli* 0157:H7 (n=2), non-cholera *Vibrio* (n=2), *Clostridium difficile* (n=2), rotavirus (n=1), and astrovirus (n=1). Co-infections were common, with 143 positive results among the 66 positive panels (average of 2.2 pathogens/positive panel). In conclusion, more than 70% of children presenting with GI illness had a positive pathogen identified, with a higher percentage of positive results in those 5 years of age and younger. This pediatric population had a high rate of coinfections, with EPEC, EIEC, and EAEC most common. As EPEC and EAEC are frequently found in asymptomatic children, the significance of these pathogens in symptomatic children should be attributed more to the co-pathogen detected, with treatment focused on the other pathogen.

0334

ESTIMATING THE ECONOMIC BURDEN OF TYPHOID IN CHILDREN AND ADULTS IN BLANTYRE, MALAWI: A COSTING COHORT STUDY

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Typhoid fever continues to cause high morbidity and mortality in low and middle-income countries. WHO recommends using typhoid vaccine to control endemic disease in countries with high typhoid fever burden. Information on disease burden, cost of illness, delivery costs, and cost-effectiveness is relevant for policymakers' informed decision-making on national vaccine introduction. This study aimed to estimate the cost of typhoid fever illness among children and adults in Blantyre district, Malawi. A prospective facility-based cohort study was undertaken. The cost of illness was the sum of direct and indirect costs borne by typhoid patients and their families plus the direct cost of managing a confirmed case of typhoid fever at the three health facilities. One hundred and nine patients with confirmed typhoid fever were recruited. Forty-four (40%) participants were admitted overnight to the hospital, with a mean length of hospitalization of 8.4 (SD 6.7) days. Sixty-three of the one hundred and nine participants (58%) were less than 15 yrs old. The mean household and health facility cost, catastrophic cost and the total mean cost of

typhoid fever will be calculated. These results contribute to the further economic analysis of typhoid fever vaccination introduction in Malawi and other sub-Saharan African countries.

0335

NASOPHARYNGEAL COLONIZATION, ASSOCIATED FACTORS, AND ANTIMICROBIAL RESISTANCE OF STREPTOCOCCUS PNEUMONIAE AMONG CHILDREN UNDER FIVE YEARS OF AGE IN THE SOUTHWESTERN COLOMBIA

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This work aims to evaluate the factors associated with *Streptococcus pneumoniae* nasopharyngeal colonization and antimicrobial susceptibility among pediatric outpatients in the southwestern-Colombia, 2019. A cross-sectional study was carried out using survey-based interviews and the collection of nasopharyngeal-swab specimens for microbiological characterization and antimicrobial susceptibility testing. Logistic regression analyses were performed for factors associated with nasopharyngeal carriage. A total of 452 children under-5 years of age were examined and 41.8% carried *S. pneumoniae*. Higher pneumococcal carriage frequencies were observed among participants <2-years and in individuals belonging to indigenous communities, which were lacking established PCV-10 immunization schemes. Additionally, children attending child-care institutions were also highly colonized by pneumococci. *S. pneumoniae* showed 57.7% non-susceptibility to benzylpenicillin (meningitiscut); 45.5% intermediate-sensitivity to benzylpenicillin (oralcut) and 21.7% to cefotaxime; and resistance to erythromycin (40.7%), tetracycline (36.0%), trimethoprim/sulfamethoxazole (24.9%), clindamycin (24.3%) and ceftriaxone (27.0%). The 41.8% of participants carrying *S. pneumoniae* show a scenario with the presence of multi-drug and extensively-drug resistant strains, which constitutes important reservoirs of bacterial transmission by children <5-years in Colombia, leading to an onset of pneumococcal diseases. Hence, there is an urgent need to expand conjugate pneumococcal immunization in the community and ensure compliance with established immunization schedules.

0336

POINT OF CARE ULTRASOUND IN MANAGEMENT OF LASSA FEVER AT IRRUA SPECIALIST TEACHING HOSPITAL, NIGERIA - A PILOT STUDY

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Point-of-care ultrasound (POCUS) is a standard of care in emergency medicine and has also proven to be useful in diagnostics and management of infectious diseases. In the management of patients with high-impact pathogens, where access to other medical imaging is limited, POCUS may be a useful tool in patient management. In this pilot-study we evaluated potential pathologies identifiable through POCUS in patients with Lassa fever. This observational study was conducted at the Irrua Specialist Teaching Hospital, Nigeria. We applied a modified eFAST protocol to identify typical pathologies of Lassa fever. Patients were evaluated using a DP50 machine (Mindray) with linear and curved array probes after admission to the isolation ward. Pathologies were recorded systematically and videos stored for assessment by an independent reviewer. The prevalence of specific pathologies was determined, and POCUS findings were correlated with laboratory parameters and clinical outcome. In total, 46 patients were evaluated using the modified POCUS assessment. Signs of serositis were found in 37 patients (80%) with pericardial effusion being the most common finding (67%), most of these were however minimal. Effusions at more than one site, reflecting the presence of polyserositis, were seen in eight patients (17%). Hyperechoic kidneys were observed in 5 patients (11%). All patients with hyperechoic kidneys had a creatinine >2g/dl and among all patients with a creatinine > 2g/dl only one individual did not show signs of renal pathology on POCUS examination. Of the five patients with renal pathology on POCUS two died (40%) compared to 12% of those without renal pathology. Pathological findings on POCUS examination were common in patients with Lassa fever evaluated in this pilot study. Assessment of specific pathologies such as hyperechoic kidneys by POCUS at admission may have an immediate impact on patient management. Further studies including larger sample sizes are needed to correlate findings with clinical parameters, clinical outcomes to better appreciate the value of POCUS for the clinical management of Lassa fever patients.

0337

ENTEROBACTERIACEAE PRODUCING EXTENDED-SPECTRUM Β-LACTAMASES IN A SPECIALIZED CLINIC IN THE CITY OF MONTERÍA- CÓRDOBA-COLOMBIA

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Extended spectrum β-lactamases (ESBLs) are bacterial enzymes that are phenotypically characterized by conferring resistance to penicillins and cephalosporins, including those of the third and fourth generation. ESBLs-producing Enterobacteriaceae are of great clinical interest, due to the high probability of therapeutic failure they generate, increasing the periods of hospitalization of patients and the costs of treatment in health care institutions. The objective of this study was to determine the prevalence of ESBLs-producing enterobacteria in a specialized clinic in the city of Montería. A descriptive study was carried out, in which the results of the antibiograms carried out on the Enterobacteriaceae isolated from the patients admitted between the years 2017 and 2018 were analyzed. The variables included in the statistical analysis were: isolated microorganism, origin of the sample, service isolation and susceptibility profile. The data were analyzed using the statistical software SPSS Statistics version 2018. During the study period, a total of 1,292 isolates were recorded, from patients admitted to different services of the clinic, where the ICU was identified as the area with the highest frequency of bacterial isolation, 974 / 1,292 (75.3%) corresponded to species of the Enterobacteriaceae family, and of these 739/974 (75.8%) belonged to *Klebsiella* spp, *Escherichia coli* and *Proteus* spp. The ESBLs-producing Enterobacteriaceae isolates corresponded to 103/739 (13.9%) and non-producing to 636/739 (86.1%). In this study, no carbapenemase-producing strain was found, however, it is recommended to confirm it by molecular biology studies. The most frequently isolated resistant strains correspond to the species of *K. pneumoniae* and *E. coli*. The results obtained in this study are consistent with those recorded globally. The prevalence of ESBLs-producing Enterobacteriaceae is a growing problem that demands more studies that contribute to the optimization of empirical therapy and the continuous monitoring of this phenomenon.

0338

PREVALENCE OF RESISTANT METICILIN STAPHYLOCOCUS AUREUS (MRSA) PRESENT IN POLI-TRAUMA PATIENTS AT A SPECIALIZED CLINIC IN MONTERÍA- CÓRDOBA- COLOMBIA

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is increasingly responsible for infections in hospitalized patients, affecting a large percentage of poly-trauma patients. A descriptive study of the type retrospective was carried out in a specialized clinic in the city of Montería during the years 2017, 2018 and 2019, where the databases of the area corresponding to Microbiology were analyzed, specifically the patients admitted positive for the isolation of *Staphylococcus aureus*. The determination of the susceptibility profile included the Oxacillin resistance test with the Kirby-Bauer methods (agar diffusion method) and Micro Scam with the Cefoxitin MIC incorporated. The study population

consisted of 1,674 polytraumatized patients admitted to the different medical services of this clinic between 2017 and 2019. Of the 16,541 patients admitted to the clinic in the study years, 10% (1,674) were polytraumatized patients of which 81.6% belonged to the male sex, possibly due to their type of occupation; The service with the highest number of hospitalized patients with multiple trauma was the ICU with a total of 680 patients, which represents 40.6% of the 1,674 hospitalized patients. During the study period 46/178 (25.8%) samples were reported as positive for MRSA. The sample where this bacterium was most frequently isolated was wound secretion with a total of 24/46 positive cultures, followed by bone sample with 9/46, bronchial secretion with 5/46 and blood culture 2/46. During the three years of the study, patients with infectious processes due to MRSA and whose anatomical location corresponded to skin, soft tissues and bone, were treated with antibiotic management guided by Antibiogram, most of them were treated with Trimethoprim Sulfa 160/800 each. 12 hours, taking into account that 98% of the isolated strains were sensitive to this antimicrobial. Patients with bacteremia were managed with Vancomycin at a dose of 1 gram every 12 hours, and in patients with pneumonic processes, Linezolid 600mg was given every 12 hours x 14 days.

0339

ANTIBIOTICS IN THE MANAGEMENT OF UNCOMPLICATED SEVERE ACUTE MALNUTRITION IN BURKINA FASO: A RANDOMIZED TRIAL COMPARING AZITHROMYCIN TO AMOXICILLIN

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Given the challenge of diagnosing infection in malnourished children, many national nutritional guidelines recommend provision of a broad-spectrum antibiotic like amoxicillin in the management of uncomplicated severe acute malnutrition (SAM). However, evidence on the role of amoxicillin for uncomplicated SAM is mixed. Azithromycin is a potential alternative to amoxicillin in the management of SAM as it can be administered as a single dose, has a long half-life, and has efficacy against pathogens responsible for common childhood infections. In this pilot randomized trial, we aimed to compare the efficacy of azithromycin to amoxicillin on weight gain in children with uncomplicated SAM in Burkina Faso. Between June and October 2020, 301 children were enrolled and randomized to receive a single dose of oral azithromycin or a 7-day course of oral amoxicillin (standard of care). Both groups received ready-to-use therapeutic food and the standard package of care for uncomplicated SAM in Burkina Faso. Children were followed weekly until nutritional recovery and again at 8 weeks for a final study visit. The primary outcome of weight gain velocity (g/kg/day) was compared by arm over the study period. Overall, 282 children (93.6%) completed the 8-week visit. Average weight gain velocity (g/kg/day) was 2.5 (SD 2.0) in the azithromycin group and 2.6 (SD) 1.7) in the amoxicillin group (Mean Difference -0.1, 95% CI -0.5 to 0.3, $P = 0.63$). Fewer adverse events were reported in the azithromycin group (Risk Ratio 0.50, 95% CI 0.31 to 0.82, $P = 0.006$). With the ease of dosing and the potential for fewer adverse events, azithromycin may be a viable alternative to amoxicillin in the management of uncomplicated SAM.

0340

DIFFERENCES IN THE EXTENT OF BREASTFEEDING BETWEEN HAITIAN CREOLE- AND SPANISH-SPEAKING HOUSEHOLDS IN THE DOMINICAN REPUBLIC

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Exclusive breastfeeding (EBF) rates in the Dominican Republic (DR) are lower than many other low- and middle-income countries and dropping. To further examine this problematic pattern, this study examined whether different groups in the DR have different breastfeeding rates. Specifically, it examined whether Haitian-Creole speaking households in the DR have a higher breastfeeding rate than Spanish speaking households. This is hypothesized based on Haitians in Haiti having much higher breastfeeding rates than the DR overall. However, as these two populations may vary on other potentially relevant factors for breastfeeding, this study considered potential confounders. Data from the 2014 DR Multiple Indicator Cluster Survey (MICS) was used for this study. MICS data contains key maternal and child health variables on a representative national sample. Subsamples of infants under six months of age at the time of the survey with complete data included 163 and 1487 Creole- and Spanish-speaking households, respectively. More Creole than Spanish speaking children were EBF, 11.0 vs. 4.1%. A greater percentage of Creole households were in the lowest wealth quintile and mothers in Creole-speaking households had lower education levels than the Spanish-speaking sample. In the final multivariate model, household language was no longer related to the extent of breastfeeding, but the wealth index was such that the group belonging to the poorest quintile retained a significant independent relationship with more breastfeeding. That living in Creole-speaking households was not independently related to breastfeeding may suggest a high degree of acculturation for this sample, which may be reflected in the relatively low rate of EBF compared to Haitians in Haiti. Future studies should examine variables such as duration of time of Haitian immigrants in the DR, in a larger sample size, to further examine possible acculturation mechanisms contributing to lower EBF in the DR.

0341

DIFFERENCES IN EARLY INITIATION OF BREASTFEEDING BETWEEN CUBA AND THE DOMINICAN REPUBLIC

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Early initiation of breastfeeding (EIBF) may decrease mortality and improve health of infants and young children. EIBF rates vary significantly between countries, however, contributors to these variations are poorly known. Examining and comparing practices in different countries, while considering potential confounding factors, may help identify contributors to EIBF differences. This study aimed to determine whether there are differences in EIBF rates between Cuba and the Dominican Republic (DR), and whether an association persists between country and EIBF rates when controlling for potential confounders. Cuba and the DR are both countries on Caribbean islands with similar populations but markedly different health care systems. The study used data from the Multiple Indicator Cluster Surveys from 2014 that collected data on nationally representative samples from both countries. For analysis, EIBF was defined as ≤1 hour post-delivery. A series of chi-square tests, Breslow-Day statistics, and logistic regression models were used for analysis. Cuba had a significantly higher rate of EIBF than the DR, 63.0% vs. 46.9%, respectively. The association between country and EIBF persisted even after controlling for cesarean section delivery (higher in the DR), place of residence, and maternal education (higher in Cuba). Although bivariate analysis identified that cesarean sections may have a less adverse impact on EIBF in Cuba compared to the DR, an interaction term examining this in the final multivariate model was not significant. Additional inquiry is required to understand what factors might explain higher EIBF rates in Cuba beyond those variables considered in this study. This may include examining health education and promotion of EIBF, determining the extent of support

for breastfeeding especially in the immediate postpartum period, and exploring maternal attitudes about breastfeeding in Cuba relative to other countries.

0342

FREQUENCY OF TYPHOID CARRIER IN PATIENTS UNDERGOING CHOLECYSTECTOMY FOR GALL BLADDER DISEASES USING REAL TIME PCR

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Annually millions of new typhoid cases are reported globally despite advancement in treatment and preventive measures. One of the major cause of typhoid endemicity is gallbladder carriage of typhoid etiological agent, *Salmonella enterica subsp. enterica, serovar Typhi (S. Typhi)* and *Salmonella Paratyphi (S. Paratyphi)*. This study is aimed to determine the frequency of typhoid carriers in patients who underwent cholecystectomy for gall bladder diseases. A crossectional study conducted at Aga Khan University Hospital and Jinnah Postgraduate Medical Center, Karachi from December 2018-February 2020. All individuals of age ≥ 10 years were included. Multiplex real time polymerase chain reaction (PCR) was performed on gall bladder specimens including gallstones, bile and gallbladder tissue and sera of same patients were screened against YncE IgG via ELISA. Out of 989 enrolled participants, 34 (3.4%) were carriers of either *S. Typhi* (2.3%) or *S. Paratyphi* (1.1%). It was found that most of the carriers harboring organism in their gallstones 24/34 (70.6%) while 20/34 (58.8%) in tissues and 11 (32.4%) in bile. ELISA was performed on sera of 34 PCR positive and 34 age and gender matched PCR negative samples (controls) to measure anti-*S. typhi Vi* IgG. Our results showed no association of Vi and YncE with PCR positive carriers. The mean age of participants was 40 (± 14.3 SD) years. The reason of cholecystectomy in 949/989 (96%) of them was gallstones. Among typhoid carriers, majority 25/34(73.5%) of them were females. History of typhoid fever was not significantly associated with typhoid carrier. We found higher rates of salmonella carriage in gallstones compared to tissue and bile. To control the typhoid fever cases in the region, we should try to interrupt the transmission cycle by typhoid carriers by developing a cost effective, specific and non-invasive diagnostic tools to improve typhoid carrier identification.

0343

DEPRESSION AND QUALITY OF LIFE AMONGST FILARIAL LYMPHOEDEMA PATIENTS: DETERMINING DEMOGRAPHIC, SOCIO-ECONOMIC AND PHYSICAL RISK FACTORS, AND THE IMPACT OF ENHANCED SELF-CARE INTERVENTION

Carrie Barrett

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Lymphatic filariasis (LF) is a major cause of disfiguring and disabling lymphoedema. This study aims to: i) determine the prevalence of and factors associated with depression and low quality of life (QOL) in lymphoedema patients; ii) understand if implementation of enhanced self-care (ESC) affects depression and QOL. A prospective cohort of ~150 patients in two regions of Malawi (North/South) was conducted over six months. Lymphoedema patients were surveyed before being trained in ESC (at baseline), involving hygiene, deep-breathing, massage and leg exercises. Follow-up surveys were at 3 and 6 months. Depression was assessed using Patient Health Questionnaire (PHQ-9) and QOL was assessed using an adapted LF Specific QOL Questionnaire (LFSQQ). Questionnaires used a normalized Likert scale scoring system. Beta regression was used to identify sociodemographic factors and lymphoedema severity associated with depression and QOL in individuals. Data were stratified by the three survey time periods. Baseline observations from 309 lymphoedema patients indicate that 23% (95%CI, 18%-28%) display mild/moderate depression (Likert score >5). The LFSQQ identified 31% (95%CI, 26%-37%) of patients with a moderately/severely low QOL score (Likert score <70). Factors significantly associated with higher depression and lower QOL scores included patients requiring a caregiver ($p<0.001$) and higher number of secondary bacterial infections (i.e. acute attacks) in last 6 months ($p<0.001$). In addition, patients requiring family support ($p= 0.004$); higher number of sick days off work ($p<0.001$) were also significantly associated with lower QOL scores. Preliminary PHQ-9 results from the North region indicate depression scores significantly reduced from baseline mean score of 2.5 to 0.7 in 3-month survey following ESC (t-test $p<0.001$). Surveys are due to be completed in August 2021. Filarial lymphoedema is associated with a high prevalence of depression and lower QOL. ESC is a promising intervention in reducing disfigurement and disability caused by lymphoedema and may play a role reducing depression and improving quality of life for those affected.

0343

DEPRESSION AND QUALITY OF LIFE AMONGST FILARIAL LYMPHOEDEMA PATIENTS: DETERMINING DEMOGRAPHIC, SOCIO-ECONOMIC AND PHYSICAL RISK FACTORS, AND THE IMPACT OF ENHANCED SELF-CARE INTERVENTION

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0344

TOWARDS THE DEVELOPMENT OF A CLINICAL MANAGEMENT DECISION TREE FOR DIAGNOSIS OF PATIENTS WITH ACUTE FEBRILE ILLNESS IN BANGLADESH: A PRELIMINARY ANALYSIS

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Acute febrile illness (AFI) is a common reason for seeking medical care among patients in Bangladesh and may be caused by dengue, typhoid,

and other bacterial and viral diseases. Since AFI patients present with similar symptoms, clinicians may need additional guidance to identify the cause of AFI to guide care. The aim of this study was to develop a preliminary decision tree for common AFI etiologies that could inform the future development of decision trees for AFI patients. We conducted surveillance for patients presenting to government hospital outpatient care with AFI in Bangladesh between May 2019 and March 2020. Using a 5-fold cross-validated chi-squared automatic interaction detection (CHAID) algorithm, we developed a preliminary decision tree with data on symptoms and vital signs of AFI patients with common causes. These patients were identified using blood cultures, polymerase chain reaction assays, and rapid diagnostic tests. Co-infected patients and patients under 5 years were excluded. The most common etiologies identified among the 629 patients enrolled with AFI were dengue (24, 19.4%), *Escherichia coli* urinary tract infections (UTIs) (37, 29.8%), and typhoid (63, 50.8%). Among the 124 patients with one of these three etiologies,65.3% (81) were male; 54.0% (67) were between 5 to 19 years and 12.1% (15) were above 35 years. The most common symptoms were headache (73.4%), lack of appetite (62.1%) and fatigue (54.8%). The CHAID algorithm used sore throat, elevated blood pressure, bone pain, and fatigue to distinguish dengue, *E. coli* UTI, and typhoid. Sore throat was the most significant factor in distinguishing dengue patients from *E. coli* UTI and typhoid patients. The CHAID model had an accuracy rate of 62.5% and a kappa statistic of 0.4. The sensitivity of the model was 64.7% for dengue patients, 11.5% for *E. coli* UTI patients, and 91.1% for typhoid patients. This analysis is limited by sample size and few prevalent etiologies. These results could guide future development of clinical management decision trees and should be validated on a larger dataset with additional clinical and demographic data.

0345

IS EDUCATION ENOUGH? AN INTERVENTION TO IMPROVE SCREENING FOR CHAGAS DISEASE AT AN ACADEMIC SAFETY NET HOSPITAL IN THE US

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Chagas disease, caused by the parasite *Trypanosoma cruzi,* causes significant morbidity and mortality related to late-stage cardiac and other sequelae. In the United States, Chagas disease remains vastly underdiagnosed though it is prevalent among individuals originating from continental Latin America. We aimed to increase knowledge of and testing for Chagas disease at a large academic safety-net hospital in Massachusetts. An interactive, one-hour educational program was developed and disseminated from April to July 2019 in five multi-disciplinary settings (departmental Grand Rounds, clinic conferences, etc.) that addressed the need for Chagas disease screening. We tracked the number of *T. cruzi* assays ordered from March 2016 – December 2019. A total of 299 tests were ordered overall, with transplant clinicians ordering the most (n = 71, 23.7%). In the five months pre-intervention, 86 tests were ordered (46 in transplant clinic and 40 in other settings including cardiology clinic and inpatient medicine services); among 45 patients with a recorded country of origin, 16 (35.6%) were from endemic regions. In the five months post-intervention, 53 tests were ordered (1 in transplant clinic and 52 in non-transplant settings); among 24 patients with a recorded country of origin, 16 (66.7%) were from endemic regions. Overall, Chagas disease screening in non-transplant settings increased slightly post-intervention; analyses are underway to see if this change was sustained. Testing of patients from non-endemic regions was common but decreased following the intervention. The high pre-intervention rates amongst transplant clinicians might reflect the temporary hiring of a transplant ID physician (March-September 2019), suggesting that in-person reminders from a peer are effective in influencing medical practice. In summary, education is one component of a strategy to address Chagas disease but must be integrated with other interventions to realize substantive increases in screening and to ensure testing of individuals from endemic areas.

0346

AN UNUSUALLY LATE CLINICAL PRESENTATION

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A 71-year-old Chinese immigrant presented with 4-months of weight loss, anorexia, constipation and abdominal pain. He reported no travel in 5 years. His past medical history included biopsy proven IgG4 disease of the pancreas and kidneys and 3 episodes of gram-negative bacteremia in the last 4 years initially attributed to gallstone cholangitis and later suspected pancreatic cancer. He had received multiple courses of immunosuppression for recurrent flares of IgG4-disease including rituximab, MMF and repeat courses of high-dose steroids. This admission, the patient had *E. coli* bacteremia. CT imaging showed new extensive bilateral parenchymal lung abnormalities with dilated stomach and small bowel loops. IV ertapenem was administered, with clearance of bacteremia. 7 days later, he developed *E. faecium* bacteremia treated with IV vancomycin. *Strongyloides* hyperinfection syndrome (SHS) was considered but deemed less likely given prior history of steroid use. *Strongyloides* serology was negative and stool microscopy was not sent due to constipation. The patient died from overwhelming sepsis from suspected aspiration pneumonia. Autopsy revealed *Strongyloides* hyperinfection as the cause of death, with multiple round worms, 350-600x15µm, found in stomach and pancreatic tissue. Immunosuppression can trigger SHS, leading to life threatening consequences and mortality rates up to 85-100%. Our patient received various immunosuppressants for treatment of IgG4 disease over a 4-year period. It was unusual that SHS did not occur earlier and only resulted in death at the most recent hospitalization. We presumed this was a result of a remote infection given the lack of occupation risk and recent travel. SHS is described with corticosteroid usage irrespective of dose, route of administration, or duration, with cases reported as early as 6-17 days. There have been only two cases reported in kidney transplant patients presenting with delayed SHS 2-6 years after receiving immunosuppression. In rare cases, SHS can be delayed when using immunosuppression. This increases the risk of a delayed or unrecognized diagnosis leading to fatal consequences.

0347

ENVIRONMENTAL ENTERIC DYSFUNCTION: HISTOPATHOLOGICAL & IMMUNOHISTOCHEMICAL ANALYSIS OF DUODENAL BIOPSIES FROM PAKISTANI MALNOURISHED CHILDREN

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Environmental Enteric dysfunction (EED) is a causative factor for malnutrition and further aggravates it. To gain insight in to its pathophysiology, this study examines expression of selected proteins and their association with duodenal histology. For histopathological workup, 63 biopsies from undernourished children refractory to nutritional intervention were processed for H & E staining. Slides were examined by pathologists using EED histology scoring system. For expression of protein markers, immunohistochemistry (IHC) was performed using tissue microarray. The core biopsies were arrayed on recipient paraffin block and

stained on robotic Ventana Discovery Ultra instrument with antibodies for epithelial cell subtyping, lymphocyte subtyping and to evaluate protein expression of various inflammatory markers. Paneth cell (PC) count on microscopy was positively associated with PC marker defensin alpha5 (DEFA5) [r= 0.467, p<0.001] and brush border marker sucrase-isomaltase (SI) [r= 0.336, p=0.009] on IHC. Similar trend was seen for aggregated histology scores with significant negative association with PC (r= -0.361) and SI (r= -0.374) while positive correlation with Reg1B expression (r= 0.300). Duodenal slides with histology confirmed *H. pylori* reported positive correlation with dual oxidase2 (r= 0.355, p=0.007) and lipocalin (r= 0.280, p= 0.035) but not with Granzyme B (r=0.176, p=0.186). No association was seen between villous blunting and SI (brush border marker), goblet cell reduction and MUC2, intraepithelial lymphocytosis and CD45, CD3 or CD19. Based on histopathology scoring, protein expressions were compared between low (<6) and high scorer (>6). A heightened expression of Reg1B (p=0.034) and CXCL10 (p=0.029) was observed in cases with higher scores while PC marker (p=0.010) and brush border marker (p=0.032) were raised in lower scorers. Immunohistochemistry supports the findings of histopathology suggesting key role of Paneth cells in gut homeostasis. Overexpression of inflammatory markers in association with a decline in Paneth cell and brush border markers appear to be involved in pathophysiology of EED.

0348

AZITHROMYCIN FOR CHILD SURVIVAL IN NIGER: IMPLEMENTATION AND RESEARCH: AN ADAPTIVE CLUSTER-RANDOMIZED TRIAL TO DETERMINE THE OPTIMAL AGE GROUP FOR IMPLEMENTATION OF BIANNUAL ORAL AZITHROMYCIN DISTRIBUTION TO REDUCE CHILD MORTALITY IN NIGER

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In 2020, the World Health Organization (WHO) released conditional guidelines on azithromycin distribution to children aged 1–11 months in high mortality settings to promote child survival. However, distribution of this intervention to children aged 1–11 months alone remains untested. The MORDOR trial found a 14% reduction in mortality comparing communities in which children aged 1–59 months received biannual azithromycin distribution or placebo over 2 years in Malawi, Niger, and Tanzania. Although crude data from subgroup analyses showed a mortality reduction of approximately 23% in children aged 1–11 months, the trial was not powered to detect effect heterogeneity by age group and there may be indirect effects from older to younger children. AVENIR is a cluster-randomized trial designed to compare the effect of targeted azithromycin distribution to children aged 1–11 months vs children aged 1–59 months on mortality and antimicrobial resistance (AMR) in Niger. In the first stage of the study, 3,350 rural and peri-urban communities in Niger's Dosso and Tahoua regions will be enrolled. Dosso communities will be enrolled in the first year and randomized in a 1:1:1 fashion to one of three arms: azithromycin to 1–11-month-olds (placebo to 12–59-month-olds), azithromycin to 1–59-month-olds, or placebo to 1–59-month-olds. Tahoua communities will be enrolled in the second year, with the randomization allocation updated based on the probability of mortality in each arm. Response-adaptive allocation allows for a more ethical distribution, as communities will have a higher probability of receiving the intervention that reduces mortality the most. A biannual door-to-door census will occur over 2.5 years to enumerate the study population, administer treatment, and monitor mortality. AMR will be monitored in a random subset of 150 communities in Dosso at baseline and after 2 years of distributions. Census and treatment distribution began in November 2020, and the primary outcome analysis will occur in Fall 2023.

0349

ACUTE FEBRILE ILLNESS IN EGYPT: ENHANCED SURVEILLANCE FOR ETIOLOGIES CAUSING THE SYNDROME

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Acute febrile illness (AFI) is a non-specific syndrome caused by many pathogens distributed differently according to the geographical area. This study aimed to describe the epidemiology and common etiologies of AFI cases presenting to Mansoura University Hospital, Nile Delta, Egypt. Blood samples were subjected to standard blood culture testing, as well as qPCR for the detection of *Salmonella spp*., *Brucella spp*., *Dengue* virus, *Chikungunya* virus, *West Nile* virus, *Plasmodium spp., Rickettsia spp., Leptospira spp., Yellow Fever* virus, *S. pneumoniae, Coxiella burnetii* and *Rift Valley Fever* virus. From June 2019 to March 2021, 287 subjects were enrolled, and their data analyzed. Demographics showed that 49% were male and the median age of patients was 44 years. Median illness duration was 4 days. Other than fever, abdominal tenderness (21%) and sore throat (14%) were the most commonly associated signs and symptoms. Only a single pathogen was detected in 38 (13.2%) patients and two (0.7%) cases showed concomitant infections. *Rift valley* virus, *Salmonella, E. coli* and *S. aureus* were identified in 9 (3.1%), 7 (2.4%), 4 (1.4%) and 4 (1.4%) cases, respectively. This study reports the detection of *Leptospira spp* in Egypt using qPCR, in contrast to previous studies reporting *Leptospira* antibodies only. Antibiograms of *E. coli* showed that all isolates were resistant to 3rd and 4th generation cephalosporins and were Extended Spectrum Beta-Lactamase (ESBL) producers, but sensitive to gentamicin and fluoroquinolones. As well, 75% of *S. aureus* isolates were Methicillin-resistant *Staphylococcus aureus* (MRSA). In 2019 the detection rate of AFI pathogens showed a significant decrease that started in November compare to June to October 2019 and in 2020 there was a peak of pathogen detection rate in August. In conclusion, our data provided information regarding the epidemiology of AFI in Egypt, however, the testing panel will be broadened to minimize the percentage of the negative specimens.

0350

NON-INVASIVE DNA AMPLIFICATION AND SEX DETERMINATION OF CULTURED BRUGIA MALAYI LARVAE

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The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) based technology is currently being used to develop a genetic toolkit and methods for the study of transcriptional and post-transcriptional regulation in all lifecycle stages and tissues of *Brugia malayi*. To genotype transgenic parasites, it is necessary to detect the genomic DNA of *B. malayi* in a non-invasive way. *B. malayi* third stage infective L3 were cultured with 1x105 Bovine Embryo Skeletal Muscle (BESM) cells/well and Minimal Essential Media (MEM) containing 20% fetal bovine serum (FBS). Following the collection of the molting media for day 8, DNA was extracted using the DNeasy® Blood & Tissue Kit and the Dynabeads™ M-280 Streptavidin method. A single distinct band of approximately 210-220 bp was obtained from molting media samples that contained one singe L3 larvae using the toY-nest and shp2-nest nested primers. A single distinct band of approximately 420 bp was obtained from the same media samples using the rps12-nest nested primers. This study provides important tools to genotype and to optimize the sex ratio for the backcrossing of transgenic parasites.

0351

DIAGNOSTIC CHALLENGES IN THE LYMPHATIC FILARIASIS POST-ELIMINATION ERA IN SRI LANKA: A CASE OF CONJUNCTIVITIS CAUSED BY BRUGIA SP. FEMALE ADULT

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Sri Lanka eliminated lymphatic filariasis in 2016 as a public health issue, yet sporadic cases of bancroftian and brugian filariasis (anthroponotic and zoonotic) are being reported. The majority of worms collected from eyes were *Dirofilaria repens* in Sri Lanka; however, rarely, *Brugia ceylonensis* and *Wuchereria bancrofti* were also reported. Therefore, a detailed discussion on a diagnosis of a rare case is presented here. A 35-year-old female patient with a history of allergies presented with irritation and redness of the right eye for two weeks. She was treated for allergic conjunctivitis for a week due to chemosis and conjunctival injection near the inferior limbus. However, symptoms remained, and a live subconjunctival worm with rapid wriggling movements was visualized in the follow-up visit after a week. The worm was near the lateral limbus with significant chemosis. Suspecting dirofilariasis, urgent extraction was performed via a conjunctival incision under topical anaesthesia. The worm was white, thread-like, 32 mm long and 95 µm wide. A distinct head bulb, two uterine tubes with unfertilized ova uniting to form a single vagina which looped just posterior to the vulva was noted. The vulva was 608 µm from the anterior end and was in relation to the posterior half of the oesophagus, which was 1026 µm long. Ovaries were placed 752 µm from the rounded posterior end. It was identified as a young but mature, unfertilized female filarial worm. Considering the epidemiology, dimensions of the worm and the position of the vulva in relation to the oesophagus, it is likely to be an adult *Brugia* sp. Differentiation of females of the genera *Wuchereria* and *Brugia* is difficult based on light microscopic morphology alone. Species confirmation is being done by PCR. No microfilaraemia was detected by night-thick-blood smear or membrane filtration. She was negative for the circulatory filarial antigen of *Wuchereria bancrofti* and eosinophilia. The diagnostic challenges will be discussed in detail once the molecular diagnosis is available.

0352

OPPORTUNISTIC MAPPING AND SURVEILLANCE FOR ONCHOCERCIASIS ELIMINATION PROGRAMS IN NIGERIA USING A MULTIPLEX BEAD ASSAY

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Over the past 31 years, the Nigeria Federal Ministry of Health in collaboration with other governmental and non-governmental organizations, has reduced the prevalence of onchocerciasis through annual mass administration of medicines (MAM) with ivermectin. Despite great progress, much of the country remains under MAM and many states lack recent data on the status of transmission of onchocerciasis. Site specific surveillance can be used to determine prevalence but is expensive and labor intensive. Resources could be used more efficiently if multiple diseases could be evaluated simultaneously. In 2018, Nigeria conducted a cross-sectional, nationally-representative, household-based survey to assess the distribution of HIV in the country. Along with survey data, blood samples, Global Positioning System (GPS) information, and consent for future analysis for other diseases of public health importance were collected. A multiplex bead assay (MBA) was used to measure IgG to multiple pathogens in stored blood samples. Antibody reactivity to OV-16 was used as a marker of onchocerciasis exposure in 31,459 children aged 0-14 years. The prevalence of OV-16 seropositivity was highest in Kogi State at 7.7% (95% confidence interval (CI) 4.6% - 11.8%), followed by Ondo at 6.6% (95% CI 3.8% - 10.8%), Ebonyi at 5.9% (95% CI 3.2% - 10.0%), and Cross River at 5.3% (95% CI 3.4% - 8.0%). Prevalence was lower in the 5 states where MAM had been stopped (0.9-2.8%). Clusters of seropositivity were identified at the sub-local government area level using GPS coordinates. MBA testing of existing samples allowed the Nigeria onchocerciasis control program to gather critical data on onchocerciasis transmission across the nation, including in areas where no recent surveillance had occurred. Linking these data to breeding sites may allow for identification of potential hotspots of transmission in both treated and untreated areas. The addition of geospatial analyses enhanced data interpretation in a program-relevant way. Opportunistic use of blood samples collected for other purposes may be an efficient way to gather critical surveillance data for NTD programs.

0353

POST-TREATMENT SURVEILLANCE FOR LYMPHATIC FILARIASIS IN HAITI: RESULTS FROM TRANSMISSION ASSESSMENT SURVEY IN SAUT D'EAU AND LA TORTUE

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Haiti is one of four countries in the Americas endemic for lymphatic filariasis (LF), a chronic neglected tropical disease commonly known as elephantiasis and transmitted by mosquitoes. In 1997, the World Health Organization (WHO), called for elimination of LF as public health problem. Since 2000, Haiti implemented annual mass drug administration (MDA) with albendazole and diethylcarbamazine in all 140 districts (*communes*) of the country. Currently, 122 (87%) communes do not require MDA, including two communes, Saut d'Eau (Centre Department) and La Tortue (Nord-Ouest Department), which stopped MDA in 2014 and 2004, respectively. After stopping MDA, WHO recommends a minimum of four years of post-treatment surveillance, including repeated transmission assessment surveys (TAS) at two- to three-year intervals to determine if LF parasite prevalence remains significantly beneath putative transmission thresholds (<2% antigen prevalence in areas with *Culex quinquefasciatus* the primary vector). From August 22 to September 8, 2020, children 6-7 years old were tested for circulating filarial antigen (CFA) by filariasis test strip (Abbott) in community-based TAS-3, with each commune considered an evaluation unit (EU). In each EU, the target sample size was 911 and the critical cut-off was 11, which corresponds to the 2% antigen threshold. In Saut d'Eau, 5 (0.54%) of 928 children tested in 62 localities were CFA-positive. In La Tortue, 3 (0.33%) of 914 children in 55 localities were CFA-positive. Results demonstrate that each commune 'passed' TAS-3, as the number of CFA-positive children in each EU was less than the critical cut-off. However, the presence of antigen positive children indicates that LF transmission may not be fully interrupted. Follow-up surveys are needed to assess the risk of on-going transmission in these areas and determine the need for targeted interventions to achieve elimination.

0354

COVERAGE AND IMPACT OF A SINGLE DOSE OF IVERMECTIN, DEC AND ALBENDAZOLE FOR LYMPHATIC FILARIASIS: RESULTS FROM A COMMUNITY STUDY

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A community study was performed in several villages in Yadgir district India (endemic for bancroftian filariasis) to assess the impact of mass drug administration (MDA) with a triple drug treatment (ivermectin, DEC, & albendazole, IDA). Household enumeration and community sensitization were carried out prior to MDA in the selected villages. Consenting residents aged > 5 years, not pregnant and not seriously ill were targeted for treatment. Participants were screened for microfilaraemia (Mf) at baseline (60 µl night blood smears). Baseline Mf prevalence was higher in males (8.0%) vs. females (5.9%) and in adults (≥20 years, 9.2%) vs. children (<20 years, 3.6%). Directly observed treatment with IDA was provided to 4758 persons with overall population adherence of 70.5% out of eligible population. Adherence was approximately 20% lower in adult males than in females. A second cross-sectional survey was performed one year after MDA. The ratio of males to females who participated in the second survey was 0.84. Since males have higher Mf prevalence than females, underrepresentation of adult males in assessment surveys may lead to overestimation of the impact of MDA. Mf prevalence (6.9 to 3.6%, 47% reduction) and geometric mean Mf density per 60µl blood (0.19 to 0.09, 52.6% reduction) were significantly reduced after MDA, but CFA prevalence (26.3%) was not reduced. Percent reductions in community Mf prevalence were greater in females (52.5%) than in males (42.5%). A separate efficacy study in the area had shown that a single dose of IDA cleared Mf in 84% of persons with Mf at baseline. Reductions in community Mf prevalence were consistent with this efficacy estimate and the MDA adherence data. These results show that high adherence will be required for the superior impact of MDA with IDA to be fully realized in India. Special effort and community specific strategies will be needed to increase adherence in adult males.

0355

MONITORING THE IMPACT OF TWO ANNUAL ROUNDS OF MASS DRUG ADMINISTRATION USING A 3-DRUG REGIMEN ON LYMPHATIC FILARIASIS IN AMERICAN SAMOA

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In 2018, the American Samoa Department of Health (DOH) began mass drug administration (MDA) for lymphatic filariasis with a 3-drug regimen of ivermectin, diethylcarbamazine and albendazole (IDA). After round one, surveyed drug coverage was high (76.7%), but circulating filarial antigen (CFA) prevalence was 2.7%, above the 1% MDA stopping threshold. DOH completed round two of IDA MDA in November 2019 and conducted a targeted impact survey after round two. In Sep-Oct 2020, DOH surveyed 831 people (≥5 years) in 8 villages with high prevalence (defined as having ≥3 CFA or ≥1 microfilariae (Mf) positive people in 2019). People were asked about participation in both MDAs. We used fingerstick blood samples to assess CFA, and tested CFA positive participants for Mf. All CFA and Mf positive people were offered treatment with IDA. Those positive for Mf were examined 7 days after treatment for Mf clearance. CFA prevalence in the 8 villages declined from 4.3% (95% confidence interval [CI] 2.8-6.4) in 2019 to 2.0% (95% CI 1.2-3.0) in 2020, but Mf prevalence was similar (2019: 1.2% (95% CI 0.6-2.7); 2020: 1.1% (95% CI 0.5-2.1). Drug coverage increased from 78.3% (95% CI 74.7-81.6) to 90.0% (95% CI 87.5-92.2). Of those surveyed, 9.0% (95% CI 6.8-12.0) did not take the medicines either year. Of 47 CFA positive participants, 46 were examined for Mf; 9 were positive. Six (66.7%) Mf positive participants took the medicines both years; 3 took them only once. Of the 9 Mf positive people, 6 were available for follow up after treatment in 2020; Mf had cleared in all. CFA prevalence was higher in males (3.2%) than females (0.8%, p<0.01) and those ≥10 years (2.9%) compared to children 5-9 years (0.8%, p=0.84). Similarly, Mf prevalence was higher in males (1.0%, p<0.001) and those ≥10 years (1.0%, p<0.05); no children 5-9 years were positive. Despite high coverage and low systematic noncompliance, CFA >1% in these villages indicates the need for continued MDA and suggests that targeted treatment might be required to meet stopping thresholds. Significantly higher active infection among older people, particularly men, suggests this may be an appropriate group to monitor in American Samoa.

0356

PROGRESS TOWARD ELIMINATION OF LYMPHATIC FILARIASIS IN CAMEROON FOLLOWING SUCCESSFUL TAS2 IN 41 HEALTH DISTRICTS OF THE EAST, FAR-NORTH, LITTORAL, SOUTH AND WEST REGIONS

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Cameroon is endemic for lymphatic filariasis (LF) in 137 of 189 health districts (HDs). The country aims to eliminate LF as a public health problem by 2025 by conducting annual mass drug administration (MDA), among other activities, as per World Health Organization recommendations. By 2018, all endemic HDs had received at least five effective rounds of MDA (≥65% epidemiological coverage) and passed a pre-transmission assessment survey (pre-TAS). From 2014 to 2018, 136 of 137 HDs met the criteria to stop MDA by passing TAS1, and one HD has not yet been assessed due to insecurity. Between 2018 and 2020, 41 out of 136 HDs successfully completed TAS2 in the East, Far-North, Littoral, South and West regions. These HDs were grouped into 14 evaluation units (EUs) according to their epidemiological profile, geographic location and population size. The Survey Sample Builder was used to calculate sample sizes and select clusters. The sampled population consisted of children aged 6-7 years. The survey was conducted in communities in all regions (schools were closed in some regions due to the COVID-19 pandemic). The Filariasis Test Strip (FTS) was used to detect *Wuchereria bancrofti* infection. Data were captured on smartphones and processed using electronic data collection system with control measures. Field teams performed day-time calibrated blood smears in *Loa Loa* co-endemic areas. Children testing positive were all confirmed by a second FTS test. All field activities were completed using appropriate COVID-19 prevention measures. Overall, 21,512 children in 498 clusters were tested and only three children were confirmed positive. All 14 EUs passed the TAS2. Children testing positive received a single dose of Mectizan® and albendazole and their parents were sensitized on the necessity of treating LF. In summary, these 41 HDs have completed TAS2 and successfully sustained interruption of LF transmission. This is a significant progress towards the elimination of LF in Cameroon.

0357

REPEAT PRE-TRANSMISSION ASSESSMENT SURVEY FOR LYMPHATIC FILARIASIS IN HOTSPOT DISTRICTS IN SIERRA LEONE

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All 16 districts in Sierra Leone were endemic for lymphatic filariasis (LF). Annual mass drug administration (MDA) with ivermectin and albendazole to interrupt LF transmission reached national geographic coverage in 2009. To date, nine districts have stopped MDA and transitioned to surveillance. Six districts failed pre-transmission assessment survey (pre-TAS) in 2013 and 2017 and Western Area Rural (WAR) failed the pre-TAS in 2017. The national program implemented enhanced strategies to improve MDA quality: sub-district-level reporting; updated census data for drug allocation; supportive supervision and mop-ups; community drug distributor (CDD) stipends; and adapting social mobilization materials for hard-to-reach communities. In 2020, repeat pre-TAS was conducted in all 7 districts using convenience sampling at sentinel sites selected from the baseline survey and spot check sites selected based on local knowledge of LF cases, hard-to-reach, likelihood of low MDA coverage and/or vector efficacy. Blood samples from 310 people aged five years and above were tested per site using the Filariasis Test Strip (FTS). Enhanced pre-survey community engagement with stakeholders was conducted to reduce participation hesitancy due to COVID-19 and risk mitigation measures were implemented during field activities. All FTS positive results were confirmed by a second FTS test and remote confirmation by a supervisor. The site antigenemia prevalence was 4.1%, 2.6%, 5.8% and 7.7% in Bombali and Karene, 1.3%, 4.8% and 0.6% in Koinadugu and Falaba, 0.7%, 1.0%, and 1.6% in Kailahun, 0.0% and 0.6% in Kenema, and 0.0% and 0.6% in WAR. Three districts successfully passed pre-TAS and will move on to conduct TAS1 in 2021. Four districts failed to meet the criteria for conducting TAS1 (>2% in one or more sites) for the third time and will conduct two more rounds of MDA. Compared with the results in 2017 (prevalence ranging from 7.5% to 25.9%), there was a significant drop in LF antigenemia prevalence suggesting that the revised strategies were effective and with continued programmatic progress, Sierra Leone is on course to achieve LF elimination.

0358

ASSESSING ACCEPTABILITY OF MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS AS PART OF ROUTINE MONITORING AND EVALUATION: FEASIBILITY AND OPERATIONAL IMPLICATIONS

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Population level acceptability of mass drug administration (MDA) for lymphatic filariasis will improve efforts to eliminate LF as a public health problem. A recently validated metric of acceptability demonstrated a more nuanced understanding of community participation than MDA coverage survey. Here we embedded an acceptability metric within a monitoring and evaluation survey in four districts of East New Britain Province, Papua New Guinea to assess acceptability on a larger scale than previously conducted. The acceptability survey included nine questions forming the composite acceptability score using a common 4-point Likert scale (range 9-36; threshold of acceptability > 22.5). To understand factors related to acceptability we used population-averaged linear regression to assess key demographic and operational variables, controlling for clustering at the village level from 3,350 persons aged 18 or older. We found acceptability scores were high (mean = 31.71 ± 4.29) which corresponded to overall MDA coverage of 81.1% in the province. Regression modeling found respondents living in two districts (Gazelle and Rabaul districts) had higher acceptability scores (2.16; $p = 0.0283$ and 3.69; $p < .0001$ respectively) than those living in Pomio or Kokopo. Higher acceptability scores were also associated with higher levels of education (Completed Middle School: 0.54; $p = 0.0308$, Completed Higher Education: 0.88; $p = 0.0142$ compared with those with no schooling), and MDA coverage (2.76; $p < .0001$). Sex, age, socioeconomic variables, and use of vector control measures were not associated with acceptability. Acceptability is influenced by regional differences and significant associations between MDA coverage reinforce the validity of the acceptability metric. Acceptability may provide further detail for targeting the MDA approach to account for regional and sub-population differences. Enumerators were not burdened by inclusion of the acceptability questions. Continued assessment of acceptability over consecutive M&E surveys would allow for trends to be assessed over time, further enriching the operational value of the metric.

0359

PROGRESS IN NATIONWIDE MAPPING OF ONCHOCERCIASIS ENDEMICITY IN ETHIOPIA USING SEROLOGICAL AND ENTOMOLOGICAL INDICATORS OF TRANSMISSION

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Onchocerciasis in Ethiopia was mapped by the African Programme for Onchocerciasis Control using nodule rates, indicating ivermectin mass drug administration (MDA) in portions of western Ethiopia. After declaring a goal of eliminating transmission in 2015, Ethiopia embarked on (re) mapping the untreated parts of the country using a combination of entomological and serological studies. Guided by recommendations from the Ethiopia Onchocerciasis Elimination Advisory Committee (EOEEAC), the Federal Ministry or Health began by excluding districts ecologically unsuitable for transmission, namely those without streams or rivers, whether seasonal or otherwise, that would support vector *Simulium* flies. This was done using a variety of maps and satellite imagery. Next, entomologists used the Rapid Epidemiological Mapping of Onchocerciasis approach to identify a minimum of three "first line" or high-risk villages for sampling. Consenting residents gave a dried blood spot (DBS) for laboratory testing using Ov16 ELISA at the Ethiopia Public Health Institute. The age group of interest shifted from children under 10 to adults 20 and older over the course of the initiative, based on EOEEAC's consideration of new guidance from the WHO Onchocerciasis Technical Subcommittee. The current threshold triggering MDA is an aggregate ≥2% positive adults from a minimum sample of ≥300 adults from ≥3 villages in a district. The mapping effort focused on 650 districts of unknown endemicity, starting with those adjacent to districts under treatment. Investigators found 158 to be ecologically unsuitable for transmission. DBS have been collected from 492; to date, 141,905 DBS have been collected and 134,705 have been analyzed. Fifty-two of districts have met transmission thresholds and MDA has begun for 5.5 million people across the country, and another 2.5 million await initiation of treatment. Entomological prospections of rivers and streams at more than 3000 sites demonstrate broad yet varied

presence of vector flies across the country, with mixed relationships to level of parasite exposure observed in humans. Only 17 districts remain to be mapped in Ethiopia.

0360

EPSTEIN BARR VIRUS CENTRAL NERVOUS SYSTEM INFECTION IN PATIENTS PRESENTING WITH SUSPECTED MENINGITIS IN BOTSWANA

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Epstein-Barr virus (EBV) central nervous system (CNS) infection in immunocompromised hosts and amongst meningitis cohorts is well recognised. The clinical significance of EBV CNS infection however is poorly understood. We determined the prevalence of EBV CNS co-infection amongst a cohort of patients with meningitis and explored associations between EBV co-infection and in-hospital mortality. Data were collected as part of the Botswana Nationwide Meningitis Surveillance study. EBV CNS infection (CSF BioFire FilmArray multiplex positive) prevalence was described and associations between baseline covariates and EBV CNS infection reported. Logistic regression modelling was conducted to investigate the association between EBV infection and in-hospital mortality. 601 participants with suspected meningitis were recruited: 54% male, median age 29 (IQR 1-42), 50.1% HIV positive. Cryptococcal meningitis was the most common infectious meningitis (11%, 66/601), 7.5% (45/601) had either probable/possible tuberculous meningitis. EBV CNS infection was common (25%); co-infection (16%) exceeded EBV mono-infection (9%). Amongst participants with EBV CNS infection there was evidence that a higher CNS EBV viral load was associated with greater CSF inflammation (p = 0.001). There was no association overall between EBV CNS infection and mortality aOR 1.27 (95% CI 0.76-2.12, p 0.36), however there was weak evidence (p = 0.099) that the association between EBV co-infection and mortality varied according to meningitis diagnostic group. Amongst participants with cryptococcal meningitis, EBV co-infection was associated with reduced odds of mortality aOR 0.15 (95% CI 0.02-0.93, p 0.03). Conversely, amongst patients with tuberculous meningitis, EBV co-infection was associated with increased mortality (aOR 4.91, 0.85 – 28.5, p 0.04). Overall EBV CNS infection was not associated with in-hospital mortality amongst this heterogeneous cohort. Larger longitudinal studies are required to understand the association between EBV, CSF inflammation and outcome, and to investigate how mechanistic pathways may differ across meningitis sub-groups.

0361

EPSTEIN-BARR VIRUS AND CYTOMEGALOVIRUS CENTRAL NERVOUS SYSTEM CO-INFECTIONS AND MORTALITY RISK IN PATIENTS WITH HIV-ASSOCIATED CRYPTOCOCCAL MENINGITIS

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Cryptococcal meningitis case fatality in sub-Sahran Africa remains devastatingly high at 24-45%. Recent data suggest that co-prevalent opportunistic infections may contribute to poor patient outcomes. No studies to date have investigated whether Epstein-Barr virus (EBV) and/or cytomegalovirus (CMV) central nervous system (CNS) co-infection is associated with mortality amongst adults with HIV-associated cryptococcal meningitis. We analysed CSF samples - including molecular EBV and CMV testing - and clinical data collected prospectively from adults with confirmed HIV-associated cryptococcal meningitis, recruited into one of two meningitis studies in Botswana and Tanzania. We compared 10-week survival rates among those with and without evidence of EBV and/or CMV CNS co-infection using multivariate Cox models. Of 140 participants, 60% (84/140) were male, median age 38 years, median CD4 count 37 cells/μL. EBV CNS co-infection was detected in 58.6% (82/140), CMV CNS co-infection in 3.6% (5/140). EBV CNS co-infection was associated with increasing age (p = 0.01), and CSF pleocytosis (p = 0.004). On multivariate analyses, EBV co-infection was associated with lower cumulative 10-week mortality (26.9% versus 44.3%, aHR 0.43, 95% CI 0.23 – 0.78). Conversely participants with CMV co-infection had higher cumulative 10-week mortality (80.0% versus 32.3%, aHR 4.59, 95% CI 1.56 – 13.49). EBV and/or CMV CNS co-infection is common amongst adults with HIV-associated cryptococcal meningitis. EBV CNS co-infection - which was strongly associated with CSF pleocytosis and improved survival - is a probable "by-stander" infection, and of doubtful clinical significance. Conversely, CMV co-infection was associated with a 4-fold increased risk of death; anti-CMV therapy warrants further investigation as a potential intervention to reduce mortality in HIV-associated cryptococcal meningitis.

0362

LIBERIA ADHERENCE AND LOSS-TO-FOLLOW-UP IN HIV/AIDS STUDY: A RETROSPECTIVE COHORT OF ADOLESCENTS AND ADULTS

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To describe the progression of newly enrolled patients along the care cascade and quantify factors associated with LTFU, retention, and treatment adherence, we conducted a retrospective cohort study using facility-level patient records. We collected patients' care and treatment records from 28 urban and rural facilities from the 5 subregions of Liberia. HIV+ patients 15 years and older, enrolled between January 2016 – December 2017, were selected and followed for 2 years. Patients were stratified into 2 cohorts based on the year of enrollment. We illustrated the operational outcomes of the care cascade and used separate univariable and multivariable logistic regression models to explore factors associated with LTFU and retention. 4185 patient records were assessed, 1905 and 2280 for 2016 and 2017, respectively. In 2016, 845 patients were LTFU as well as 906 in 2017. The probability of poor adherence for 2016 and 2017 cohorts were 0.102 (SD 0.176) and 0.072 (SD 0.148), respectively. Multivariable analysis showed that being pregnant during the last visit (OR 1.66, p= 0.02, 95%CI: 1.08 – 3.72), drug side effects (OR 2.00, p= 0.02, 95%CI: 1.08 – 3.72), and WHO stage 3 (OR 2.62, p<0.01, 95%CI: 1.42 – 4.80) were associated with increased risk of LTFU among patients in 2016. In 2017, drug side effects (OR 1.54, p<0.01, 95%CI: 1.11 – 2.14) combined with WHO stage 2 (OR 2.65, p<0.01, 95%CI: 2.16 – 3.65) and stage 3 (OR 2.81, p<0.01, 95%CI: 2.16 – 3.65) were predictors of LTFU. In 2016, age 25 – 34 years (OR 1.35, p=0.01, 95%CI: 1.05 – 1.73), 35 – 44 years (OR 1.74, p<0.01, 95%CI: 1.32 – 2.29) and 45 years and older (OR 2.10, p<0.01, 95%CI: 1.59 – 2.75) in a rural facility (OR 1.50, p<0.01, 95%CI: .990 – 2.29) were associated with retention. In 2017, patients aged 25 – 34 years (OR 1.54, p=0.034, 95%CI: 1.03 – 2.32) and 35 – 45 years (OR 1.92, p<0.01, 95%CI: 1.27 – 2.90) and 45 years and older (OR 2.52, p<0.01, 95%CI: 2.07 – 3. 05) were likely to remain in care. LTFU and treatment adherence is still a major challenge and newer strategies are needed to improve patient tracking. Retention is higher in older patients, whereas stronger engagements and dedicated services are needed for youths.

0363

PREGNANT WOMEN KNOWLEDGE ABOUT THE INTERVENTIONS RECEIVED AT THE ANTENATAL CLINIC IN RURAL AFRICA

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Current prevention and treatment guidelines for HIV in Mozambique require that pregnant women living with HIV (PWLHIV) self-administer five tablets on average from different medications daily. However, HIV-related maternal and neonatal mortality is still high, suggesting that compliance with the medication might be low. To optimize this compliance, we explored pregnant women's knowledge about common diseases in pregnancy, their prevention and the indication of prescribed medications given at the antenatal care (ANC) to HIV-infected pregnant women in Manhiça District, Mozambique. This qualitative study collected data through semi-structured exit and in-depth interviews with pregnant women attending the ANC clinic during December-2019 to October-2020. The ability to distinguish the different tablets was assessed by presenting them without any identification to the pregnant women. Forty PWLHIV were enrolled. We found a high level of knowledge on the most common diseases in pregnancy and their preventive measures. HIV, malaria, sexually transmitted diseases and tuberculosis were all considered serious infectious in pregnancy. Interviewed women used the container, format, color, size and stamping to identify the tablets. The stamping of a figure at the antiretroviral tablet was the recognition marker. Cotrimoxazole was frequently confused with paracetamol and isoniazid. Few women recognized mebendazole. Knowledge about the indication of the drugs prescribed was limited, and it was specially referred to antiretroviral drugs and ferrous sulfate. Participants tend to several indications to each drug. Interestingly, it was generally perceived that all the drugs prescribed could serve to treat malaria infection. African pregnant women attending the ANC in a rural area showed a high level of knowledge of the most common diseases in pregnancy and their prevention. Their poor recognition of the drugs prescribed, and their indication may cause medications errors and affect compliance. Strategies are needed to increase the information provided to pregnant women at ANC clinics considering the high number of medications presctibed.

0364

PREVALENCE OF CERVICAL DYSPLASIA BY VISUAL INSPECTION ACETIC ACID (VIA) IS INCREASED IN WOMEN WITH FEMALE GENITAL SCHISTOSOMIASIS; A CROSS-SECTIONAL STUDY IN ZAMBIA

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Female genital schistosomiasis (FGS) is a disabling gynaecological disease caused by the waterborne parasite *Schistosoma haematobium*. Evidence on the relationship between FGS and cervical cancer is limited. Visual inspection with acetic acid (VIA) is widely used in Sub-Saharan Africa to screen for cervical dysplasia. We aimed to assess the association between FGS and cervical dysplasia by VIA positivity. Women were recruited from Livingstone, Zambia as part of a home-based FGS diagnostic study nested within the HIV prevention trial HPTN 071 (PopART). Procedures included polymerase chain reaction (PCR) to detect parasite DNA from genital samples, urine CAA and microscopy, demographic and reproductive health questionnaires, and available HIV serostatus. All women were offered VIA to test for cervical dysplasia. VIA, and FGS association were explored through multivariable analysis. In 237 women, positive FGS-PCR and VIA positivity were strongly correlated (OR: 6.08, 95% CI: 1.58-23.37, p=0.016). FGS diagnosed by imaging, urine CAA or microscopy were not associated with VIA positivity (OR: 0.58, 95% CI: 0.20-1.69, p=0.303, OR 2.21, 95% CI 0.83-5.89, p=0.129 respectively). This is the first study to find an association between genital FGS-PCR and cervical dysplasia, diagnosed by VIA. More studies are urgently needed to further investigate the role of FGS in cervical cancer to define better diagnostic and treatment algorithms across Sub-Saharan Africa.

0365

THE COST-EFFECTIVENESS OF A FAMILY-BASED ECONOMIC EMPOWERMENT INTERVENTION (SUUBI+ADHERENCE) IN IMPROVING ADHERENCE TO ANTIRETROVIRAL TREATMENT AND OUTCOMES IN ADOLESCENTS LIVING WITH HIV IN SOUTHERN UGANDA

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A randomized controlled trial conducted in southern Uganda over 24 months demonstrated the efficacy of a family-based economic empowerment (FEE) intervention, Suubi+Adherence, in improving adherence to antiretroviral therapy (ART) among adolescents living with HIV (ALHIV). To aid policy makers to consider the integration of FEE as part of routine HIV care in Uganda, this study assessed the longer-term costs and health outcomes of the Suubi+Adherence intervention using decision analytical modelling. A Markov model was developed to compare the costs and health benefits of the FEE intervention to the bolstered standard of care (BSOC) in a hypothetical cohort of 1,000 ALHIVs in Uganda (median age 12) from a health care provider's perspective. The model included 3 mutually exclusive health states: undetectable viral load (<40 copies/ml), detectable viral load (>=40 copies/ml, <=1000copies/ml), and virological failure (>1000 copies/ ml) and was run for 8 years, until ALHIVs reached the age of 20 and were no longer eligible for the intervention. We used cost, probability, and intervention efficacy data from the Suubi+Adherence trial and the published literature. All costs were adjusted to 2020 US dollars, and a 3% discount rate was used for costs and health effects. Uncertainty was assessed using deterministic and probabilistic sensitivity analyses. The incremental cost-effectiveness ratio (ICER) was $3,440 per disability-adjusted life year (DALY) averted (95% credible interval = -$19,710 to $34,540). Our results showed that the Suubi-Adherence intervention was cost-effective with a 51% probability against a cost-effectiveness benchmark of 3 times Uganda's gross domestic product per capita based on WHO guidelines. ICERs were most sensitive to the baseline transition probability from undetectable to detectable viral load under ART, intervention efficacy, and per-child intervention cost. The results suggest the feasibility of integrating FEE-based interventions into routine HIV care as a long-term cost-effective solution to improve treatment adherence outcomes and overall health of ALHIVs in resource-poor settings.

0366

HIGH MORTALITY IN ADULT PATIENTS WITH HIV ADMITTED WITH FEVER TO HOSPITALS IN MALAWI, MOZAMBIQUE, AND ZIMBABWE - RESULTS FROM THE FIEBRE STUDY

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Despite important advances in HIV diagnosis and roll-out of antiretroviral therapy (ART) in sub-Saharan Africa, HIV-associated conditions continue to be a major cause of hospitalisation and death. The aim of this study was to compare mortality and causes of death among HIV-infected and uninfected adults hospitalised with febrile illnesses in Malawi, Mozambique, and Zimbabwe. This is a preliminary analysis including patients aged ≥15 years who were admitted to seven hospitals in Malawi, Mozambique and Zimbabwe and enrolled into the FIEBRE (Febrile Illness Evaluation in Broad Range of Endemicities) study evaluating infectious causes of fever. Participants provided detailed clinical information and samples were collected for laboratory diagnosis of infections. A follow-up visit was conducted at 28 days to assess patient outcome. Causes of death were assessed by combining information from hospital records, family reports, and the FIEBRE study data. Among 940 adult patients admitted to hospital, the median age was 35 years (IQR 26-49) and 498 (53.0%) were female. HIV status was determined in 926 (98.5%); 383 (41.4%) were HIV-infected Outcome information was available for 889 patients and of those 122 (13.7%) died. Mortality at 28 days was 22.4% and 7.1% (p<0.001) in patients with and without HIV, with 69.8% of the deaths occurring among patients with HIV. The most common causes of death among HIV-infected individuals in whom a cause of death was available (n=73) were tuberculosis in 29 (39.7%) and other conditions associated with advanced HIV infection in 37 (50.7%). In patients without HIV infection, 16 (51.6%) and 15 (48.4%) of deaths were attributed to infectious and non-infectious causes, respectively. Mortality among adult patients with HIV was three times higher than in HIV uninfected patients. Tuberculosis and other preventable and treatable conditions associated with advanced HIV represented major causes of death in our population. This highlights the need for improved HIV diagnosis, monitoring, therapy and retention in care to decrease HIV-associated mortality. Comprehensive data from all sites will be presented in November.

0367

CHANGES IN THYROID STATUS OF PEOPLE LIVING WITH HIV AFTER HAART ATTENDING ANTI-RETRO VIRAL THERAPY CENTER OF A TERTIARY CARE HOSPITAL OF BANGLADESH

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The aim of this prospective observational study was to study the changes in thyroid function amongst people living with HIV (PLHIV) attending anti-retroviral therapy (ART) center of Bangabandhu Sheikh Mujib Medical University (BSMMU), Bangladesh.We enrolled 61 HIV patients before starting treatment. Baseline investigations along with thyroid function tests were done including TSH, FT_3, FT_4, Anti-thyroglobulin antibody and Anti-thyroid peroxidase antibody. Patients were in follow up for six months and again investigated for the above mention thyroid investigations. Due to COVID-19 pandemic situations we could not complete follow up of all 61 patients. Until now we have completed follow-up of 40 patients and amongst them, four patients expired.Majority patients were male, hailing from Dhaka division. Unprotected promiscus sexual activity and use of commercial saloon was the commonest source of transmission. Unexplained weight loss (79%) was the commonest mode of presentation and majority presented with stage 2 or stage 3 of disease severity. Syphilis and tuberculosis was the commonest co-infection. We found that 14% of patients had thyroid dysfunction before starting HAART, and 20% patients develop thyroid dysfunction 6 months after treatment with HAART, this change was not statistically significant (p=0.7531). This study also revealed that 6% of patient had positive auto antibody before starting HAART and it reached to25% six months post treatment. This increment was statistically significant (p=0.0457) and indicates an underlying IRIS. We also found that both of these changes were common amongst patients presented with advanced disease (WHO clinical stage 4). Total four patients died during the whole study period and three of them were due to severe COVID. In conclusion, thyroid dysfunctions particularly development of auto antibody are common amongst PLHIV. However large scale robust follow up is mandatory to draw any final conclusion.

0368

ACCEPTABILITY AND PENETRATION OF A SUPERVISED AND PEER-LED HIV SELF-TESTING INTERVENTION AMONG UNIVERSITY STUDENTS IN EASTERN ZIMBABWE

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Given the low HIV testing rates among young people, inventive interventions are needed to reach them with HIV services. Despite the elevated risk of HIV acquisition among youths, a 2015 Zimbabwean survey revealed that young people were less likely to go for HIV testing mainly due to facility-based and provider-initiated testing-related barriers like stigma and lack of privacy. This study aimed to assess the penetration and acceptability of a supervised, peer-led oral HIV self-testing (HIVST) intervention among young adults at a university in Zimbabwe. A formative assessment was conducted by utilizing administrative data reviews; a baseline survey, three community dialogue sessions with the students; and recruitment of peer counselors. The implementation phase (August 2018 to February 2019) involved three brainstorming sessions by a team of multi-disciplinary experts of researchers, health workers, government agents. and student board representatives at Africa University. Peer counselors and university clinic health workers were trained on HIVST and the distribution process. Peer counselors disbursed the HIVST kits under the supervision of the nurses. The HIVST information leaflets were printed in English, French, and Portuguese to accommodate the enrolled students from 29 African countries. An end-line survey and in-depth interviews were conducted post-implementation. Intervention penetration was measured using administrative data to measure the kit uptake while acceptability was assessed by self-reported use of the HIVST kit. All the 744 HIVST kits were collected within the six months of the intervention. The initiative managed to increase the HTS uptake rate from 6.3% during the 12 months of 2017 to 49.6% during the six months of the intervention. Overall, the acceptability of HIVST was high with the use of trained peer counselors, multi-lingual pre-packaged leaflets, college seniority, and previous HIV test/s being associated with willingness to test. However, post-test services were poorly utilized. The study findings support the acceptability of scaling up HIV testing services among university students in Zimbabwe.

0369

INNATE-LIKE T CELL RESPONSES IN HIV EXPOSED UNINFECTED MALAWIAN INFANTS

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Each year, an estimated 1.5 million HIV+ women give birth. Increased access to antiretroviral therapy (ART) ensures most infants born to HIV+ women do not contract the infection vertically, and thus remain HIV exposed but uninfected (HEU). During their first year of life, HEU infants exhibit increased rates of lower respiratory tract infections and diarrheal diseases, compared to HIV unexposed (HU) infants. It is hypothesized that exposure to HIV (and ART) before birth perturbs the developing fetal immune system by increasing inflammation at the fetal-maternal interface and induces long term effects that contribute to the increased infectious morbidity in HEU infants. The specific immunologic mechanisms behind this clinical outcome remain unclear. Among the subsets that may be perturbed by increased inflammation, innate-like T cells (γδ, MAIT, NKT cells) may play an important role against pathogens in early life. We hypothesize that prenatal HIV exposure results in early life activation of innate-like T cell subsets. Utilizing a well-characterized cohort of Malawian infants, we compare three groups of infants born to women with: 1) HIV infection but undetectable viremia through pregnancy (HEU-lo); 2) HIV infection diagnosed at mid-gestation or later, with high viral loads (HEU-hi); 3) no HIV infection (HU). A preliminary analysis of cord blood Vδ2 T cells suggests that production of Th1 cytokines in response to polyclonal stimulation is increased in HEU neonates. The frequency of TNFα+ Vδ2 T cells is highest in HEU-hi neonates, while the frequency of polyfunctional (IFNγ+TNFα+) Vδ2 cells is highest in HEU-lo neonates. These findings, if confirmed, would suggest that exposure to replicating maternal HIV results in more robust TNFα production, while prolonged exposure to ART in HEU-lo neonates may contribute to the observed differences in Vδ2 cells polyfunctionality. This study provides an opportunity to assess immune perturbation of innate-like subsets in HEU infants, contributing to our understanding of immune responses and mechanisms of increased infectious morbidity in this vulnerable population.

0370

PREVALENCE, INCIDENCE AND PREDICTORS OF CHRONIC KIDNEY DISEASE IN PERSONS WITH HUMAN IMMUNODEFICIENCY VIRUS RECEIVING PROTEASE-INHIBITORS IN RURAL TANZANIA

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Ritonavir-boosted protease inhibitors (bPI) in people living with HIV (PLWH) have been associated with chronic kidney disease (CKD). Limited data are available from rural sub-Saharan Africa where people are at increased risk for CKD.Using data from the Kilombero and Ulanga Antiretroviral Cohort Study (KIULARCO) in rural Tanzania from 2005-01/2020, we assessed i) the prevalence of CKD (estimated glomerular filtration rate <60 mL/min/1.73m^2) at the time of switch from first-line ART to bPI-regimen and ii) the incidence of CKD while being on bPI-regimens. We used logistic and Cox regression models to assess the relationship between clinical and demographic characteristics and CKD prevalence at the time of switch to bPI and subsequent incidence. CKD was present in 52/687 PLWH (7.6%) at the switch to bPI. Among 556 participants with normal kidney function at switch 41 (7.4%) developed CKD after a median time of 3.5 (IQR 1.6-5.1) years (incidence 22/1,000 person-years (95%CI 16.1-29.8)). Factors associated with CKD at switch were older age (adjusted odds ratio (aOR) 1.55 per 10 years; 95%CI 1.15-2.11), body mass index (BMI) <18.5 kg/m^2 (aOR 2.80 versus ≥18kg/m^2; 95%CI 1.28-6.14) and arterial hypertension (aOR 2.33; 95%CI 1.03-5.28). The risk of CKD was lower with increased duration of ART use (aOR 0.78 per one-year increase; 95%CI 0.67-0.91). The CKD incidence under bPI was associated with older age (adjusted hazard ratio 2.01 per 10 years; 95%CI 1.46-2.78). This study shows a high prevalence of CKD at the time of switch from first-line ART to bPI and a high incidence of CKD after switch to bPI-regimens. Strong associations between CKD and older age, BMI, arterial hypertension and time on ART before the switch were seen. Given the need for lifelong ART, these data support the implementation of universal routine monitoring of renal function and improved pharmacovigilance monitoring.

0371

A SYSTEMATIC REVIEW OF THE EPIDEMIOLOGY AND CLINICAL FEATURES OF HIV AND TRYPANOSOMA CRUZI (CHAGAS DISEASE) CO-INFECTION

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Chagas disease (caused by *Trypanosoma cruzi*) can behave as an opportunistic infection in patients with HIV, leading to reactivation, with atypical manifestations such as multiple space-occupying lesions and necrohaemorrhagic meningoencephalitis. There are significant differences in the way co-infection is screened for and managed in different countries and there has been little advance in our understanding of its epidemiology and clinical features. We present the results of our systematic review on the epidemiology and clinical features of HIV and *Trypanosoma cruz*i co-infection. Medline, Embase, Global Health, Global Index Medicus, Web of Science and Scopus databases were searched, with no language or date limits, giving a total of 3628 records. Following abstract and full-text screening, 151 articles were included for data extraction. Study details, population demographics, medical history, clinical presentations, investigations, management, and outcomes were extracted for over 700 patients with co-infection. In the majority of patients, HIV status was already known, and they were acutely unwell at presentation. Mean age at presentation was 37 years and 64% were male. Common presentations included fever, headache, weight loss, seizures, syncope, neck stiffness and focal neurological signs. Toxoplasmosis was the most common opportunistic infection also present. The most common neuroimaging finding was a single lesion; in around half these were ring-enhancing. Approximately half the patients with co-infection died, and in those who survived, the most common disability was hemiparesis. As the most conclusive and only up-to-date systematic review on HIV and Chagas disease co-infection, our findings will inform clinical and public health management, screening policy, healthcare service planning and identify future research priorities.

0372

EPIDEMIOLOGY OF TB/HIV CO-INFECTION IN THE GAMBIA, MARCH 2019 - FEBRUARY 2020

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Tuberculosis (TB) still remains a major public health problem causing ill-health among millions of people around the world. HIV co-infection, together with drug-resistant TB and other co-morbidities are the growing problems that are fueling the epidemics of TB. HIV/AIDS is the leading risk factor for the reactivation of latent tuberculosis. TB on the other hand is also regarded as the most common opportunistic infection and the leading cause of death among people infected with HIV. The two diseases (TB and HIV) have a synergistic interaction; each promotes the progression of the other. Tuberculosis in The Gambia mainly affects people in the productive age group (15 - 59 years), with males being the majority of the patients. The aim of this study is to determine the prevalence of HIV co-infected TB patients in The Gambia.A retrospective study was carried out from March 2019 to February 2020. The inclusion criterium was all smear positive TB patients within the stated time span. Data was extracted from the National Leprosy and Tuberculosis Program and was reviewed, analyzed and presented into proportion and rates using excel, out of which a total of 2359 smear positive tuberculosis patients were screened for HIV. Out of which the majority were males. Less than quarter of the sample tested seropositive for HIV. However, more than half of the HIV positive cases were females. Moreover, the regional distribution shows that West Coast-2 has the highest number cases and Lower River Region registered the lowest. In conclusion, the prevalence of HIV co-infection among TB patients still remains a burden in the Gambia especially among female and in the West Coast Regions. This emphasizes the need to strengthen the routine surviellance in the two regions to ensure timely detection and management of cases. The use of Isoniaziad preventive therapy among HIV seropostive patients without TB infection is also needed. This will help to minimize the risk of reactivation of latent TB thereby reducing the prevalence TB/HIV co-infection. Further studies should be done to investigate the factors responsible for high burden of TB/HIV co-infection among females.

0373

CO-INFECTION OF DISSEMINATED HISTOPLASMOSIS AND MYCOBACTERIUM TUBERCULOSIS IN A PATIENT WITH AIDS: A CASE REPORT

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It is estimated that the prevalence of histoplasmosis and Mycobacterium tuberculosis (MTB) are equivalent, in Latin America$_1$. In a Colombian case series, up to 70% of patients with disseminated histoplasmosis (DM) were co-infected with MTB$_2$. This case examines the diagnostic barriers encountered in Latin American countries and management of co-infections. We present a case of a Panamanian 35-year-old male who presented to the emergency room with a 48-hour history of fatigue, confusion, somnolence, and fever. He had recently been diagnosed with HIV (CD4 count 4). Based on his imaging (CT chest with a diffuse miliary pattern), his labs (pancytopenia), and profound immunosuppression the empiric diagnosis of DH was made. Yeast compatible with histoplasmosis was confirmed in a peripheral smear. He was started on amphotericin deoxycholate with mild improvement. Due to his persistent encephalopathy, fevers, and generalized weakness MTB became a concern. He was started on rifampin, isoniazid, pyrazinamide, and ethambutol. His clinical status began to improve and his mental status returned to baseline. His sputum AFB and CSF MTB PCR have been negative, leaving the MTB an empiric diagnosis. The patient continued on amphotericin deoxycholate past the 2-week transition point due to the scarcity and high prices of itraconazole. At the time of this patient's care, the histoplasma reagent was not available, which leads to a delay in diagnosis unless it can be identified in culture or tissue biopsy. DH is treated with amphotericin for the first 2 weeks then itraconazole for 12 months$_2$. In mild to moderate cases, histoplasmosis is treated with itraconazole$_2$. The scarcity and price of itraconazole is a barrier for outpatient treatment in some countries. Hospital Santo Tomas has developed an ambulatory amphotericin deoxycholate infusion clinic for weekly infusions to continue treatment. This case has a third layer of complexity due to co-infection of DH and MTB. Currently, there are no guidelines for treatment of co-infections and the medication interactions require frequent monitoring as rifampin can decrease itraconazole serum levels.

0374

RECONSTITUTION OF CATALYTIC ACTIVITIES OF TWO ESSENTIAL LEISHMANIAL CYTOCHROME P450 ENZYMES REVEALS NOVEL AND DISTINCT BIOCHEMICAL ROLES IN THE ERGOSTEROL BIOSYNTHESIS

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CYP51 and CYP5122A1 are two cytochrome P450 (CYP) enzymes essential for the survival of *Leishmania donovani*, a major causative agent of human visceral leishmaniasis. Leishmanial CYP51 acts as a sterol C14α-demethylase during ergosterol biosynthesis, and it has been considered as an attractive target for developing new antileishmanial drugs. CYP5122A1 is also involved in the leishmanial ergosterol biosynthesis pathway. However, its exact biological role remains unknown. We have proposed that CYP5122A1 act as a sterol C4-demethylase. To determine its exact biochemical function, it is important to reconstitute its catalytic activity with endogenous substrates and redox partner. In this study, recombinant *L. donovani* CYP51 and CYP5122A1 and *Trypanosoma brucei* NADPH-CYP reductase (*Tb*CPR) were expressed and purified from *Escherichia coli*. Purified CYP51 and CYP5122A1 exhibited characteristic UV-Vis spectrophotometric properties of CYP enzymes and were reduced by *Tb*CPR in the presence of NADPH. Using lanosterol as the substrate, CYP51 and CYP5122A1 were reconstituted *in vitro* with *Tb*CPR and NADPH. Their catalytic activities showed a pH-dependence, with maximum activities at pH 6.2. LC-MS/MS-based sterol analysis confirmed that leishmanial CYP51 catalyzes lanosterol C14α-demethylation to form FF-MAS. However, CYP5122A1 catalyzes hydroxylation of lanosterol at a position different from the methyl group at the C14 position, yielding a mixture of alcohol, aldehyde, and acid metabolites. Furthermore, when probed with several intermediate sterols (4,14-dimethylzymosterol, FF-MAS and T-MAS) in the ergosterol biosynthesis pathway, CYP51 and CYP5122A1 showed selectivity to 14-methylated and 4-methylated sterols, respectively. These results support that CYP5122A1 plays a different biochemical role than CYP51 during ergosterol biosynthesis. In conclusion, this study improved our understanding of the biological roles of CYP51 and CYP5122A1 in *Leishmania* parasites and provided new insights to target ergosterol biosynthesis for developing novel therapeutics against leishmaniasis.

0375

ANALYSIS OF RELEASED PEPTIDASES AND THEIR ROLE IN THE TRANSMISSION BIOLOGY OF AFRICAN TRYPANOSOMES

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Molecules released by African trypanosomes have an identified role in parasite virulence and quorum-sensing and may also be important at various stages of the parasite life cycle. Some of these secreted proteins include peptidases, with functions in creating the density-sensing signal in the bloodstream and potentially interacting with the mammalian immune system or during infectivity of the tsetse fly. Here, we analysed proteins released/secreted by parasites in the bloodstream and early during differentiation to the tsetse midgut procyclic form. Using detailed mass spectrometric analysis of the *T. brucei* secretome,

we have identified twelve peptidases released by the parasites during these developmental stages. Each was then validated for their release from parasites using individually epitope-tagged lines. Systematic ectopic overexpression and gene knockout using CRISPR/Cas9 of each peptidase gene was then used to identify that two peptidases (oligopeptidase B and metallocarboxypeptidase 1) significantly contribute to the generation of the trypanosome's quorum-sensing signal. Knockout of each peptidase gene reduced development of bloodstream parasites to stumpy forms, whereas add back experiments restored normal stumpy formation for oligopeptidase B, with metallocarboxypeptidase 1 add back experiments in progress. Furthermore, combined knockout of both peptidases demonstrated their additive contribution to the generation of the quorum-sensing signal. This systematic analysis of parasite released molecules identifies that two major peptidase activities released by the parasite dominate the generation of the quorum-sensing signal. Their further analysis will identify their oligopeptide products, pinpointing the specificity that drives the parasite's developmental response in preparation for transmission.

0376

HOMOLOGIES IDENTIFIED BETWEEN TRYPANOSOMA CRUZI ANTIGEN 36 AND THE TRIM FAMILY OF PROTEIN GENES

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We have previously reported on a partial homology between the gene for *Trypanosoma cruzi* Clone 36 repetitive antigen ("Antigen 36"), and human TRIM21, which codes for the ubuiquitin dependent ligase that regulates Interferon transcription production factors or degradation in innate immunity. TRIM21 is a member of the TRIM family of vertebrate proteins that has a tripartite domain structure, where the three domains are RING, B box, and coiled-coil structures. We compared the Antigen 36 gene, GenBank M21331, with 47 mammalian TRIM genes using the Needleman-Wunsch algorithm for genes alignment at https://usegalaxy.org. TRIM40 showed 10% partial gene homology; TRIM73 and TRIM74 showed 6% homology; TRIM6, TRIM7, TRIM10, TRIM43, TRIM61, TRIM64 showed 5% homology; TRIM15, TRIM17, TRIM21, TRIM31, TRIM47, TRIM48, TRIM50, TRIM60, and TRIM75, showed 4% partial homology; and TRIM11, TRIM27, TRIM34, TRIM49, TRIM52, and TRIM65, showed 3% homology. Twenty-three other TRIM genes showed homology ranging from 0-2.3%. The variation in gene size, from TRIM40 having only 777 nucleotides, to TRIM28 with 13,247 nucleotides, may account for differences in the percent homology observed. The Antigen 36 gene has two stretches of bases that are duplicated, and these may facilitate its partial homology to multi-domain genes such as the TRIM genes. The Antigen 36 gene is duplicated at least three times in T. cruzi. Its mRNAs, exported to the host cell cytoplasm in exosomes, for example, may recognize host TRIMs' mRNAs, possibly silencing these genes. This could impact the host innate immune response to the parasite, or the host's cellular growth.

0377

ENZYMATIC AND MOLECULAR CHARACTERIZATION OF ANTI-LEISHMANIA MOLECULES THAT TARGET THE LEISHMANIA EUKARYOTIC TRANSLATION INITIATION FACTOR 4A, LEIF

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The two structural analogues 7-aminocholesterol and 6-aminocholestanol, and the rocaglamide, were previously described as anti-*Leishmania* molecules, but no experimental data were provided for their mode of action. However, we demonstrated that 6-aminocholestanol was an inhibitor of the *Leishmania* eIF4A-like protein, LeIF. In this study, we investigated the mode of action of these molecules on the enzymatic activity of LeIF. We compared their biochemical effects and molecular interactions on the RNA-dependent ATPase activity of LeIF and its mouse ortholog eIF4A$_{Mus}$.Michaelis–Menten kinetics of the ATPase activity of LeIF and eIF4AI$_{Mus}$ were conducted using increasing concentrations of ATP and saturating whole yeast RNA. Kinetic measurements of the inhibition of the ATPase activity were also done using increasing concentrations of the compounds. Moreover, their anti-leishmanial activity was confirmed in *L. infantum* promastigotes. The Michaelis–Menten ATPase parameters in the absence of compounds measured a K_m of 151 µM for eIF4A$_{Mus}$, which showed a similar binding affinity to what we previously measured with LeIF (150 µM). However, the k_{cat} of eIF4$_{Mus}$ was 0.65 min^{-1}, which was 3.7-fold lower than our previously measured value for LeIF (2.4 min^{-1}). The kinetic analyses showed that 7-aminocholesterol inhibited LeIF with a higher affinity (K_i = 8.594 µM) than for eIF4A$_{Mus}$ (K_i = 30.14 µM). We previously found that in the presence of 6-aminocholestanol, the K_i values were similar for LeIF and eIF4A$_{Mus}$ (19.5 µM and 13.5 µM, respectively). Cholesterol, a negative control, showed no effect on LeIF nor on eIF4A$_{Mus}$ ATPase activity. In contrast, rocaglamide increased the ATPase activities of both proteins to different extent (6.2-fold for eIF4A$_{Mus}$ vs 0.64-fold for LeIF). All tested compounds affected the promastigote viability. We are currently using docking simulations and model analyses to investigate the molecular interactions and mechanisms underlying the observed results. In conclusion, the tested compounds affected LeIF and eIF4AI$_{Mus}$ differently. The inhibitory effects probably involve different binding pockets on the two proteins.

0378

DEEP SEQUENCING OF THE MINI-EXON GENE IMPROVES GENOTYPING OF MULTICLONAL TRYPANOSOMA CRUZI INFECTIONS ASSOCIATED WITH CONGENITAL CHAGAS DISEASE

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It is estimated over 1.1 million women of childbearing age in Latin America are infected with *Trypanosoma cruzi*, and there are approximately 8668 new cases of congenital Chagas disease (CD) each year. Lacking treatment, approximately 30% of infected infants will develop potentially fatal forms of chronic disease later in life and girls can chronically harbor the parasite and transmit it to their own children during their reproductive years. Genetic variability of the parasite, which has been divided into 7 discrete typing units (DTUs) named TcI-TcVI and TcBat, has been suspected to impact vertical transmission, but data from our group suggests parasite haplotypes are more closely associated with congenital transmission rather than specific DTUs. Common methods for genotyping *T. cruzi,* including PCR and Sanger sequencing, are only capable of identifying a single dominant haplotype or strain and are insufficient for detecting multiclonal infections, thus limiting our ability to understand how sequence diversity impacts congenital transmission. This study aimed to fully characterize the *T. cruzi* profile among women and newborns through next-generation sequencing of the mini-exon gene among 90 clinical samples (57 maternal blood, 33 umbilical cord blood) originating from Argentina (n=32), Honduras (n=31), and Mexico (n=27). Multiple mini-exon haplotypes were identified across all samples and ranged in frequency from a single sequence to 19 haplotypes per sample. Infections with multiple DTUs were observed in the majority of cases, and phylogenetic analyses showed a predominance of infections with TcII-V-VI DTUs, as well as parasites from the TcI DTU. In addition, the diversity of dominant haplotypes (>1%) from each DTU varied depending on the country of origin. These data provide a more thorough understanding of the makeup of *T. cruzi* strains associated with congenital transmission and validates our sequencing methodology for parasite genotyping and detecting multiclonal infections among congenital CD cases.

0379

DEVELOPMENT OF QUANTITATIVE RAPID ISOTHERMAL AMPLIFICATION ASSAY FOR ABSOLUTE QUANTIFICATION OF LEISHMANIA DONOVANI

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Quantification of pathogen load is of paramount importance for accurate diagnosis and clinical management of a range of infectious diseases. In a point-of-care testing (POCT) scenario such as in resource-limited settings, this is highly challenging with regards to availability, establishment, and operation of real-time quantitative PCR (qPCR) based applications. We devised a method to examine the absolute quantification ability of fluorophore-based isothermal recombinase polymerase amplification (RPA) assay. As for test of principle, two 10-fold dilution series of *Leishmania donovani* (LD) genomic DNA extracted from culture broth (CB) and culture-spiked peripheral blood (PB) were prepared, and a modified 15-minute assay protocol was performed in replicates. The quantification algorithm minimized the sample-to-sample variation in noise and defined the exponential amplification phase. The threshold times (Tt) in seconds thus obtained were plotted against the initial load of parasite genomes. The best-fitted regression models were found linear with mean r^2/RMSE values of residual points estimated as 0.996/8.063 and 0.992/7.46 for replicated series of CB and PB, respectively. In both series, the lower limit of detection reached less than 1 parasite equivalent genomic DNA. Inter-assay mean coefficients of variation were found to be 1.19% (95% CI: 0.07-2.32%) and 1.84% (95% CI: 0.46-3.21%) for CB and PB series, respectively. The method was blindly evaluated against qPCR in DNA samples that were extracted from visceral and post Kala-azar dermal leishmaniasis case specimens and stratified into high, moderate, and low Ct values. Absolute agreement between the two methods was found for test positivity, and strong positive correlations were observed between the Tt and Ct values ($r = 0.89$; $p<0.0001$) as well as between the absolute parasite loads ($r = 0.87$; $p<0.0001$) quantified by respective assays. The findings in this very first quantitative isothermal assay for leishmaniasis are suggestive of its potential in monitoring of LD load upon need (e.g. test of cure) and for the development of quantitative RPA assay for other infectious diseases.

0380

MILTEFOSINE FAILURE AND SPONTANEOUS RESOLUTION OF CUTANEOUS LEISHMANIASIS BRAZILIENSIS

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Cutaneous leishmaniasis (CL) is the most common manifestation of leishmaniasis, and in South America is most often caused by *Leishmania braziliensis* (*L. braziliensis*), but treatment failures do occur. Here, we present a case of miltefosine failure in a patient with CL followed by spontaneous resolution. A 19-year-old previously healthy female traveled to Peru. One month after she returned, she developed a single lesion on her ankle. Clinical examination revealed a 1.5 cm erythematous plaque with central ulceration. A skin biopsy was sent to the CDC, which was positive for *L. braziliensis* on RT-PCR. The patient completed a 28-day course of miltefosine, however the lesion never fully healed, and a second proximal plaque developed 1.5 months later. An additional biopsy was performed of the initial plaque which was positive for *L. braziliensis* by culture. After consideration of other treatment options, the patient decided to wait and both lesions spontaneously resolved over 9 months. Despite this, the risk for mucosal spread remains. For treatment to be considered curative, the lesion should decrease in size by more than 50%, begin reepithelializing, and new lesions should not develop by 4-6 weeks after completion. If not fully epithelialized after 3 months, treatment failure should be considered. The effectiveness of miltefosine against *L. braziliensis* CL in clinical trials ranges from 33-75% depending on the country. Cutaneous lesions do have a propensity to spontaneously heal, and it can take 6-15 months for *L. braziliensis* specifically. In 2-10% of untreated cases of New World CL, mucosal involvement occurs. It causes lesions that can be disfiguring and life-threatening, so systemic treatment is recommended for all cases of New World CL. The risk of mucosal spread is highest within 2 years, but can take decades to develop, so specific questioning about the development of new mucosal symptoms should be assessed at all subsequent visits. This case highlights the challenges associated with treatment of *L. braziliensis* infection and need for greater understanding of factors leading to treatment failure and/or self-resolution.

0381

EVALUATION OF CHAGAS DISEASE TESTING AND MANAGEMENT PRACTICES IN A TEXAS SAFETY-NET HEALTHCARE SYSTEM

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Chagas disease, a chronic infection caused by the protozoan parasite *Trypanosoma cruzi,* is a neglected tropical disease of the Americas; 6-7 million people are estimated to be infected globally, including 300,000 in the US. 20-30% of infected people develop cardiac or gastrointestinal sequela. US medical provider knowledge of *T. cruzi* screening and diagnosis is key to decreasing morbidity. Implementation of screening protocols is important in regions with large populations of people with Chagas disease risk factors, such as Texas. The objective of this project is to evaluate Chagas disease screening and management practices among medical providers in a county healthcare system with a large at-risk population. The Harris Health System is a safety-net system that cares for >1 million patients annually; 58% identify as Hispanic. Using the SlicerDicer tool of Harris Health's Epic electronic health record, we retrospectively identified patients ≥18 years old evaluated between 2010-2020 who had (1) undergone *T. cruzi* testing (identified via lab test codes) or (2) received a Chagas disease diagnosis (identified via ICD-9/10 codes). This resulted in 463 patient records, including 75 (16.2%) with a Chagas disease diagnosis. A systematic chart review is underway. In a preliminary analysis of 205 patient records, the average age was 47.5 years (SD=14.50); regions of birth include Central America (n=99, 48.3%), North America (n=91; Mexico = 30.7%, US = 13.7%), and South America (n=8, 4.0%). 25 (12.2%) had positive results by initial single *T. cruzi* IgG test. Only 9 underwent appropriate confirmatory testing. Of these, 9 (3.9%) were confirmed positive, and 7 received treatment (with benznidazole). Medical provider specialties ordering initial *T. cruzi* testing included Internal Medicine (n=91, 44.4%), Infectious Diseases (n=37, 18.0%), Cardiology (n=30, 14.6%), and other (n=47, 23.0%); no tests were ordered by Obstetrics. Once data analysis is complete, we will develop recommendations for potential interventions to improve Chagas disease screening and management practices and identify targets for patient and provider education and research.

0382

UTILITY OF SKIN BIOPSIES IN DIAGNOSIS AND PROGNOSIS OF CUTANEOUS LEISHMANIASIS

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The recurrence rate & the number of patients with partially healed cutaneous leishmaniasis (CL) lesions in Sri Lanka is just the tip of an iceberg. The major challenge in curing a cutaneous leishmaniasis patient completely in a Sri Lankan hospital setting is ensuring adherence to the weekly treatment sessions. This study evaluated the findings from skin biopsies obtained from patients with CL in relation to diagnosis & as a prognostic marker. 116 CL suspected patients were recruited to the study & 3mm punch skin biopsies were obtained from the edge of their lesions. Fresh biopsies were gently rolled over the microscopic slides to create impression smears. Histological examinations were made using the FFPE tissue blocks. Patients were followed up until the end of treatment & the number of sodium stibogluconate (SSG) doses required for complete healing was recorded. Biopsy impression smears had a positivity rate of 86.2% indicating 100 positive patients while standard slit skin smears (SSS) had a positivity rate of 88.8% reporting 103 patients. The number of SSG doses required for complete healing ranged from 7 to 21 doses with a mean of 12.2±0.622 doses (nearly 4 months). Patients with heavy parasite loads required more than 13 treatment doses for complete cure *(p=0.001)*. Intensity of the inflammation (*p=0.008)*, number of macrophages (*p=0.001)* & epidermal atrophy (*p=0.033*) were associated with the parasite load. Granuloma formation & epidermal acanthosis were features of low parasite loads. Higher parasite loads and epidermal acanthosis were more frequent in delayed responders. Detection rates of impression smears were similar to slit skin smears suggesting impression smears to be a reliable diagnostic tool in settings where technicians lack expertise in obtaining SSS. Histology findings may be used to predict delayed responders to treatment which can thus be applied to monitor patients more closely to assess the need for alternative treatment.

0383

INCREASED LEVELS OF CHEMOKINES AND CYTOKINES MEDIATORS OF EOSINOPHIL AND T CELL RECRUITMENT IN SUBJECTS WITH CHRONIC CHAGAS DISEASE WITH HYPERSENSITIVITY REACTIONS TO BENZNIDAZOLE

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Chagas disease is caused by the intracellular parasite *Trypanosoma cruzi* and it is the main cause of infectious myocardiopathy in the world. Benznidazole and nifurtimox are the two drugs approved for the treatment of Chagas disease. A suspension rate of approximately 17-30 percent primarily due to the appearance of severe dermatitis is observed during treatment with benznidazole in chronic Chagas disease. In this study, 49 adult subjects with diagnosis of *T. cruzi* infection were recruited prescribed for etiological treatment. The treatment consisted of benznidazole administered at 5 mg/kg body weight per day for 30 days. All subjects with severe dermatitis discontinued benznidazole therapy. Through cytometric bead assays and ELISA capture techniques, we measured the levels of pro-inflammatory cytokines, pro-apoptotic molecules and mediators of activation, differentiation and migration of eosinophils and T cells in sera of *T. cruzi*-infected subjects treated with benznidazole who exhibited skin adverse events (n=23) and compared with those without adverse events (n=26). The amounts of IL-5, soluble FAS-L, IP-10 and MIG significantly increased at 7-30 days post-treatment and decreased thereafter, in subjects with dermatitis but not in subjects without dermatitis, compared with uninfected subjects. Additionally, granzyme B levels increased in a proportion of the treated subjects who had skin adverse events. In contrast, normal levels of eotaxin were observed in treated subjects with or without dermatitis. The levels of different hematologic parameters, cholesterol and IgE were not found to be predictors of adverse drug reaction. These results support a delayed type hypersensitivity reaction to drugs, with eosinophils and T cell recruitment and release of cytotoxic molecules.

0384

PROFILE OF COVID-19 POSITIVE PATIENTS ADMITTED FROM MARCH TO JUNE 2020 AT THE SINO-CONGOLESE FRIENDSHIP HOSPITAL IN N'DJILI FROM KINSHASA, DEMOCRATIC REPUBLIC OF THE CONGO

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The recent emergence of COVID-19 worldwide, including in the Democratic Republic of Congo has led to a better understanding of the mode of transmission, severity, clinical features, and risk factors of the infection. Th study was undertakenTo determine the epidemiological, clinical, paraclinical and therapeutic profile of COVID-19 positive patients followed at the Sino-Congolese Friendship Hospital. A descriptive study carried out on a group of on a group of 83 COVID-19 positive patients from March 10 to June 30, 2020. The mean age was 46 years of while 63% are under 50. Sixty-two patients (75%) were not severe cases, and twenty-one patients (25%) were severe cases. Male gender was predominant (63%) and the sex ratio (M/F) was 1.68%. Thirty percent of the patients had comorbidities of which high blood pressure was predominant (20.5%). Symptoms were dominated by the tetrad "fever-respiratory distress signs-headache-cough" of which fever was predominant (39.8%). The radiographic sign was basal interstitial lung disease (2.4%) and the CT sign was ground glass (1.2%). The case fatality rate was 12.3%. Fifty patients (60%) were treated with (Chloroquine +azithromycin + vitamin C + zinc) and twenty-three patients (27%) treated with (amoxicillin-clavulanic acid + azithromycin) were cured. This study, carried out in the context of the pandemic, showed that age over 50 years appears to be strongly associated with the development of acute respiratory disease.

0385

IN VITRO ACTIVITY OF MYCOBACTERIOPHAGES AGAINST MYCOBACTERIUM TUBERCULOSIS BIOFILMS

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Tolerance of *Mycobacterium tuberculosis* to antibiotics has been associated with its tendency to grow in cell aggregates also known as biofilms. These biofilms are thought to favour the persistence of *M. tuberculosis* in the body of their hosts by physically shielding the infectious bacilli from antibiotics and the host immune defences; providing a platform for quorum sensing, as well as harbouring mycolic acids, an important

carbon source in the 'persister state'. This highlights the need for targeting biofilms when treating persistent tuberculosis. This study evaluated the activity of mycobacteriophages (viruses of mycobacterium) against biofilms of *M. tuberculosis*. Mycobacteriophages (phages) were isolated from soil and sewage collected along the Nakivubo sewage channel in Kampala, Uganda. The phages were characterized based on their plaque morphologies, adsorption time, latent period and burst size. The spot test was used to determine the mycobacteriophage host range against 60 clinical isolates of *M. tuberculosis,* obtained from both the pulmonary and extra-pulmonary compartments. Phage-susceptible *M. tuberculosis* isolates were cultured on MBEC® plates for one and two weeks in order to establish active and mature biofilms respectively. These biofilms were infected with a cocktail of three broad-host-range phages at multiplicities of infection (MOI) 100, 10, 1, 0.1 and 0.01. Phage anti-biofilm activity was expressed as a percentage reduction in biofilm formation in the no phage treated biofilms. Mycobacteriophages reduced biofilm formation up to 40% in active biofilms and up to 30% in mature biofilms. Anti-biofilm activity of the mycobacteriophages was directly proportional to the MOI used. Although pulmonary *M. tuberculosis* isolates were more susceptible to phage infection compared to the extra-pulmonary, there was no significant difference in anti-biofilm activity against pulmonary and extra-pulmonary isolates. This study has demonstrated the potential of mycobacteriophages as anti-biofilm agents. However, the underlying mechanisms for the anti-biofilm activity reported here are yet to be understood.

0386

EPIDEMIOLOGICAL ANALYSIS OF TUBERCULOSIS IN ADULTS IN OTTAWA, CANADA FROM 2009-2018

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All adult cases of tuberculosis (TB) in Ottawa are referred to the Regional Tuberculosis Clinic at The Ottawa Hospital (TOH) for treatment and management. Prior to this study, there was no comprehensive database of local TB data at TOH. This study reported informative data to improve the quality of care for TB patients. A retrospective chart review of TB cases at TOH from 2009-2018 was completed to report on demographics, TB risk factors, clinical history, diagnostics, antimicrobial resistance (AMR), treatment, and clinical outcomes. A total of 399 cases (77.4% foreign-born; 8.5% Indigenous) were seen at TOH. The average annual incidence was 40 cases (4.2/100,000). HIV co-infection was present in 4.3%. Diabetes was present in 12.5%. Pulmonary-involved TB comprised 55.1% of cases. AMR was identified in 9.3%. Directly observed therapy (DOT) was implemented in 86.2%. TB rates in Ottawa are slightly lower than the national average. Foreign-born patients comprised a majority of TB cases. Out of Canadian-born cases, Indigenous peoples were disproportionately affected by TB. Cases with diabetes and HIV had increased mortality and morbidity. Antimicrobial stewardship and DOT remained essential.

0387

COMPLETE GENOME SEQUENCE OF BORDETELLA PERTUSSIS STRAINS IN THE AMHARA REGIONAL STATE, ETHIOPIA

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Pertussis, or whooping cough is an acute respiratory tract disease caused by *Bordetella pertussis*. In 2014, globally, there were an estimated 24.1 million pertussis cases and 160,700 deaths from pertussis in children younger than 5 years. Due to vaccine pressure there have been genotype changes in the circulating *B. pertussis* strains. The aim of this study was to determine distribution of *B. pertussis* strains in the Amhara Regional State, Ethiopia. Methods: Cross-sectional study design was employed on clinically suspected pertussis patients. Sixteen DNA samples these positive for IS*481* (Ct<28 cycles) were sequenced using next generation illumina whole genome sequencing. The sequenced data was analyzed using software's and sequenced data was compared with reference strains using BLAST and phylogenetic tree. The ptxP1, fim4 and prn1 alleles were found in 11/15 (73%) of sequenced samples. The remaining four samples (27%) carried the ptxP3, fim2 and prn2 alleles. None of the samples carrying the ptxP1 allele was found to carry the fim2 or prn2 allele and none of the samples carrying the ptxP3 allele was found to carry the fim4 or prn1 allele. The ptxP1 allele carrying *B. pertussis* was the commonest strains circulating in the Amhara Regional State, Ethiopia. The increased number of ptxP3 strains in Ethiopia indicates that the shift from ptxP1 to ptxP3 strains. This ptxP allele shift needs further investigation whether it is associated with the type of vaccine used or not.

0388

SETTING UP A TUBERCULOSIS RESEARCH DATABASE IN ZIMBABWE THROUGH THE NATIONAL TUBERCULOSIS PROGRAM

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Zimbabwe is a high-burden country for Tuberculosis (TB), Multi-drug resistant tuberculosis (MDR-TB) as well as Tuberculosis/ Human Immunodeficiency Virus (TB/HIV) co-infection. The Government of Zimbabwe is committed to ending TB by 2030. By 2025, the National Tuberculosis Program (NTP) aims to reduce TB incidence and mortality by 80%. To achieve this goal, a Tuberculosis Research Technical Working Group (TBR TWG) was created to support the NTP through working in consultation with various stakeholders to scale-up evidence-based programmes through operational in-country research. The TBR TWG facilitated stakeholder meetings to establish an inventory of current TB research to identify gaps, avoid duplication of research and allow smooth coordination on mapping the way forward. This process involved creating a TBR database which the country never had before. The TBR database was created through a literature extraction which was done to capture published work. Other methods which will be employed to feed into the TBR database include conducting an online survey and seeking for researches done through the Medical Research Council of Zimbabwe (MRCZ) to capture program work in operational research which has not published. A literature extraction was conducted by searching two major databases PubMed and Google Scholar, to identify articles pertinent to Tuberculosis in Zimbabwe from 2010 to date. Over 90 publications were found and computed into the TBR database. The database includes various information such as authors, titles, thematic areas, study setting, year research was done, year of publication, funding and publication link. We went on to develop the online survey using google forms. A stakeholder mapping exercise will be done first, to send the survey via email and the social media WhatsApp platform. Information obtained from both the survey and MRCZ will be consolidated and merged into the TBR database. We will eventually create an online repository, by hosting the TBR database on a server under the Ministry of Health and Child Care of Zimbabwe. The online repository will be accessible to everyone interested in TB research in Zimbabwe.

0389

EFFECT OF INDOOR AIR QUALITY ON NEONATAL DEATH DUE TO PNEUMONIA: FINDINGS FROM BANGLADESH DEMOGRAPHIC AND HEALTH SURVEY DATA

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Improving child survival in low and middle-income countries faces a major difficulty due to death of newborn child within first 28 days of their birth. In Bangladesh neonatal mortality rate higher than global, 41 vs 31 per

1000 live births. Pneumonia is an inflammatory condition of the lungs caused by infection, which could be triggered by higher exposure to the particulate matter (PM2.5) and is the third leading cause of neonatal death in Bangladesh. Among the top 10 countries with the highest level of Ambient PM2.5, produced by all types of combustion common in urban and rural places, Bangladesh is in the ninth position. Household cooking fuel is one of the prime sources of PM2.5 in indoor settings. We explored association between neonatal death in pneumonia and mothers' and neonates' exposure to PM2.5 in indoor settings. Exposure to PM2.5 is measured by the type of cooking fuel used in the household. An indoor cooking with unimproved biomass fuel is defined as high exposure to PM2.5. We designed an unmatched case-control study from Bangladesh Demographic and Health Survey 2017-18. Deaths due to pneumonia were extracted from verbal autopsy module and then, randomly selected controls from the same geographic area where death occurred. We fitted a logistic regression model to estimate the association between neonatal death and exposure to PM2.5. Findings shows that, among the neonates, who died due to pneumonia is 1.2 (95% CI 0.6-2.7) times more likely to have higher exposure to PM2.5 while adjusting by potential covariates. The major portion of the confidence interval is above one which indicates a higher risk, though the association is statistically not significant due to smaller sample size. The results suggests that neonatal death due to pneumonia could be explain by higher exposure to PM2.5 in indoor settings. A larger longitudinal study to quantify level of PM2.5 exposure during pregnancy and after birth in the household setting could reveal more insights about the association. The result presented here will serve as an important basis for such future research.

0390

FACTORS ASSOCIATED WITH COMMUNITY ACQUIRED SEVERE PNEUMONIA AMONG UNDER FIVE CHILDREN IN DHAKA, BANGLADESH: A CASE CONTROL STUDY

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Pneumonia is the leading cause of death in children globally with the majority of these deaths observed in resource-limited settings. The annual incidence of clinical pneumonia in children under 5 years of age is approximately 152 million each year worldwide, mostly in the low- & middle-income countries. Of these, 8.7% progressed to severe pneumonia requiring hospitalization. However, data to predict children at greatest risk to develop severe pneumonia from pneumonia are limited. Secondary data analysis was performed after extracting relevant data from a prospective cluster randomized controlled clinical trial; children of either gender, aged two months to five years with pneumonia or severe pneumonia acquired in the community were enrolled over a period of three years in 16 clusters in urban Dhaka city. The analysis comprised of 2,597 children aged 2-59 months. Of these, 904 & 1693 were categorized as pneumonia (controls) and severe pneumonia (cases), respectively based on WHO criteria. The median age was 9.2 months (range, 5.1-17.1) and 1,576 (60%) were male. After adjustment for covariates, children with temperature ≥38 °C, duration of illness ≥3 days, presence of crepitation, male gender, and severe stunting showed a significantly increased likelihood of developing severe pneumonia compared to those with pneumonia. Severe pneumonia in children occurred more often in older children who presented commonly from wealthy quintile families, and who often sought care from private facilities in urban settings. Male gender, longer duration of illness, fever, crepitation, and severe stunting were significantly associated with development of WHO-defined severe childhood pneumonia in our population. The results of this study may help to develop intervention targets to reduce morbidity and mortality. 1

0391

WHICH IS BETTER TREATMENT OPTION IN SEVERE PNEUMONIA: INTRAVENOUS AMPICILLIN OR AMOXICILLIN? AN OPEN-LABEL, RANDOMIZED, CONTROLLED TRIAL IN BANGLADESH

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To treat severe pneumonia, intravenous ampicillin needs to be administered at a six-hourly interval, which requires frequent nursing intervention and bed occupancy for 5-7 days, limiting its utility in resource-poor settings. We compared the efficacy of a potential alternative drug, intravenous amoxicillin, which requires 12 hourly administration and less frequent nursing intervention and can be switched to oral form after clinical improvement. We conducted an unblinded, randomized, controlled, non-inferiority trial in the Dhaka Hospital of icddr,b. Children from 2-59 months of age presenting with WHO-defined severe pneumonia with respiratory danger signs were randomly assigned 1:1 to either 50 mg/kg ampicillin or 40 mg/kg amoxicillin per day 7.5mg/kg gentamicin. The primary outcome was treatment failure as per the standard definition of persistent danger sign(s) of severe pneumonia beyond 48 hours or deterioration within 24 hours of therapy initiation. We undertook an intention-to-treat analysis to compare treatment failure. The trial is registered in Clinicaltrials.gov (NCT03369093) and received institutional ethical approval. We enrolled 308 participants, 154 in each arm, between 1st January 2018 to 31st October 2019. Baseline characteristics were similar among the two groups. Sixty-two (20%) children ended up with treatment failure, 21 (14%) in amoxicillin, and 41 (27%) in ampicillin arm. A significantly lower proportion of children in the amoxicillin arm experienced treatment failure than the ampicillin arm (relative risk [RR] 0.22, 95% CI 0.23-0.81; p=0.004). We reported 14 deaths for serious adverse events, 4 (3%) and 10 (6%) among amoxicillin and ampicillin arm, respectively, which was not statistically significant. Intravenous amoxicillin plus gentamicin combination is not non-inferior to intravenous ampicillin plus gentamicin in treating severe pneumonia in under-five children in Bangladesh. Considering the less frequent dosing and more compliance, parenteral amoxicillin is a better choice for treating children with severe pneumonia in resource-limited settings.

0392

ASPIRATION PNEUMONIA REVIEW: CASE REPORTS FROM KENYA CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE NETWORK (CHAMPS) PROGRAM

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Aspiration pneumonia (AP), thought to be related to swallowing difficulties, is common in children with neurological or complex medical conditions. Children with AP have longer hospitalizations, higher intensive care unit admission and readmission rates compared to children with non-aspiration pneumonia, but data characterizing AP deaths in Africa are lacking. We describe AP among child deaths enrolled in the Kenya Child Health and Mortality Prevention Surveillance (CHAMPS) Network. CHAMPS is a multi-country surveillance program that systematically identifies causes of under-five mortality from defined catchment areas. Between May 2017 and December 2020, causes of death (COD) were determined by a panel of experts for 325 deceased children 0-59 months in Kenya using data from post-mortem minimally invasive tissue specimen

testing, clinical records, and verbal autopsy. Twenty-five (7.7%) had AP in the causal chain (15 as immediate COD and 10 as underlying COD). Four (16%) AP cases were in neonates, 13 (52%) in infants, and, 8 (32%) in children (12-60 months). Ten (40%) cases died in the community while 15 in the hospital. Fifteen of 19 (79%) children with AP as immediate COD had a condition that likely increased aspiration risk or poor outcome from pneumonia, including: gastroenteritis (3), malnutrition (2), chronic malaria (2), down syndrome (1), cleft palate and lip (1), cerebral palsy (1) and convulsive disorder (1). Of the 24 cases with lung tissue findings, the most common included interstitial inflammation (21/24, 88%), increased alveolar macrophages (18/24, 75%), hyaline membranes (5), alveolar neutrophilic infiltrate (4) and intra-alveolar hemorrhage (3). Four cases had super-imposed pneumonia with bacterial (2) and viral (2) etiologies. Only eight (50%) children had a diagnosis of AP ante-mortem. CHAMPS revealed AP—associated pediatric deaths that would have otherwise been unreported. Given medical complexity of managing children with AP, a high index of clinical suspicion and routine clinical audits of child deaths should be encouraged to identify and manage aspiration pneumonia cases.

0393

GENOMIC SURVEILLANCE OF SARS-COV-2 IN A PACIFIC MILITARY POPULATION

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged as a novel respiratory virus in December 2019. It is important to characterize variant strains to assess the mutagenesis and evolution of the virus circulating in geographical locations where our service members are stationed. Routine clinical diagnostics are unable to differentiate variant SARS-CoV-2 strains; therefore, whole genome characterization is critical for surveillance. In this study we characterized positive nasopharyngeal specimens submitted to Tripler Army Medical Center (TAMC) on Oahu, Hawaii. Genomes were analyzed using the Illumina Next-Generation Sequencing (NGS) on the MiSeq platform to align sequence reads to the SARS-CoV-2 reference, NCBI RefSeq NC_045512, and construct consensus sequences from the mapped reads. Initial analyses were conducted with Geneious Prime v2021.1. Clade assignments were performed using Nextclade, and lineages established using Pangolin. Thus far, 74 full SARS-CoV-2 genomes of samples collected from March 2020 to April 2021 have been sequenced. Clades 20A, 20B, 20C, 20G, and 19B were represented, with nonsynonymous mutations relative to the reference. The G614D amino acid (AA) and P681H AA mutations were the dominant nonsynonymous changes in the spike protein, found in almost all genomes sequenced. Identified variants of concern include B.1.1.7 and B.1.429. Multiple B.1.526.2 variants were also present. Of note, a potential novel unassigned lineage containing the critical N501Y substitution in the spike protein, along with a large ORF8 deletion, was also observed. These data demonstrate that NGS is an important tool to investigate the role of viral adaptation in the transmissibility and pathogenicity of SARS-CoV-2. Increased genetic surveillance capabilities in unique geographical locations like TAMC will significantly shorten the turn-around-time for rapidly informing the public health community of any concerning genetic changes.

0394

COMPARATIVE DIAGNOSTIC POTENTIAL OF SCHISTOSOMA MANSONI-DERIVED ANTIGENS FOR CHRONIC INTESTINAL SCHISTOSOMIASIS IN LOW ENDEMIC AREAS

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The study aimed to determine the schistosomiasis diagnostic potential of crude antigens prepared from different stages in *Schistosoma mansoni* life cycle. *S. mansoni* egg antigen (SEA), *S. mansoni* worm antigen (SWA) and schistosomula crude antigen (SCA) were prepared from the eggs, adult worms and schistosomula stages respectively. The diagnostic potentials of SEA, SWA and SCA were evaluated using ELISA test. Either Kato-Katz or saline gradient method or both were employed as the diagnostic reference. The areas under the ROC curve were 0.90 (CI 0.82 - 0.98, $p < 0.0001$), 0.96(CI 0.90-1.00, $p < 0.001$), and 0.95 (CI 0.88-1.01, $p < 0.0001$) for SEA, SWA and SCA respectively. The sensitivity of SWA and SEA was similar (90%) but was lower than that of SCA (96.7%). The human IgG-specific responses against the S. mansoni-derived antigens were significantly higher in *S. mansoni* infected individuals compared to the non-infected population ($p < 0.0001$). Our study showed that SEA, SWA, and SCA are reliable diagnostic tools for chronic schistosomiasis.

0395

NEW AVENUES FOR THE VISUAL DIAGNOSIS OF FEMALE GENITAL SCHISTOSOMIASIS

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Schistosoma haematobium infection may lead to Female Genital Schistosomiasis (FGS) in women and girls. FGS is associated with abnormal vaginal discharge, ectopic pregnancies, and HIV. Due to the range of symptoms, FGS is a differential diagnosis for many gynecological conditions. In rural health care settings, where syndromic diagnosis is performed, FGS is consistently misdiagnosed and treated as cancer or sexually transmitted infections. The reference standard for diagnosis of FGS is a thorough inspection for lesions on the cervix, fornices, and the vaginal walls with a stationary colposcope. However, the colposcope is not practical in resource-limited settings as it is expensive, requires extensive training, and electricity. Our aim was to systematically review handheld equipment which could potentially be used for point-of-care diagnosis of FGS, and to assess the quality of the evidence. We searched Medline and Embase 2015–2020 for handheld devices used in cervical cancer screening and FGS diagnosis. We excluded studies that did not compare the device to standard-of-care colposcopes or histopathology. We found 11 studies that fit the criteria, where four handheld colposcopes, two smartphones, and one compact digital camera could be evaluated. Some requirements for FGS diagnosis were unaddressed by the studies, such as vaginal wall inspection and adequate lighting for FGS lesions. No device provided automatic lesion recognition. However, the Gynocular and Mobile ODT seemed best suited. For patients from schistosomiasis endemic areas, we suggest that handheld devices could be used in FGS diagnosis. Cervical cancer screening should be done simultaneously. Studies are needed to

determine which of the two devices is most adequate for FGS diagnosis. Further studies are needed to explore computer-assisted diagnosis for FGS, and effective management of FGS.

0396

THE PRAZIQUANTEL IN PRESCHOOLERS TRIAL: STUDY PROTOCOL FOR A PHASE II PK/PD DRIVEN RANDOMISED CONTROLLED TRIAL OF PRAZIQUANTEL IN CHILDREN UNDER FOUR YEARS OF AGE

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Over 200 million individuals worldwide are infected with *Schistosoma* species, with over half of infections occurring in children. Despite most children experiencing first infections early in life impacting their growth and development, praziquantel (PZQ) the only available drug for the treatment of schistosomiasis only has regulatory approval among adults and children over the age of four, but frequently used "off label" in endemic settings. Furthermore, pharmacokinetic/pharmacodynamics (PK/PD) evidence suggests the standard PZQ dose of 40 mg/kg is insufficient in preschool aged children (PSAC). Our goal is to understand the best approaches to optimising treatment of PSAC with intestinal schistosomiasis. We are conducting a randomised, controlled Phase II trial in a *Schistosoma mansoni* endemic region of Uganda and a *Schistosoma japonicum* endemic region of the Philippines (the praziquantel in pre-schoolers (PIP) trial; NCT03640377). Six hundred children, 300 in each setting, aged 12-47 months with *Schistosoma* infection are being randomised in a 1:1:1:1 ratio to receive either (1) 40 mg/kg PZQ at baseline and placebo at six months, (2) 40 mg/kg PZQ at baseline and 40 mg/kg PZQ at six months, (3) 80 mg/kg PZQ at baseline and placebo at six months, or (4) 80 mg/kg PZQ at baseline and 80 mg/kg PZQ at six months. Following baseline treatment, children will be followed up three times over a period of 12 months. The primary outcome is drug efficacy assessed by cure rate and egg reduction rate at four weeks. Secondary outcomes include drug efficacy assessed by novel antigenic endpoints at four weeks, actively collected adverse events and toxicity for 24 hours post-treatment, morbidity and nutritional outcomes at 6 and 12 months, biomarkers of inflammation and environmental enteropathy and PZQ PK/PD parameters. Recruitment has started in Uganda in April of 2021. In conclusion, the PIP trial will provide valuable information on the safety and efficacy of the 80 mg/kg PZQ dose in PSAC, and on the impact of six-monthly versus annual treatment, in this vulnerable age group.

0397

MULTIPLE ANTIGEN BLOT ASSAY AS AN ANTIBODY SCREENING TECHNIQUE FOR SCHISTOSOMIASIS MANSONI IN RESOURCE-LIMITED SETTINGS

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Schistosomiasis is a leading cause of morbidity in Sub-Saharan Africa and South America, with about 207 million estimated cases per year. Like other helminthiasis, human exposure is related to occupations usually performed by low-to-middle income populations. Therefore, rapid and inexpensive testing for schistosomiasis in limited-resource settings is paramount. Multiple Antigen Blot Assay (MABA) is a simple, reproducible, and inexpensive technique that can detect IgG against up to 26 antigens simultaneously, including *Schistosoma mansoni* and can be used for screening in asymptomatic individuals. 122 African medical students who arrived in Venezuela for an educational exchange program were tested for serum IgG against 3 antigens of *Schistosoma mansoni* (adult worm soluble antigen, soluble egg antigen and the synthetic peptide IMT180/1086 from Sm31 cathepsin B protein). Briefly, the antigens were loaded onto a nitrocellulose membrane and were exposed to serum from the subjects. After washing, the membranes were incubated with secondary antibodies and detected using chemiluminescence. Reactivity against any of the three antigens was considered as a positive result. All individuals were screened using both SMP-ELISA and MABA, and positive cases were confirmed with Circumoval Precipitin Test (COPT) (gold standard). 78 individuals were seropositive by SMP-ELISA and 12 were positive by MABA. All individuals that had positive serology by MABA were also SMP-ELISA-positive. Using COPT as the confirmatory test for active infection, 6 patients were confirmed to carry *Schistosoma mansoni*. MABA appears to be an inexpensive and reliable method for screening of schistosomiasis in resource-limited settings, even more so than SMP-ELISA. Discordance between SMP-ELISA and MABA results needs further research, but the latter appears to be more specific based on our results. The simultaneous evaluation of multiple antigens (synthetic peptides, crude antigens and purified or recombinant proteins) in MABA makes it more appealing for massive screening purposes, which is needed to adequately manage a disease with such severe implications.

0398

DEVELOPMENT OF A REAL-TIME PCR ASSAY FOR DETECTION OF SCHISTOSOMA JAPONICUM IN HUMAN STOOL

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Schistosomiasis is a debilitating Neglected Tropical Disease estimated to infect at least 230 million people worldwide. Thus, its elimination requires sensitive diagnostic tools to accurately determine prevalence of infection in endemic areas. *Schistosoma japonicum*, the most virulent of the schistosomiasis-causing parasites, has historically been detected using the Kato-Katz technique, a method shown to radically underestimate levels of infection. However, the ever-improving capabilities of next-generation sequencing and bioinformatic analysis tools have enabled the development of sensitive molecular diagnostic assays with nucleic acid targets. Utilizing Tandem Repeat Analyzer (TAREAN) and RepeatExplorer2, we performed a cluster-based analysis of the *S. japonicum* genome to identify a tandemly arranged genomic repeat which was selected as the target for a real-time PCR diagnostic assay. Upon optimization, this species-specific assay consisting of a primer/probe set can reliably detect as little as 200 ag of *S. japonicum* genomic DNA and as little as 1 egg per gram of human stool. While additional validation studies using field samples are required, this extremely sensitive diagnostic assay should facilitate the accurate detection of *S. japonicum*, particularly in regions with low levels of endemicity, appropriately aiding disease control and elimination efforts.

0399

STUDIES RELATED TO FIBROSIN, A CYTOKINE

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We have identified a novel heparin binding cytokine. Fibrosin. It was originally identified from activated T cells from granulomas of mice infected with S. mansoni. It affects hepatic fibrosis in infected mice and other pathologies. Binding studies using biotinylated fibrosin identifies bound ligands with a molecular mass of 25 to 40 kD. These could be receptor ligands and may have therapeutic applications in fibrosis and other autoimmune disease. We are also interested in developing biomarker related studies.

0400

THERAPEUTIC ACTIVITY OF A SALMONELLA-VECTORED SCHISTOSOMA MANSONI VACCINE IN A MOUSE MODEL OF CHRONIC INFECTION

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Schistosomiasis is an important fresh-water-borne parasitic disease caused by trematode worms of the genus *Schistosoma*. With >250 million people infected worldwide and approximately 800 million people at risk, the World Health Organization considers schistosomiasis to be the most important human helminth infection. Several prophylactic non-living vaccines are in pre-clinical and clinical development, but only one has been assessed for therapeutic effect in an animal model with modest results. Live attenuated *Salmonella* have multiple potential advantages as vaccine vectors. We have engineered an attenuated *Salmonella enterica* Typhimurium strain (YS1646) to produce a vaccine that targets the parasite digestive enzyme Cathepsin B (CatB). A multi-modality immunization schedule was used in chronically infected mice that included three oral (PO) doses of this CatB-bearing YS1646 strain on days one, three, and five as well as an intramuscular (IM) dose of recombinant CatB on day one. Parasite burden (worm count, intestinal and liver egg numbers) were 46.5 – 50.3% lower than in control animals 1 month post-vaccination and relative reductions further increased to 63.9 – 73.3% at 2 months. Serum anti-CatB IgG increased significantly after vaccination with the development of a more balanced T_H1/T_H2 pattern of response (ie: a shift in the IgG1:IgG2c ratio). Compared to control animals, a broad and robust CatB-specific cytokine/chemokine response was seen in splenocytes isolated 1-month post-vaccination. A vaccine that has both prophylactic and therapeutic activity would be ideal for use in conjunction with mass treatment campaigns with praziquantel in schistosome-endemic countries.

0401

MORPHOLOGICAL AND GENOMIC CHARACTERIZATION OF PARAGONIMUS FROM CAMEROON INDICATE A DISTINCT CLADE OF LUNG FLUKES

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Paragonimus lung flukes are important food-borne trematodes that cause serious diseases. More than 30 *Paragonimus* species have been described from Asia, Africa and the Americas, and about one third of them is confirmed to infect humans with an estimated 21 million infections. *Paragonimus* species are widely distributed in wildlife in Africa, but only *P. uterobilateralis* and *P. africanus* have been documented to infect humans and only a 445 bp DNA sequence of *P. africanus* is available in GenBank. In order to improve diagnostics, to better understand the epidemiology of paragonimiasis and to develop new intervention strategies, detailed phylogenetic and phylogeographic information is crucial. Therefore, we isolated metacercariae from freshwater crabs collected in areas endemic for human paragonimiasis in the South West Province of Cameroon, infected rats and recovered adult flukes. Detailed morphological examination of several specimens showed that the flukes are most similar to *P. africanus* with characteristic antler-shaped testis and medium size eggs measuring 102 X 66 µm. Genomic DNA was extracted from 4 specimens and sequenced using Oxford Nanopore and Illumina technology. A mitochondrial genome of 16.6 Kb assembled as a single scaffold contained 12 protein and 24 tRNA coding sequences as well as 3.2 Kb non-coding repetitive DNA comprised of two distinct repeat units. The 934 Mb nuclear genome had a BUSCO score completeness of 84.5%. Based on the ITS2 sequence our specimens were most similar to *P. africanus*, the only African *Paragonimus* species in the database. Comparison of the mitochondrial genomes and selected nuclear sequences of several *Paragonimus* species revealed that our *P. africanus* is more similar to *P. kellicotti* from North America than to *P. westermani* and other Asian species. This study fills a molecular phylogenetic gap in *Paragonimus* species from Africa, links this information with a detailed morphological description, and informs the ongoing comparative genomic analysis with an improved taxonomic sampling to better understand the global diversity of this important pathogen.

0402

SCHISTOSOMA MANSONI - NO LOWER GENITAL TRACT LESIONS{ A STUDY IN CONSECUTIVE CASES WITHOUT S. HAEMATOBIUM

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Female genital schistosomiasis (FGS) constitutes four different lesions known to be caused by *Schistosoma haematobium* ova deposited in the genital tract. Both *Schistosoma haematobium* and *S. mansoni* have been found to be associated with HIV infection. The mechanism for HIV transmission has been thought to be through FGS lesions, such as the sandy patches, rubbery papules and friable blood vessels. *Schistosoma mansoni* ova have been found in the genital tract, however, it is not known if *S. mansoni* also causes the typical FGS lesions. The study was conducted in eight villages along the shores of Lake Victoria, Western Kenya. Stool and urine samples collected from women of reproductive age on three consecutive days were analysed for *S. mansoni* and *S. haematobium*. Willing participants, aged 18 - 50 years, *S. mansoni* positive and *S. haematobium* negative, were invited to answer a questionnaire (demographics, symptoms), undergo a gynaecological examination by an FGS expert and cytology specimen collection. Gynaecologic investigations were conducted in 147 *S. mansoni*-positive women who had a mean infection intensity of 253.3 epg (95% CI: 194.8-311.9 epg). Nearly 90% of them used Lake Victoria as their main water source. None were found to have grainy sandy patches or rubbery papules, 49/147 (33.3%) had abnormal blood vessels, homogenous yellow patches were found in 12/147 (8.2%). Women with homogenous yellow patches were significantly older (47 years) than the rest (34 years, $p = 0.001$). No association was found between intensity of *S. mansoni* infection and homogenous yellow patches ($p = 0.70$) or abnormal blood vessels ($p = 0.14$). *S. mansoni* infection intensity was not associated with genital discomfort, genital itch, post-coital bleeding, bloody or malodorous vaginal discharge. We did not find grainy sandy patches in any women. The homogenous yellow patches could potentially have been a result of an old *S. haematobium* infection. *S. mansoni* infection was not associated with lower genital tract lesions or symptoms typical of FGS.

0403

HEPATIC FIBROSIS BIOMARKING BY PLASMA CYTOKINE PROFILING DURING HUMAN SCHISTOSOMIASIS

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Human and preclinical studies have now convincingly showed that hepatic fibrosis occurs and progresses dissimilarly in Schistosomiasis diseased-individuals with same egg burden, with similar biosocial determinants and subjected to similar environmental conditions. This suggests that parasite-

independent and currently poorly defined host intrinsic factors might play a defining role in the regulation of liver fibrosis. A screening of the literature allow us to report in a review article a library of host regulators of hepatic fibrosis during Human Schistosomiasis. Among those factors, cytokines were the most represented. We then hypothesized that cytokines could play a crucial role in driving hepatic fibrosis (HF) upon infection and could be used as biomarkers to diagnose hepatic fibrosis in Schistosomiasis endemic areas. A cross sectional study was performed in five villages in a previously identified endemic area for *Schistosoma mansoni* (*Sm*) in the Centre region of Cameroon. In total 1,002 participants, all schoolchildren were screened for *Sm* eggs by Kato Katz and for HF in 642 consenting participants using ultrasound. We then clustered our participants in four groups based on their infectious and HF status: i) non infected for *Sm* eggs without HF (controls); ii) non infected for *Sm* eggs with HF; iii) infected for *Sm* eggs without HF; iv) infected for *Sm* eggs with HF. Subsequently, blood samples were collected from 350 compliant participants, screened for other liver affecting diseases prevalent in the study area prior to Luminex analysis. Subsequent to Luminex screening, we identified four cytokines that plasma levels associate with HF and correlated with the severity of fibrosis. Correlation and PLSDA analyses further confirmed the potential of those cytokines as good biomarkers for the diagnostic of hepatic fibrosis. Receiver Operating Characteristic analysis, showed sensitivities above 80.0% for each biomarker and up to 95% when biomarkers were combined in a same model. These results imply that the identified cytokines could be used as a tool for the rapid diagnostic of HF in Schistosomiasis endemic areas.

0404

IDENTIFICATION OF NOVEL MODULATORS OF A SCHISTOSOME TRANSIENT RECEPTOR POTENTIAL ION CHANNEL ACTIVATED BY PRAZIQUANTEL

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Given the worldwide burden of neglected tropical diseases, there is need to develop new anthelmintic agents to strengthen the pipeline of drugs to combat these burdensome infections. Many diseases caused by parasitic flatworms are treated using praziquantel (PZQ),a drug employed for decades as the key clinical agent to treat schistosomiasis. PZQ activates a flatworm transient receptor potential (TRP) channel within the melastatin family ($TRPM_{PZQ}$) to mediate sustained Ca^{2+} influx and worm paralysis. As a druggable target present in many parasitic flatworms, $TRPM_{PZQ}$ is a promising target for a target-based screening campaigns with the goal of discovering novel regulators of this channel complex. Here, we have optimized methods to miniaturize a Ca^{2+}-based reporter assay for *Schistosoma mansoni* $TRPM_{PZQ}$ (*Sm*.$TRPM_{PZQ}$) activity enabling a high throughput screening (HTS) approach. A pilot screen of ~16,000 compounds yielded a novel activator of *Sm*.$TRPM_{PZQ}$, and numerous potential blockers. The new activator of *Sm*.$TRPM_{PZQ}$ represented a distinct chemotype to PZQ, and is a known chemical entity previously identified via phenotypic screening. The fact that a compound prioritized via phenotypic screening is revealed to act, like PZQ, as an *Sm*.$TRPM_{PZQ}$ agonist underscores the validity of $TRPM_{PZQ}$ as a druggable target engaged by antischistosomal compounds.

0405

CHARACTERIZING SPATIAL AND TEMPORAL CHANGES OF MALARIA TRANSMISSION FROM PREGNANT WOMEN AT ANTENATAL CARE VISITS

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Estimating malaria trends directly from passive detection of clinical malaria cases at health facilities remains difficult due to challenges associated with availability of access to care and the estimation of denominator population. Estimates of burden from nationally-representative household surveys are expensive, so that these surveys are typically carried out every 2-3 years and lack power to estimate prevalence and to detect trends when transmission declines to low levels. We present a study comparing the spatial and temporal changes of transmission estimated from pregnant women at first antenatal care visit with those estimated from clinical cases and cross-sectional surveys. The study was conducted in southern Mozambique, comparing data from pregnant women (9,000 samples), cross-sectional surveys (18,000 samples) and from clinical cases between November 2016 and October 2019. Multigravid women had higher *P. falciparum* positivity rates (estimated by qPCR) than primigravid women in high transmission settings, but the difference between both populations decreased for lower transmission levels. Data from pregnant women allowed to detect temporal changes of transmission that are consistent with estimates from clinical cases and at the same time characterise the spatial patterns of transmission identified from cross-sectional surveys. We have calculated the Pearson correlation coefficients (PCC) between the parasite positivity rate estimations from pregnant women and from the cross-sectional surveys. The high values of PCC suggest that malaria transmission patterns in pregnant women represent those from the general population. In high transmission levels, primigravid women show better consistency with children 2-10 years old, but all pregnant women give good consistency with children for low transmission levels, giving more precise measurements and finer time granularity of the estimations than those from cross-sectional surveys.

0406

SCHOOL-AGED CHILDREN ARE THE MOST IMPORTANT CONTRIBUTORS TO THE HUMAN INFECTIOUS RESERVOIR FOR MALARIA IN AREAS OF HIGH AND LOW PLASMODIUM FALCIPARUM TRANSMISSION INTENSITY IN UGANDA

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To determine how transmission intensity influences the infectious reservoir, we quantified parasite carriage and contribution to transmission to mosquitoes longitudinally in two settings of markedly different malaria transmission intensity in Uganda. Cohorts of Ugandan children and adults were recruited from an area under highly effective malaria control in Nagongera, Tororo district (n=531) and from an area of intense malaria transmission spanning the border between Tororo and Busia districts (border area; n=636). Cohorts were followed by continuous passive surveillance and routine assessments every 28 days for 2 years (Nagongera) or 6 months (border area). *Plasmodium falciparum* total parasites and gametocytes will be quantified using molecular techniques; membrane

feeding assays were performed to assess infectivity to mosquitoes. We found that clinical malaria incidence was 0.04 episodes/per person per year (PPY) in Nagongera and 1.3 episodes/PPY in the border area. Parasite carriage at densities above 100 parasites/uL, a level previously associated with transmission potential, was observed in 1.2% of samples in Nagongera and 14.2% in the border area. When mosquitoes fed on blood from these parasite carriers, 1.2% (446/37,404; Nagongera) and 1.8% (215/11,831; border area) of mosquitoes became infected. When feeding on blood from clinical malaria cases, 0.3% (4/1,586; Nagongera) and 1.3% (58/4,499; border area) of mosquitoes became infected. Considering both transmissibility of infections and their relative frequency, school-aged children (5-15 years) comprised 58.7% of the human infectious reservoir in Nagongera and 74.1% in the border area. Adults were considerably less important for the infectious reservoir (15.6% and 0%, respectively in preliminary analyses). Despite marked differences in transmission intensity and clinical malaria incidence, children were responsible for most mosquito infection events in both settings. This suggests that interventions targeting infections in children, including older school-aged children, will have a pronounced effect on community-wide malaria transmission.

0407

SPATIAL AND TEMPORAL CLUSTERING OF MALARIA INCIDENCE AMONG CHILDREN AGED 5 TO 14 YEARS IN KOULIKORO, MALI

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Despite the significant decrease in malaria incidence reported worldwide during the last decade, malaria remains a serious public health problem, particularly in Sub-Saharan African countries, where it is a leading cause of morbidity and mortality. Identifying spatial and temporal variation of malaria hot spots over one transmission season and associated risk can provide evidence-based guidance for efficient implementation of control strategies. This study aims to detect clustering of *Plasmodium (P.) falciparum* malaria cases from the start to the end of malaria transmission in two health districts of Koulikoro: Kati and Kangaba. As part of a cluster- randomized trial, malaria infection incidence was assessed in 20 communities (N=1,100 children aged 5 to 14 years) from July to December 2020. Patient visits were performed every four weeks, and all children with fever or history of fever were tested using rapid diagnostics. Clusters of high and low risk for malaria incidence were analyzed and quantified using the Kulldorff scanning method. Temporal clustering analysis was based on the incidence of malaria infection per person per week in relation with climatic variables (cumulative rainfall, relative humidity, and minimum and maximum temperature). Malaria hot spots showed significant spatial and temporal variation during the malaria transmission season. The risk of malaria infection within clusters varied from August to November, with the highest risk occurring in September and November (RR=5.7 and RR=5.4, respectively). Risk analyses revealed a positive correlation between the number of malaria cases and relative humidity (P<0.001) and a negative correlation between the number of malaria cases and maximal temperature (P<0.001). Testing for spatial and temporal variation of clustering using incidence data is a valuable tool for assisting researchers in understanding the variability of malaria distribution within and between local communities and guiding targeted control interventions.

0408

MALARIA IMPORTATION INTO AN EPIDEMIC-PRONE SETTING IN ARID NORTHWEST KENYA

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Parasite importation can undermine malaria elimination efforts in low-transmission settings. The arid Turkana region in Northwest Kenya has traditionally been unsuitable for sustained transmission, but increased development, land-use changes, and travel to the region are enhancing the ability to permit transmission. In this study, we investigated *Plasmodium falciparum* importation events and their impact on local transmission, and we hypothesized that importation of malaria genotypes was driving local transmission in the region. To do this, we collected parasites in two groups, using active case detection in passengers on the few weekly bus trips and plane flights into the region (n = 1891) and using re-active case detection following RDT-positive infection at 6 clinics in index patients (n = 1891) in their household members in and around Lodwar (n = 3314). All participants provided detailed travel histories. We genotyped all parasites using PCR amplification and amplicon deep sequencing of polymorphic *P. falciparum* targets *pfcsp* and *pfama1,* for which we inferred haplotypes using established methods. Despite some individuals traveling from areas of high malaria transmission, the prevalence of *P. falciparum* was much lower among bus and plane passengers (6.72% [95% CI: 5.65% - 7.96%]) compared to local household members (30.7% [29.2% - 32.3%]) as measured by PCR. Overall, we observed 72 *pfcsp* haplotypes and 88 *pfama1* haplotypes. The mean (standard deviation) multiplicity of infection based on *pfcsp* in bus and plane passengers was 3.3 (2.9), significantly higher than that of individuals residing in Turkana (1.8 (1.5)), with similar patterns observed for *pfama1*. A small number of facility cases and household members also reported travel in the month prior to sample collection (157/5205). Using computational modeling methods and parasite genotype data, we detected *P. falciparum* importation events due to travel and estimated their contributions to local transmission. These analyses can inform the design and management of interventions to mitigate parasite importation and control local malaria transmission.

0409

IMPACT OF FOURTEEN-DAY PRIMAQUINE RADICAL CURE ON PLASMODIUM VIVAX RELAPSE RATES AMONG PUBLIC SECTOR PATIENTS IN CAMBODIA: AN INTERRUPTED TIME SERIES ANALYSIS

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Cambodia has accomplished an 86% decline in malaria cases from 2010 to 2020 and aims to eliminate all malaria by 2025. However, a significant proportion of remaining infections are due to *Plasmodium vivax* (90% of total cases in 2020), historically treated using the ACT first-line treatment which does not cure the liver stage of parasite development, risking relapse. In November 2019, Cambodia introduced 14-day primaquine for treatment of *P.vivax* infections (known as radical cure) in G6PD-normal males across four provinces to fully treat *P. vivax* and prevent relapse. An interrupted time series analysis was conducted to estimate the impact of radical cure implementation on relapse cases compared to a control

group of five provinces with similar *P.vivax* incidence. Patient records were compiled from January 2018 to December 2020 to match key patient identifying fields (name, sex, age and village of residence) by calculating a Jaro-Winkler similarity score. If a matched patient had received a prior diagnosis within one to nine months, they were considered a relapse case. Matches with a difference of 30 days or fewer were excluded to account for potential treatment failure. Of a total 31,175 *P.vivax* patient records with complete identifying field information, 13,047 (41.8%) met the criteria for relapse cases. Post-intervention, the trend in relapse cases decreased by 15.8 ($p<0.001$) cases per month in the intervention group relative to the control group. In the fourteenth month following rollout the average monthly relapse cases in radical cure provinces were 286 fewer than would have been expected if radical cure had not been implemented, representing a 90% reduction in relapse cases. The proportion of observed relapse rates decreased from 59.2% pre-intervention to 53.3% post-intervention in radical cure provinces; however, this may underestimate the effect of radical cure and with a 9-month relapse period it should take additional time to see the impact reflected in observed data. As Cambodia scales up radical cure to all provinces in 2021, radical cure treatment may serve as a catalyst for progress towards national elimination of *P.vivax* infections.

0410

DELETIONS OF PLASMODIUM FALCIPARUM PFHRP2 AND PFHRP3 GENES FROM PERSONS PRESENTING TO HEALTH FACILITIES IN THE DEMOCRATIC REPUBLIC OF THE CONGO, ETHIOPIA, KENYA, MADAGASCAR, AND RWANDA, 2016-2018

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Histidine-rich protein 2 (HRP2) and HRP3 proteins are highly expressed throughout blood stage *Plasmodium falciparum* infection, with diagnostic antibodies binding to repeat epitopes on each. Thus, HRP2-based rapid diagnostic tests (RDTs) are widely used throughout sub-Saharan Africa (SSA) to diagnose *P. falciparum* malaria. However, multiple SSA countries have reported *pfhrp2* and *pfhrp3* (*pfhrp2/3*) gene deletions, which threaten the validity of HRP2-based RDTs. To evaluate the presence of *pfhrp*2/3 deletions, we used 1109 dried blood spot (DBS) samples from Democratic Republic of Congo (DRC), 147 from Ethiopia, 332 from Kenya, 620 from Madagascar, and 218 from Rwanda collected as part of routine therapeutic efficacy studies (TES) conducted between 2016-2018 from individuals presenting to health facilities with microscopically confirmed, uncomplicated *P. falciparum* mono-infection. Using multiplex antigen detection, DBS samples from day of enrollment were screened for low HRP2 expression compared to pan-LDH and pan-aldolase antigens; *Pfhrp2/3* genotyping was carried out based on antigen profile. No deletions were found in Kenya. *Pfhrp2* single deletions were identified in DRC, Ethiopia, and Madagascar in 0.3%, 2.0%, and 0.6% of screened samples, respectively. No *pfhrp2* deletions were found in Rwanda. *Pfhrp3* single deletions were found in 0.3%, 2.7%, 0.2%, and 0.5% of screened samples from DRC, Ethiopia, Madagascar, and Rwanda, respectively. Dual *pfhrp2* and *pfhrp3* deletions were observed only in Ethiopia (2.0% of samples). Although our study was not designed or powered for precise prevalence estimates, we found low levels of *pfhrp*2/3 deletions (<5%) in the samples from each of the TES study sites in four SSA countries. In Pawe, Ethiopia, we detected deletions in 3.4% of samples, which suggests that close monitoring of *pfhrp2/3* deletions in this region is needed. TESs are routinely conducted throughout SSA and provide a set of samples from children with microscopically confirmed infection. Testing samples from TESs for *pfhrp2/3* deletions may be a useful screening approach.

0411

PLASMODIUM FALCIPARUM MALARIA SURVEILLANCE IN GUYANA USING WHOLE-GENOME AND MICROHAPLOTYPE ANALYSIS

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Malaria control in South America is challenged by a resurgence of cases within Venezuela and cross-border spillover via migrant workers and refugees. Molecular surveillance is especially urgent in neighboring Guyana, where gold mining activities are proliferating through remote malaria-endemic regions and, alarmingly, *de novo* emergence of artemisinin resistance-associated kelch13 mutation (C580Y) has been detected in a clonal subpopulation of *Plasmodium falciparum* parasites. The present study applies a new multiplexed amplicon sequencing protocol (128 *Plasmodium falciparum* microhaplotypes simultaneously amplified in a single PCR reaction) for rapid molecular surveillance in this context. We apply the new tool to monitor the persistence and possible expansion of C580Y-carrying parasites in >1000 *P. falciparum*-infected blood samples collected from individuals in Guyana in 2020-21. We intersect the amplicon sequencing results with whole-genome sequencing (WGS) data from 2016-17 (>800 samples) and find that different clonal clusters predominate between the two time points, suggesting stochastic boom-bust dynamics that can skew haplotype estimates inferred from cross-sectional sampling. The spatiotemporal dynamics we describe also inform maps of drug resistance marker distribution and malaria source-sink dynamics among mining and non-mining communities. Additionally, we make various comparisons of microhaplotype vs. WGS-based epidemiological inference, assessing how well geographic attribution correlates with travel history metadata and how pairwise relatedness and complexity of infection (COI) estimates differ between the two data types. By joining amplicon sequencing and WGS data from over 1800 *P. falciparum* samples collected in Guyana over a period of five years (periodically representing ca. 5-10% of cases nationwide), we have created the most comprehensive genomic epidemiological profile of any parasite population in the Americas, leading to insights about factors that may contribute to the *de novo* emergence and potential disappearance of drug resistance mutations in the region.

0412

COMPREHENSIVE PAIRWISE COMPETITIVE GROWTH ASSAYS TO ASSESS THE FITNESS IMPACT OF INTERACTING CHLOROQUINE RESISTANCE LOCI

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In addition to the large effect of *pfcrt* on *Plasmodium falciparum* chloroquine resistance, additional transporters, such as *pfmdr1*, have been shown to influence the level of resistance in some genetic backgrounds. These resistance-modulating mutations may share some physiological overlaps with *pfcrt* natural function and thus could impact the fitness cost of mutant *pfcrt*. We generate experimental genetic crosses using human liver-chimeric mice (FRG huHep/huRBC mouse) and measure phenotypes of the resulting recombinant progeny to determine the interactions of specific loci. Using a cross between the lab-line NF54 (chloroquine-sensitive, CQS) and a recent Southeast Asian isolate NHP4026 (chloroquine-resistant, CQR), we first took a bulk segregant approach with chloroquine drug pressure followed by sequencing of the bulk population. As expected, we observed a strong selection signal at a locus on chromosome 7 that encompasses *pfcrt* as well as a second locus on chromosome 6. Subsequently, we isolated clones from this selected population and chose 16 recombinant progeny with different combinations of parental alleles at the chromosome 6 and 7 loci to investigate their fitness effects. All-on-all pairwise competitive growth assays between these 16 progeny and both parents revealed an unambiguous fitness relationship from 153 competitive growth outcomes. Notably, the CQR parent, NHP4026 ranks as more fit than NF54 (CQS), demonstrating that the inheritance of CQR *pfcrt* alone does not ensure an unfit competitor. Four progeny ranked higher in fitness than NHP4026, one of which was CQR. Therefore, the allele combination that contributes to NHP4026 being both CQR and a good competitor is inherited in some of the CQR progeny. In this analyzed set of progeny, the chromosome 6 x 7 interaction did not account for the range of observed competitive outcomes. Additional experiments show that the relationship between individual growth rates and competitive outcomes was non-linear, indicating that growth rates do not by themselves predict *in vitro* competitive outcomes. Together these data indicate multiple genes interact to generate fit CQR parasites.

0413

ILLUMINATING THE BLACK BOX OF PLASMODIUM FALCIPARUM LIVER STAGE GENE EXPRESSION

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The liver stages (LS) of *Plasmodium falciparum* (*Pf*) remain poorly characterized, limiting our knowledge to develop new effective intervention strategies. The study of the asymptomatic LS remains difficult however due to the parasites trophism for primary human hepatocytes and low infection rates. The use of a liver-humanized mouse model (FRGHuHep) for *Pf* LS infection has ameliorated some of these limitations. We have generated a *Pf* NF54 line that constitutively expresses green fluorescent protein (GFP) under the circumsporozoite protein promoter, which confers strong fluorescence to sporozoites and LS. Infection of FRGHuHep mice with this parasite enabled the isolation of LS-infected hepatocytes at different timepoints of development using cell sorting. We conducted extensive RNA-seq analysis of isolated LS, spanning development from early LS trophozoite/early schizont to late LS schizont stage. The data revealed that the LS parasite increases gene expression over time consistent with its developmental stage and biomass expansion. We identified unique LS-expressed transcripts, LS-specific processes associated with the different maturation stages such as cell cycle, metabolism and host-parasite interaction pathways. Furthermore, we found subsets of AP2 transcription factors expressed throughout LS development. Interestingly, we also observed extensive expression of variant multigene families including *var* gene transcripts. In order to establish LS translation efficiency, we analyzed how the introns retained in mature mRNAs regulate parasite liver stage gene expression. Our results provide the first comprehensive gene expression profile of *Pf* LS isolated during *in vivo* intrahepatocytic development. This will inform biological studies and the search for new interventions points that can prevent infection.

0414

LONG READ SINGLE CELL RNA SEQUENCING OF PLASMODIUM VIVAX REVEALS DIVERSITY IN TRANSCRIPT ISOFORMS

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Single cell RNA sequencing (scRNA-seq) is becoming a valuable tool for investigating malaria infections as it allows differentiating the developmental stages of the parasites present in the same blood stage infection. This technique is particularly valuable for the study of *Plasmodium vivax* for which there is no long-term *in vitro* culture system and little sequestration *in vivo*. We have previously used 10X scRNA-seq to characterize the gene expression profiles of individual *P. vivax* blood stage parasites using a non-human primate model. However, the sequence information from the 10X assay derive almost exclusively from a small portion of the 3' end of each mRNA molecule, which 1) is sometimes difficult to associate to a specific gene given the incomplete annotation of *P. vivax* untranslated regions (UTRs) and 2) does not provide any information of the actual isoform expressed. Here, we combine standard 10X scRNA-seq library preparation with long-read PacBio sequencing to characterize full-length transcripts expressed in 2,787 individual *P. vivax* blood stage parasites. Using more than three million high-quality reads, we identified 8,553 different protein-coding mRNAs, transcribed from 7,159 unique genes. Of those, 2,423 shared less than 30% amino acid identity with any annotated *P. vivax* coding sequences and represent potential new genes. Additionally, our data revealed many alternative transcription start sites, previously unidentified gene isoforms, and numerous antisense transcripts. Interestingly, some of these transcripts/isoforms appear to be expressed in a stage-specific manner. Overall, these data provide a detailed description of the variations in isoform expression throughout the *P. vivax* intraerythrocytic life cycle.

0415

DRIVERS OF DIVERSIFICATION OF THE MULTICOPY VAR GENE FAMILY IN PLASMODIUM FALCIPARUM

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Plasmodium parasites that infect humans have evolved an extremely high degree of antigenic diversity, thereby giving them the ability to maintain lengthy, chronic infections and to avoid generating an immune

response that provides protection against subsequent infections. For *P. falciparum*, the most virulent of the human malaria parasites, this diversity is exemplified by the *var* gene family that encodes the major surface antigen displayed on infected red blood cells. The parasite's ability to continuously generate sequence diversity within their genomes is key to parasite persistence in the human host. We have previously identified recombination events between subtelomeric *var* genes, notably a cascade of recombination that led to the generation of novel chimeric *var* genes in disparate subtelomeric regions. We showed that small regions of sequence identity between *var* genes served as sites for homologous recombination and the generation of free DNA end fragments that were then used for subsequent HR events and the generation of two additional chimeric *var* genes. We proposed that this recombination cascade relies on HR machinery and assessed *var* gene recombination in a parasite line where Rad51 was deleted. These parasite lines have normal growth under standard *in vitro* conditions but are sensitive to the genotoxic stress of irradiation. They have both a growth delay and persistent signs of DNA damage that lasts longer than in wildtype parasites. We have also found that these parasites are unable to repair directed DNA double strand breaks when a template is given for repair, consistent with a lack of HR. We have also generated parasite lines that are knock outs of the specialized error prone polymerases, Rev3 and Rev1. These parasite lines also display normal growth in standard *in vitro* conditions and display sensitivity to irradiation, specifically at the ring stage. HR to housekeeping genes remained intact. However, for all three parasite lines we could not induce a recombination cascade between subtelomeric *var* genes. We propose that the parasite has evolved specialized mechanisms to handle DNA damage and diversify multicopy gene families.

0416

THE ROLE OF PFCORONIN IN THE REGULATION OF ACTIN DYNAMICS REQUIRED FOR CYTOSKELETON-LINKED CELLULAR PROCESSES IN PLASMODIUM FALCIPARUM

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The frontline artemisinin (ART) drug class has been critical to malaria control. However, treatment failures of ART and artemisinin combination therapy (ACTs) in some parts of the world threaten their efficacy. ART treatment failures in Southeast Asia have been attributed to mutations in *Plasmodium falciparum* (*Pf*) *kelch13* locus. Recently, *Pf*kelch13 mutations evolved independently in South America, raising concern about the expansion of ART resistance to Africa. Thus, mitigating the spread of ART resistance to Africa, where malaria is most prevalent, remains a critical global public health priority. Previously, we sought insights into potential ART resistance in African parasites by performing *in vitro* evolution to generate ART resistance in Senegalese isolates. We found the actin-bundling protein, *Pf*coronin, to be a major driver of ART resistance *in vitro*, as measured by the ring-stage survival assay (RSA). Given the possible role of actin in endocytosis, a pathway recently found to be disrupted in *Pf*kelch13-mediated ART resistance, we explored resistance synergy between *Pf*coronin and *Pf*kelch13 mutations by generating single and double mutants in the Senegalese genetic background and measuring ART susceptibility using RSA. We found a similar RSA phenotype in *Pf*coronin/*Pf*kelch13 double mutants as in *Pf*kelch13 single mutants: *Pf*coronin-mediated resistance is masked by that of *Pf*kelch13. To functionally probe *Pf*coronin, we used CRISPR-Cas9 to generate parasites expressing HA-tagged *Pf*coronin. In pulldown experiments, we identified several interacting proteins, including *Pf*actin-1 and actin-2, tubulin alpha and beta chains, and myosin-A. These findings suggest that *Pf*coronin may regulate actin dynamics and cytoskeleton-linked cellular processes. Further studies are underway to elucidate how *Pf*coronin mutations may confer ART resistance by modifying cytoskeletal dynamics.

0417

EFFICACY OF THE RTS,S/AS01$_E$ MALARIA VACCINE ADMINISTERED ACCORDING TO DIFFERENT FRACTIONAL AND FULL DOSE REGIMENS UNDER CONDITIONS OF NATURAL EXPOSURE IN AFRICAN CHILDREN AGED 5-17 MONTHS: FIRST RESULTS FROM A PHASE 2B RANDOMIZED CONTROLLED TRIAL

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A phase 3 trial showed that RTS,S/AS01$_E$ has moderate vaccine efficacy (VE) against malaria in African children when administered according to a 0, 1, 2-month (M) primary schedule (M012) with a 4th dose given at M20. Subsequent controlled human malaria infection studies in malaria-naïve adults demonstrated improved VE of RTS,S/AS01$_E$ with a delayed 3rd and/or 4th fractional (Fx) dose. We present initial results, including the primary objective, of an ongoing phase 2b open label randomized, controlled trial (NCT03276962) evaluating the efficacy of RTS,S/AS01$_E$ Fx dose regimens under conditions of natural exposure. A total of 1500 Kenyan and Ghanaian children aged 5-17M were randomized (1:1:1:1:1) to receive RTS,S/AS01$_E$ (4 regimens) or a rabies control vaccine (M012). Children in the RTS,S/AS01$_E$ groups received 2 full doses at M0 and M1 and either full doses at M2 and M20 (R012-20) or at M2 and M14 (R012-14), Fx doses (1/5 of full dose) at M2 and M14 (Fx012-14) or at M7 and M20 (Fx017-20). The primary objective was to demonstrate superiority of the Fx012-14 regimen *vs* the full 3 dose regimen (R012-14+R012-20) against first episode of clinical malaria over 12M post dose 3. Safety and reactogenicity were also evaluated. All vaccine regimens were well tolerated with no safety signals observed. Each regimen showed statistically significant VE against first episode of clinical malaria 12M post dose 3 compared to the control group; VE ranged from 35% (Fx012-14; 95% confidence interval [CI]: 13, 51), to 47% (R012-14+R012-20; 95%CI: 31, 59), and 54% (Fx017-20; 95%CI: 38 ,66). Neither Fx dose regimen showed superior VE *vs* the full dose regimen up to data lock (M20). We did not demonstrate superior VE of Fx012-14 *vs* R012-14+R012-20 over 12M post dose 3 (incremental VE: -21%, 95%CI: -57, 7). The trial is ongoing and annual Fx or full dose boosters are given to the children per allocated vaccination group. Continued follow-up until study end (M50) may provide further insight on possible flexibility of the 4 dose regimen currently recommended by WHO and the potential of alternative regimens for public health benefit.

0418

THE R21/MATRIX-M™ MALARIA VACCINE CANDIDATE: A PHASE III TRIAL

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The last year has seen some significant progress in malaria vaccine development. R21/Matrix-M (R21/MM), a circumsporozoite protein-based vaccine, initially showed high-level efficacy in phase I/IIa controlled human malaria infection (CHMI) trials in malaria-naïve adults in the UK. Following this, a phase 1b age de-escalation trial in Kilifi, Kenya, showed good safety and potent immunogenicity in 91 adults, children and infants. A phase IIb field-efficacy study in 450 infants, aged 5-17-months, in Nanoro, Burkina Faso was initiated in 2019, administering three monthly doses prior to the malaria season. 77% efficacy was observed over 12 months of follow-up. This appears to be the first time any vaccine has reached the WHO-specified efficacy goal of ≥75% in the target population of African children. Follow-up for a second year, after a booster dose at 12 months, is ongoing and efficacy results will be updated, together with a design to assess the value of a further booster dose at 24 months. In this phase IIb trial, R21/MM had a favourable safety profile and high NANP specific IgG levels were observed, which correlated with vaccine efficacy. These antibodies were re-stored to peak levels following one booster dose at 12 months. This year, a phase III double-blind, randomised controlled trial has been initiated across five African sites in Kenya, Tanzania, Mali and Burkina Faso, with differing seasonal and perennial transmission patterns and differing malaria burdens, in a broader age-range of children. 4800 participants, aged 5-36 months, are receiving 3 vaccine doses 4 weeks apart and a booster dose one year later. The primary objectives are efficacy at 12 and 24 months following three vaccine doses and a booster at 12 months, with safety outcomes measured throughout the trial. We will report on progress of this Phase III trial of R21/MM along with updated findings from other parts of the overall R21/MM clinical development programme.

0419

CHARACTERIZATION OF MALARIA PFCSP MRNA-LNP INDUCED INHIBITORY ANTIBODIES

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Human malaria affects the vast majority of the world's population with the *Plasmodium falciparum* species causing the highest rates of morbidity and mortality. Recent approval and demonstration of efficacy of mRNA vaccines has led to increased excitement surrounding mRNA as a platform for infectious disease vaccines in humans. The immunodominant coat protein of the invasive malaria parasite stage, circumsporozoite protein (PfCSP), was selected to assess the immunogenic and protective potential of an mRNA malaria vaccine with an array of dosing factors such as formulation, dose, interval, and number of immunizations. *PfCSP* mRNA-LNP achieved sterile protection against infection in mice utilizing two PfCSP transgenic parasite strains to varying degrees depending on the *PfCSP* mRNA-LNP (TriLink/unmodified versus UPenn/nucleoside-modified). Notably, serum antibodies tested for their ability to inhibit sporozoites in the *in vitro* inhibition of liver stage development assay (ILSDA) revealed significant reductions in liver stage burden, comparable to an established reference PfCSP repeat-specific monoclonal antibody (NSF1). We will report on the relationship of *PfCSP* mRNA-LNP-induced *in vivo* protective responses in mice, the antibody inhibition observed in the ILSDA with antibody specificities to well-documented regional, junctional, and repeat-specific PfCSP epitopes. These findings may have major implications for the suitability of mRNA-LNPs for use as vaccines in malaria.

0420

EVALUATION OF PLASMODIUM VIVAX ANTIGENS AS VACCINE CANDIDATE TO PLASMODIUM SPP.

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The bottleneck of the malaria vaccine is the challenge to find multi-stage antigens conserved in different *Plasmodium spp.* Most malaria vaccines focus on antibody induction to the pre-erythrocytic stage, even though the sterile vaccine protection induced by attenuated sporozoites is directly related to cytotoxic T cells (CTLs). Our group lately demonstrated that *P. vivax* (Pv)-infected reticulocytes can activate CTLs, in an antigen-dependent manner, describing a protective mechanism against blood-stage parasites. In unpublished data, we perform the immunopeptidomics analysis aiming to identify HLA-I-associated peptides of Pv-infected reticulocytes. It was assessed that 60% of the eluted peptides are derived from 'housekeeping' proteins, which are conserved in *Plasmodium* cross-stage and cross-species. Afterward, we selected 50 peptides and their source proteins to be studied, for the first time, as a potential malaria vaccine candidate. *P. yoelii* (Py) infection is an excellent experimental model for investigating malaria immune response. First, we tested the 50 peptides in an anti-IFNγ ELISPOT assay with Py-infected mice splenocytes. Then, we produce three recombinant proteins (L30, S25, and H2A) in the *E. coli* expression system for immunization protocols. Mice were immunized three times with each protein associated with Alum + CpG as an immunological adjuvant. Twenty-one days after the last boost, mice were challenged with 10^6 PyX17NL-infected red blood cells, and the parasitemia was monitored for 30 days. We identify 23 of the 50 Pv peptides were immunogenic in the acute infection. Moreover, 12 remain in the convalescent phase, suggesting an immunological memory. Regarding the immunization assessment, we identify that 2 of 3 tested proteins can induce antigen-specific IgG Total, IgG1, and IgG2c levels, additionally, to decrease up to 50-80% the parasitemia compared to the control group. Therefore, we newly identify Pv antigens that can induce a protective response to Py infection, indicating cross-species protection and a potential vaccine candidate.

0421

EFFICACY OF PFS230D1-EPA-AS01 VACCINE, A TRANSMISSION BLOCKING VACCINE AGAINST PLASMODIUM FALCIPARUM, ASSESSED WITH A DIRECT SKIN FEEDING USING ANOPHELES GAMBIA (COLUZZI) IN CHILDREN 9-18 YEARS OLD IN MALI

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Transmission blocking vaccines (TBV) prevent mosquito infections and community spread of malaria parasites but require durable functional antibody responses and need to be safe to administer at the population level for elimination efforts. A phase 2 double-blind, 1-1 randomized, family compound, comparator-controlled trial in Mali is underway to assess safety, tolerability, vaccine activity and vaccine efficacy of Pfs230D1-EPA-AS01versus comparator administered on a 0, 1, 2, 14 month schedule, at a community level. Family compounds were aggregated by proximity and mosquito habitat into Vaccine Units (VU) that were randomized as a whole for receipt of vaccinations by all eligible subjects 5 years or older; children 1-4 years of age received AL treatment (but not vaccine), prior to their VU receipt of third and fourth vaccine doses, and then were followed for parasitemia endpoints. The trial began Apr 2019 with an age de-escalation pilot phase and proceeded to the main phase with third dose completed in Aug-Nov 2019 the first year; fourth dose was given in Aug 2020 the second year. In prior studies at this site, 9-18-year old children transmitted parasites most frequently to mosquitoes, and for this trial participated in mosquito direct skin feeding (DSF) assays as the primary endpoint of vaccine activity (reduction in the rate of positive DSF assays). DSF was performed every 2 weeks post third vaccination for a total of 8 DSFs and a total of 10 DSFs for the booster fourth dose. A total of 380 and 311 subjects respectively during the first and second year underwent at least one DSF procedure. No AEs was recorded as related to the 5946 DSF performed during the study. Using the rate of positive DSF assay in vaccines as compared to the control group, in the 1st year, the vaccine efficacy (VE) was 71.9% and 75% in the 2nd year with overall VE of 73.6% ($p<0.001$) over the 2-year trial. Parasite speciation of infected DSF samples is ongoing to confirm Pf-specific VE. These results justify further development of Pfs230D1-EPA-AS01 as a malaria vaccine candidate alone or in combination with pre-erythrocytic vaccines.

0422

PFSPZ VACCINE IMMUNIZATION IN WOMEN OF CHILDBEARING POTENTIAL AND IMPACT ON MALARIA DURING SUBSEQUENT PREGNANCIES: A TWO-YEAR STUDY

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Pregnant women are highly susceptible to *Plasmodium falciparum* (Pf) malaria, leading to substantial maternal, perinatal, and infant mortality. Sanaria® PfSPZ Vaccine, composed of Pf sporozoites (SPZ), is an advanced malaria vaccine candidate being developed for use in pregnant women based on a highly favorable safety profile and proven vaccine efficacy (VE) over at least a 6-month period. This randomized, double blind, normal saline (NS) placebo-controlled study assessed the safety and VE of two dosage regimens, $9x10^5$ and $1.8x10^6$ PfSPZ of PfSPZ Vaccine administered on days 1, 8 and 29 in Malian women of childbearing potential who reported a plan to become pregnant in the next 1-2 years but were willing to undergo pregnancy prevention during vaccinations and for 1 month post vaccination. 300 women were randomized 100/arm to receive $9 x10^5$ or $1.8x10^6$ PfSPZ or placebo before peak of the 2019 malaria transmission season. All received artemether-lumefantrine 2 weeks prior to 1st and 3rd vaccination to clear any existing parasitemia. The women were immunized July-August 2019 and followed for malaria endpoints over the course of two years (peripheral Pf; clinical malaria; placental malaria) including during pregnancy. Post-vaccination, 163 women had 180 pregnancies: 60 pregnancies in 56 women receiving $9 x10^5$ PfSPZ, 69 in 60 women receiving $1.8x10^6$ PfSPZ and 51 in 47 women receiving NS. The first pregnancy was identified September 2019; first pregnancies were detected at a similar pace in the two vaccine arms (median: 213 days post vaccination) but with a delay in the placebo arm (median 264 days). Peripheral Pf occurred at least once during pregnancy in 19/60 (31.7%) women in the $9x10^5$ PfSPZ arm, 22/69 (31.9%) in the $1.8x10^6$ PfSPZ arm, and 25/51 (49%) in the placebo arm; 5 placental malaria episodes have occurred ($9x10^5$ PfSPZ: 2; $1.8x10^6$ PfSPZ: 1; NS: 2). Further analysis, including time to first peripheral Pf, Pf frequency, and severity of malaria episodes during pregnancy will be presented, taking into account prior parasitemias, antibody and T-cell responses, seasonal variation, and IPTp dosing.

0423

GLYCOLIPID-ADJUVANTED RADIATION-ATTENUATED SPOROZOITES IMPROVES THE EFFICACY OF A HETEROLOGOUS PRIME-AND-TRAP MALARIA VACCINE

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Malaria is caused by *Plasmodium* parasites and is responsible for an estimated 250 million infections every year. Many leading candidate malaria vaccines target the sporozoite and liver stages to block the subsequent blood stage, clinical disease, and transmission. For liver stage vaccines, inducing liver-resident memory CD8+ T (Trm) cells appears to be critical for protection in mice. Liver Trm cells are induced by a 'Prime-and-Trap' vaccine that combines sporozoite antigen DNA priming with a single intravenous (IV) dose of liver-homing radiation-attenuated sporozoites (RAS) to direct and "trap" activated and expanding T cells in the liver. Although Prime-and-Trap confers durable protection in a rodent malaria model, the requirement for high doses of IV administered RAS is a potential impediment for translation to larger animal models or humans. Here, we investigated methods to improve the translational potential of Prime-and-Trap by reducing the RAS dose, eliminating the IV administration requirement, and adding a co-administered glycolipid adjuvant, 7DW8-5. In *P. yoelii* circumsporozoite protein-encoded DNA primed mice, 7DW8-5 potentiated the efficacy of otherwise sub-therapeutic doses of RAS such that Prime-and-7DW8-5-adjuvanted Trap protected mice against challenge. Additionally, we showed that intradermally-administered RAS and 7DW8-5 improved protection against challenge. These findings in mice demonstrate that Prime-and-Trap is a versatile malaria vaccine that can induce protective liver stage immunity using a single RAS dose and warrants further translation to human studies.

0424

POST-MORTEM SURVEILLANCE FOR EBOLA VIRUS USING ORAQUICK® EBOLA RAPID DIAGNOSTIC TESTS, EASTERN DEMOCRATIC REPUBLIC OF THE CONGO, 2019-2020

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The 10th Ebola virus disease (EVD) outbreak in the Democratic Republic of the Congo (DRC) lasted from 1 August 2018 to 26 July 2020 and, in DRC, was the longest, the most geographically widespread, and the largest EVD outbreak with 2,287 deaths and 1,171 survivors. Challenges in controlling this outbreak included security threats, widespread community mistrust in the response activities, low acceptance of systematic safe and dignified burials (SDBs), and long turnaround times of required RT-qPCR results to bereaved families prior to burials. To quickly return laboratory test results to families and improve community engagement and acceptance of SDBs, we trained local healthcare workers to screen cadavers' oral fluids for Ebola infections in Beni Health zone using the OraQuick® Ebola rapid diagnostic test (RDT) (OraSure Technologies, Inc., Bethlehem, PA, USA). Bodies were returned to families for traditional burial within 30-60 minutes when RDT were non-reactive. Molecular confirmation was done by RT-qPCR. Data were collected with tablets outfitted with Kobo-based data collection that operated online. The study was approved by the DRC Ministry of Health. An RDT Consortium and a Technical Working Group coordinated the RDT implementation activities. RDT testing was conducted on 443 cadavers from health facilities (77%) or the community (23%) between 1 August - 31 October 2020 by 19 local teams, each composed of a laboratorian or nurse, a hygienist, a community engagement specialist, and a supervisor. In total, 425 (96%) samples had non-reactive RDTs, 11 (2%) were invalid and 7 (2%) were reactive. Molecular confirmation yielded only 2% false positives and zero false negatives. With a 100% negative predictive value, the post-mortem OraQuick® Ebola RDT effectively complemented outbreak response efforts during the 10th EVD outbreak in DRC. Our study shows that in-depth training of the local workforce equipped with innovative Ebola RDT data collection tools adapted to low resource environment could contribute to the detection of EVD related deaths, enables quick public health actions, reduces the risk of community transmission and saves lives.

0425

TYPHINET: AN ONLINE DASHBOARD FOR GENOMIC SURVEILLANCE OF SALMONELLA TYPHI

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Typhoid fever is a faeco-orally transmitted systemic infection caused by the bacterium *Salmonella* Typhi (*S.* Typhi). Each year >10 million cases occur worldwide of which >100,000 are associated with mortalities making it a public health threat in many low- to middle-income countries with limited hygiene and sanitation infrastructure. Whole genome sequencing (WGS) and core-genome phylogenetics have become the standard for typhoid molecular epidemiology in both research and public health settings, providing insights into population structure, transmission dynamics, antimicrobial resistance (AMR) emergence and dissemination, as well as outbreak investigation and monitoring of implemented intervention strategies. Despite the relatively widespread adoption of WGS these data are not united and summarised in a single surveillance platform for maximum public health benefit. Here we present TyphiNET; an automated online surveillance dashboard (available at: http://typhi.net) developed as a MERN (MongoDB, Express, React, Node.js) stack JavaScript application. TyphiNET imports public data from Typhi Pathogenwatch (available at: https://pathogen.watch/) producing interactive visual summaries of public health utility including global and country level overviews of population structure and AMR over time. TyphiNET successfully captures previously reported changes in the *S.* Typhi population structure including the emergence of extensively drug-resistant (XDR) typhoid in Pakistan, the emergence of mutations associated with azithromycin resistance in South Asia, and replacement of antimicrobial sensitive genotypes in Malawi following the introduction of multidrug-resistant (MDR) H58 genotypes. Data and visualisations can be filtered to report data from different sampling frames, time spans and geographic locations, and can be downloaded for use in presentations and reports. Subsequently, TyphiNET provides an up-to-date overview of the global *S.* Typhi population structure to better capture the emergence and spread of AMR variants, changes in population structure, and impacts of intervention strategies worldwide.

0426

DISSECTING GENETIC DETERMINANTS OF HETEROGENEITY IN PATHOGEN-INDUCED CYTOKINE RESPONSES: COMPARISON OF EUROPEAN AND AFRICAN-BASED COHORT STUDIES

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Humans exhibit remarkable inter-individual and inter-population immune response variability upon microbial challenges. Cytokines play a vital role in regulating inflammation and immune responses, but dysregulation of cytokine responses has been implicated in different disease states. Host genetic factors were previously shown to have a strong impact on cytokine response variability mainly based on European-based studies, but it is not known whether these findings are transferable to non-European individuals. We examined both cytokine and genome-wide SNPs data of approximately 300 and 500 healthy adults of East-African ancestry from Tanzania and Western European ancestry respectively. We performed cytokine quantitative trait loci (cQTLs) mapping and functional enrichment analyses to identify genetic regulators cytokine responses in both populations. In the Tanzanians, cQTLs mapping identified 80 independent suggestive and one genome-wide significant locus (***TBC1D22A***) at chromosome 22; SNP rs12169244 was associated with IL-1β release after *Salmonella enteritidis* stimulation. Remarkably, the identified cQTLs varied significantly when compared to the European cohort, with a very limited percentage of overlap (1.6% to 1.9%). We further observed ancestry-specific pathways regulating induced-cytokine responses, with significant enrichment of the interferon pathway specifically in the Tanzanian cohort. Furthermore, contrary to the European cohort, genetic variants in the *TLR10-TLR1-TLR6* locus showed no effect on cytokine response. Our data reveals both ancestry-specific effects of genetic variants and pathways on cytokine response heterogeneity, hence arguing for the importance of initiatives to include diverse populations into genomics research.

0427

THE ROLE OF MULTIDIMENSIONAL POVERTY IN ANTIBIOTIC MISUSE IN EAST AFRICA: A MIXED-METHODS STUDY

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Antimicrobial resistance (AMR) is a 'wicked' problem that poses a particularly stark threat in low- and middle-income countries (LMICs), where antibiotics are widely available without prescriptions. Poverty has been suggested as an important upstream driver of both AMR and inappropriate antibiotic (AB) use. However, we know little about how population-level poverty inequalities relate to AB use. This mixed-methods study investigated the role of poverty in patient-reported AB use in East Africa. The Holistic Approach to Unravelling Antimicrobial Resistance (HATUA) consortium collected data (2018-20) from 6,559 patients presenting with urinary tract infection (UTI) symptoms at public clinics in Tanzania, Kenya, and Uganda. Applying hierarchical regression models, we investigated the association between multidimensional poverty (measured using the Global Multidimensional Poverty Index, capturing acute deprivation in education, living standards, and health simultaneously) and self-reported patterns of AB use. While general AB use was not socio-economically patterned, AB misuse- self-medicating with antibiotics, skipping a dose, and not completing the course- was statistically more common among the better off. These patterns were consistent across contexts, with the exception that in Tanzania, self-medication was equally common across the poverty gradient. In-depth qualitative interviews with a subset of patients (n=90) suggested that these findings are driven by the perceived inconvenience of the healthcare system, ease of access to antibiotics, and inconsistent knowledge about best practices. In Uganda and Kenya, symptoms stigma contributed to skipping doses while at work or in public. Notwithstanding potential reporting differences, the propensity to misuse ABs increases as level of deprivation decreases. Contrary to previous research in which poverty-driven behaviour has been shown to fuel inappropriate use due to inadequate access to and lack of money for effective ABs, these key findings have implications for future trends in AB use and AMR within LMICs and the development of policies to tackle the issue.

0428

COMPLEX TREATMENT-SEEKING FOR SYMPTOMS OF URINARY TRACT INFECTION IN EAST AFRICA AND LINKS TO ANTIBIOTIC MISUSE: A MIXED-METHODS STUDY

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Past research has shown that patients with urinary tract infections (UTIs) in low and middle-income countries (LMICs) struggle to access appropriate care, exacerbating the risk of antibiotic misuse and leading to antimicrobial resistance (AMR). This mixed-methods study aims to investigate the socioeconomic and attitudinal factors of patient treatment-seeking pathways (self-treatment, antibiotic use, pathway complexity) for UTI-like symptoms in three East African countries. The Holistic Approach to Unravelling Antimicrobial Resistance consortium collected quantitative data on patient pathways from 5,945 patients with UTI-like symptoms at public clinics in Tanzania (n=2,788), Uganda (n=1,701) and Kenya (n=1,456) [2018-20]. Multilevel logistic regression was used to assess the factors associated with having complex multi-step pathways, first choice of treatment/provider, and antibiotic use before coming to clinic. Qualitative patient interviews were collected from a subset (n=103) and analysed using thematic content analysis. Results showed that 55% of patients tried something else before coming to clinic: 29% had 1 step, 16% 2 steps, and 10% 3+ steps. Among those who tried something else, 74% went to other clinics, 26% self-treated with either drugs or home remedies/herbs, 40% took antibiotics suitable for UTI, and 9% used unsuitable antibiotics. Tanzanian and Ugandan patients were more likely to have complex pathways than those in Kenya. Long waiting times at clinics, not having close access to health centres, and being able to afford drugs made self-treatment more probable. Men, those who previously attended other clinics, and those who felt symptoms stigma were more likely to take antibiotics. Wealthier patients were more likely to have complex pathways and take unsuitable antibiotics. Our findings challenge common presumptions around drivers of AMR in East Africa. Our mixed methods approach was essential in demonstrating the complexity of structural factors which contribute to patient behaviours and identifying under-explored themes for further analysis, including stigma and healthcare structure/accessibility.

0429

ENHANCED PARTICIPATION OF ROHINGYA REFUGEES INCREASES IMMUNIZATION COVERAGE IN A CAMP-BASED POPULATION IN COX'S BAZAAR, BANGLADESH

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During the SARS-CoV2 pandemic routine vaccination coverage fell below 20% among Rohingya refugees in Bangladesh, creating conditions conducive to outbreaks of vaccine-preventable illness. We evaluated a pilot program implemented by and for refugees whose objective was to increase vaccinations among pregnant women and children under

age three living in two camps. Rohingya community immunization volunteers (CIVs) conducted baseline and midline census surveys and ongoing household surveillance and identified 2,877 individuals due for one or more immunizations, including 353 newly enlisted beneficiaries not previously recorded in official registries. After CIVs visited the household of each target beneficiary to explain the vaccination process half (49%) of beneficiaries were vaccinated. CIVs returned within 72 hours to visit beneficiaries who missed their initial appointments (51%) and accompanied them to vaccination sites. After excluding beneficiaries who moved outside the study area or died (2%), over 99% of eligible beneficiaries were successfully vaccinated. The CIV pilot achieved vaccination coverage that exceeded respective coverage rates estimated in this population prior to and during the pandemic, and in similar camp populations adjacent to the pilot area. Qualitative interviews with refugees and vaccination stakeholders suggested keys to success included the ability of CIVs to earn the trust of both community members and government vaccination staff, and to accompany beneficiaries as they navigated long distances, vaccine shortages and other perceived barriers to immunization. Our findings align with other evidence of community health worker effectiveness and suggest Rohingya refugees themselves can collect and manage timely information on household composition and conduct targeted outreach to dramatically increase vaccination coverage during the acute-on-chronic crisis of the SARS-CoV2. Policies that limit refugee participation in the delivery of health and other services deserve critical scrutiny for their possible adverse impacts on coverage and outcomes.

0430

IMPACT OF THE INTRODUCTION OF PCV7OR 13 ON ANTIMICROBIAL RESISTANCE IN INVASIVE PNEUMOCOCCAL DISEASE IN WESTERN REGION OF THE GAMBIA

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We describe antimicrobial resistance in invasive pneumococcal disease due to all serotype and non-vaccine type(NVT) pre and post pneumococcal conjugate vaccine(PCV) implementation in The Gambia in all age groups. We identified, serotyped and performed antimicrobial susceptibility testing using disc diffusion methods on pneumococcal isolates obtained from invasive samples collected from standardize population-based pneumococcal disease surveillance in the Bundung maternal and Child health demographic system. The study commenced May 2010. PCV7 was introduced in August 2011 and PCV13 in September in 2012. Antibiotic susceptibility was interpreted using clinical laboratory standard institute guidelines. 390 pneumococcal isolates were screened against five antimicrobial agents. There was a moderate decline in antibiotic resistance in all age groups in invasive pneumococcal disease during vaccine implementation. In the 2-23-month age group, annual counts of oxacillin, chloramphenicol, and tetracycline resistant case fell from 10-15 in 2010 and 2011 to 6-7 in 2014 and 2015. In the 24-59-month age group, there was a large fall in tetracycline resistant cases. In those under 5 years, oxacillin, chloramphenicol and tetracycline fell to zero case in 2013 and 2014. Resistance fell primarily due to reduction in vaccine serotypes 1, 5, 14 and 23F. The proportion of resistant cases increase over time, particularly in the 2-23-month age group with tetracycline resistance mainly in serotypes 10A, 12F, 11B,7C and 25A and tetracycline resistance in serotype in 12F in 2016. Isolates were generally sensitive to erythromycin but in 95-98% were resistant to clotrimazole throughout the study. Although there is an overall reduction in cases of antimicrobial resistant. We hypothesis that increased transmission of NVT after the introduction of PCV and exposure to antimicrobials agents. Ongoing surveillance is important to determine future trends in resistance as it has both clinical and public health importance in PCV era.

0431

FRONTLINE HEALTH WORKER AND COMMUNITY HEALTH VOLUNTEER EXPERIENCES WITH DELIVERY OF MASS DRUG ADMINISTRATION USING TRIPLE DRUG THERAPY FOR LYMPHATIC FILARIASIS IN PAPUA NEW GUINEA: A MIXED METHOD STUDY

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A three-drug regimen of ivermectin, diethylcarbamazine citrate and albendazole (IDA) for lymphatic filariasis has been found to be more effective for achieving sustained clearance of microfilariae than the standard two-drug regimen. This exploratory study, undertaken across four treatment-naïve implementation units of East New Britain Province (ENBP), Papua New Guinea, aimed to understand the performance, motivation, and resilience of frontline health workers (FLHW) and community health volunteers (CHV) responsible for distributing the IDA treatment regimen for the first time. Our mixed methods study included a FLHW/CHV survey, a focus group discussion (FGD), and in-depth interviews (IDIs). FLHW (n=41) and CHV (n=33) involved with the mass drug administration were purposefully selected and surveyed. The behavioural and outcome aspects of FLHW/CHV performance were measured using a 20-item Likert scale. Items on the scale assessed task and contextual performance across four domains (adherence to protocol/achievement of goals, interpersonal skills, self-efficacy/behaviour in response to challenges, and teamwork). Motivation was explored using a micronarrative survey. Resilience was measured using the Connor-Davidson Resilience Scale 25. Qualitative data were collected from one FGD and three IDIs. When surveyed, 51.4% of FLHW/CHV said they "always" delivered the medicine to the households they planned to reach each day, with 33.8% saying they "never" did. Only 45.9% felt they could "always" contact a program supervisor for help if they encountered problems or didn't know what to do. Survey data also showed that 78.4% said they "always" watched people swallow the medicines. Mean resilience scores were reported out of a total score of 100, for FLHW 67.39 (± 20.20) and CHV 73.02 (± 19.33). A short time frame for distribution, transportation issues, drug shortages, and lack of drinking water were some of the challenges mentioned in the FGD and IDIs. These results validate the high treatment coverage rate across ENBP and provide the health department information for future implementation of the new regimen in Papua New Guinea.

0432

POST-TREATMENT SURVEILLANCE FOR ONCHOCERCIASIS IN PLATEAU AND NASARAWA, NIGERIA: EVIDENCE FOR ELIMINATION OF TRANSMISSION

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Nigeria has 480 onchocerciasis-endemic districts or local government areas (LGAs), of which 12 (2.5%) are in Plateau and Nasarawa States. In 2017

these two states were the first in the country to interrupt transmission and stop mass drug administration (MDA) for onchocerciasis after 8 – 26 years of MDA with ivermectin in the 12 hyper-/meso-endemic LGAs and ivermectin-albendazole treatment for LF in 18 other LGAs non-endemic or hypo-endemic for onchocerciasis. A three-year post-treatment surveillance (PTS) entomological evaluation, required to declare transmission elimination, was completed in 2019-2020 transmission season per WHO and National Onchocerciasis Elimination Committee (NOEC) guidelines. Entomology collections for *Simulium damnosum* s.l. blackflies were carried out from mid-June through October 2019 and 2020 in 42 sites (21 sites per state) in formerly hyper-/meso-endemic areas. Collections were from known high risk LGAs as well as hypo-endemic LGAs bordering with other endemic Nigerian states. Blackflies were tested for the presence of L3 infective-stage *Onchocerca volvulus* by O-150 polymerase chain reaction. In Nasarawa State, results from 93 pools comprised of 7925 flies found 0 positive pools (0%, 95% confidence interval [CI] 0% - 0.024%). In Plateau State, 89 pools comprised of 7368 flies resulted in 0 positive (0%, 95% CI 0% - 0.026%). These results meet the 2016 WHO entomological criteria for elimination of transmission infectivity threshold of less than 0.05% (<1/2000). Other PTS activities included tracking and treating immigrants and internally displaced persons (IDPs), and health educating the communities. A total of 21,994 immigrants and IDPs were tracked and treated with ivermectin in the border villages as well as those in identified camps. Health education messages were disseminated through radio jingles, posters and face-to-face contact by community-directed drug distributors. Pending approval of the NOEC, these results indicate Plateau and Nasarawa states could be the first to eliminate onchocerciasis transmission in Nigeria.

0433

ONCHOCERCIASIS ELIMINATION STATUS IN FIVE CARTER CENTER-SUPPORTED STATES IN SOUTHERN NIGERIA: MEASURING PROGRESS TOWARDS ONCHOCERCIASIS ELIMINATION IN NIGERIA

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In 2013, Nigeria adopted a goal of onchocerciasis transmission elimination and formed the National Onchocerciasis Elimination Committee (NOEC). Between June 2019 and October 2020, we conducted cross-sectional epidemiological sero-surveys in five states (Abia, Anambra, Delta, Enugu, and Imo) to collect dried blood spots (DBS) from children aged 5 – 9 years for OV16 ELISA analysis; positive children were tested by O-150 PCR for *Onchocerca volvulus* microfilariae in skin snips. DBS were collected from 167 high-risk villages (within 5km of known breeding sites) pre-selected by the NOEC. In Delta, 2/3,380 children were OV16 positive (0.06%, upper 95% CI [UCL] 0.14%); skin snip samples from the two positive children were PCR negative. In Imo, 1/3,197 was OV16 positive (0.03%, UCL 0.09%). In Abia, 1/3,174 was OV16 positive (0.03% UCL 0.09%), skin snips were not obtained. In Enugu, 2/3,180 were OV16 positive (0.10%, UCL 0.15%); skin snips were negative. Finally, in Anambra, 3/3,167 children were OV16 positive (0.10%, UCL 0.20%); skin samples were available for two of the three positives, and PCR was positive for one of these. The WHO threshold for transmission interruption is UCL <0.1%, but guidelines permit OV16 positive/PCR negative children to be excluding from the calculations if less than 10 children are positive. In all but Anambra State recalculation of the UCL using skin snip PCR results showed <0.1% threshold was reached. An entomological study of 6,591 vector heads of *Simulium damnosum s.l* carried out in Delta state from May – November 2020 showed all pools to be O-150 PCR negative (0%, UCL 0.58). The WHO threshold is <1/2000. We believe Delta State has met the WHO criteria for 'transmission interrupted' classification, and that MDA could be stopped here in 2021. Abia, Imo and Enugu should proceed to entomology studies for a potential stop MDA decision for 2022. Anambra should wait for the outcome of the last skin PCR. These recommendations are pending decision by the NOEC May 2021 meeting. If the Delta recommendation is accepted, it will be the first state in southern Nigeria to stop onchocerciasis MDA.

0434

THE IMPACT OF MASS DRUG ADMINISTRATION WITH A TRIPLE DRUG REGIMEN (IVERMECTIN, DIETHYLCARBAMAZINE AND ALBENDAZOLE) ON LYMPHATIC FILARIASIS IN PAPUA NEW GUINEA AND IMPLICATIONS FOR POST-MDA SURVEILLANCE

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The impact of mass drug administration (MDA) with triple drug therapy IDA (ivermectin, diethylcarbamazine and albendazole) on lymphatic filariasis (LF) in treatment naïve areas has been little studied. WHO recommends two rounds of MDA with IDA in treatment naïve areas, or areas that have failed repeated rounds of two drug therapy outside of sub-Saharan Africa. Endpoints for stopping MDA in implementation units following IDA remain to be defined, but Mf surveillance may be better than surveys for circulating filarial antigen (CFA) which declines slowly after IDA and may take years to decline below <2%. We studied the impact of MDA on LF in the East New Britain Province (ENBP) of Papua New Guinea with a population of ~375,000 residents without previous MDA for LF. Prior to MDA, we surveyed 49 clusters or villages (30 randomly selected and 19 purposely selected) of ~100 persons each (n=50 ages 6-9 years; n=50 >10 years). We found 24 of 49 clusters had >2% CFA and 14 >1% Mf with overall Mf prevalence of 2.2% (95% CI:1.8, 3.2, range 0-18%). We also observed little LF infection in children. In November 2019, MDA was performed for the entire province with 82% epidemiological coverage. We used geostatistical modelling of baseline data to select 46 clusters for monitoring and evaluation following MDA. The model prioritized 23 of the 24 hot spot clusters identified at baseline and identified an additional 23 clusters likely to have high endemicity for post-MDA surveillance. Because of the low baseline LF prevalence in children, post-MDA surveys focused on adults (~100 per village). One-year post-MDA, only 2 of 23 previously surveyed hotspots had Mf >1%, and none of the other 23 clusters had Mf >1%. The overall Mf prevalence post-MDA was 0.26% (95% CI: 0.2, 0.5, range 0-3.9%). These results show that a single round of MDA with IDA can result in a dramatic decrease in Mf prevalence to a level that may not sustain transmission apart from a few hotspots. Geospatial modeling may also be useful for focusing post-MDA surveys to high-risk areas. Surveys of adults for persistence of Mf in high-risk areas is likely to be superior to CFA surveys in children for endpoint surveillance in PNG.

0435

THE IMPACT IF ANNUAL VS SEMIANNUAL MASS DRUG ADMINISTRATION WITH ALBENDAZOLE AND IVERMECTIN ON HELMINTH INFECTIONS IN HARPER DISTRICT, LIBERIA

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The World Health Organization recommends annual mass drug administration (MDA) with ivermectin plus albendazole (IA) to eliminate lymphatic filariasis (LF) in most endemic countries in Africa. In this study, we compared the impact of three rounds of annual MDA with five rounds of semiannual MDA with IA on LF and on soil-transmitted helminth (STH) infections in separate treatment areas within Harper District, Liberia. LF infections were detected by tests for circulating filarial antigenemia (CFA); persons with CFA were screened for *Wuchereria bancrofti* microfilaremia (Mf) by microscopic examination of 60 µl night blood thick smears. STH infections were detected with duplicate Kato-Katz smears. Microfilariae of *Onchocerca volvulus* were detected by examination of skin snips. The primary outcomes for the study were % reductions in infection prevalence from baseline to month 36 (12 mo after the last treatment). *W. bancrofti* CFA and Mf prevalences decreased by 78.2% (from 19.7% to 4.3%) and by 100% (from 8.6% to 0%), respectively after annual MDA; reductions in CFA and Mf after semiannual MDA were 55% (from 37.8% to 16.8%) and 94.4% (17.9% to 1%) respectively. Reductions in hookworm and *Trichuris* prevalences and intensities were slightly greater in the annual treatment area. *Ascaris* prevalences were relatively unchanged in both areas, although infection intensities decreased. Interestingly, a follow-up survey found that reductions in LF and STH infection parameters at 36 mo were sustained or improved further at 72 mo, i.e. 3 yrs after the Liberian Ministry of Health (MOH) assumed responsibility for annual MDA in the district. These results show that annual and semiannual MDA were equally effective for reducing LF and STH infection parameters over a three-year period and that these improvements were sustained for an additional three years by routine annual MDA provided by the Liberian MOH.

0436

RAPID SOCIAL SCIENCE ASSESSMENT IN LYMPHATIC FILARIASIS HOTSPOTS IN SIERRA LEONE

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Four districts in Sierra Leone failed lymphatic filariasis pre-transmission assessment survey (pre-TAS) twice despite effective reported treatment coverage. High baseline prevalence, migratory movements across West Africa, non-biomedical beliefs on disease causation and MDA hesitancy may have contributed to the failure in these hotspots. A deeper analysis of social factors including COVID-19 that may lead to MDA hesitancy was conducted to develop adaptive community engagement strategies. A rapid qualitative assessment tool was used for participatory observations, power mapping and rumor tracking. Trained teams visited nine high-risk communities planned for repeat pre-TAS. They conducted nine in-depth interviews and 18 focus group discussions (males vs females, young vs old). Data were transcribed, analyzed and coded using framework and thematic analysis. Traditional and political leaders were seen to be powerful but not necessarily trusted homogenously across communities and demographic groups. Religious leaders were the most trusted. Young men highlighted "Ataya" base while females identified mother's group as sources of information. Discussions around preference of traditional healers for LF showed more layers into it and highlighted the importance of including them in mainstream approaches. Trust in health workers and NTD programming was influenced by non-NTD interactions and rumors of adverse events in earlier MDAs. Trust in community drug distributors/ health workers was driven by sharing same local languages. COVID-19 affected trust levels, and participants reported lower attendance at health facilities. Knowledge of NTDs/MDA was high but coexisted with different explanations and channels of health seeking behavior. Social mobilization approaches should shift from a focus on health education to a more comprehensive engagement to address drivers of community mistrust of health system and seeing rumors as a window into more deeply rooted mistrust in health systems. Creating content targeting specific social groups in multiple dialects will help reduce MDA-hesitancy.

0437

POST-TREATMENT SURVEILLANCE FOR LYMPHATIC FILARIASIS IN THE DOMINICAN REPUBLIC

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The Dominican Republic is one of four countries in the Americas endemic for lymphatic filariasis (LF). Also known as elephantiasis, LF is caused by repeated infection with the parasite *Wuchereria bancrofti* and transmitted by *Culex quinquefasciatus* mosquitoes. Transmission in the Dominican Republic was limited to 19 districts clustered into three geographic foci. Following three to five rounds of annual mass drug administration (MDA) with albendazole and diethylcarbamazine, MDA stopped in the three foci after 2006 (La Ciénaga), 2007 (Southwest), and 2017 (East), respectively. The La Ciénaga and Southwest foci have completed multiple post-treatment surveillance (PTS) surveys, including successfully passing a third transmission assessment survey (TAS-3) in 2018, while the East passed TAS-1 the same year. The purpose of the current surveys was to conduct TAS-2 in the East and continued PTS in the other foci. The surveys followed the TAS protocol of testing children 6—7 years old for the presence of circulating filarial antigen (CFA) by filariasis test strip (FTS) in community-based surveys, with each focus considered a single evaluation unit. Additionally, one other individual age 15 years or older from the same household as child participants was randomly selected for FTS testing. In La Ciénaga, none (0%) of 478 children and 478 adults tested were CFA-positive in a census survey of the foci in March 2021. In the Southwest, none (0%) of 1574 children and 1591 adults tested in 56 clusters were CFA-positive in October 2020. In the East, sampling is ongoing, but none (0%) of 228 children and 223 adults tested in 36 clusters to date were positive. Household surveys also contributed information for estimating LF morbidity. A total of 41 individuals with lymphedema were identified across the three foci. These results indicate that LF transmission has been eliminated in the formerly endemic areas of the Dominican Republic and indicate the need to provide morbidity management and disability prevention services to LF patients.

0438

MALARIA DOES NOT RESPECT NATIONAL BORDERS: TACKLING MALARIA ON THE BRAZIL-GUYANA-VENEZUELA BORDERS, THE BIGGEST CHALLENGE IN THE AMERICAS REGION

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Globally, human population movement across international borders presents one of the major challenges for malaria elimination. The Brazil-Guyana-Venezuelan (BRA-GUY-VEN) border area harbors the highest concentration of malaria cases in the Americas region e. Unofficial mining activities and the VEN political and economic crisis have contributed to increased population movement. This study aims to assess malariometric trends and explore risk factors for *P. falciparum* (Pf) versus *Plasmodium vivax* (Pv) in those border areas between 2016 and 2020, using data on reported malaria cases (local and imported) from government/non-government organizations. During the study period, there were 645,096 malaria cases reported (BRA=27,852; GUY=42,118; VEN=575,126) along border areas. Most infections were due to Pv (75%), followed by Pf (21%), and mixed infections/others (4%). A malaria resurgence in the VEN border area peaked in 2018 (from 119,984 in 2016 to 160,367 cases in 2018). This increase in cases was also seen in BRA (from 2,027 in 2016 to 6,734 in 2018) and GUY (from 8,487 in 2016 to 11,016 in 2018). The Brazilian state of Roraima was the largest receiver of imported cases (15,089 cases in 2016-2020, 81.8% of these from Venezuela). Overall, during the study period reported malaria cases in border areas decreased 65% in VEN, in contrast to increases of 354% and 57% in BRA and GUY, respectively. The key affected populations were miners, followed by indigenous groups, and males over 15 years old. The design of future policies and interventions should use approaches that most effectively target these high-risk groups. There is a pressing need for coordination and multicountry strategic investments. Recommendations for resilient strategic planning for malaria elimination in border areas will be also presented.

0439

BURDEN OF MALARIA IN PREGNANCY AMONG ADOLESCENT GIRLS FROM FIVE SUB-SAHARAN AFRICAN COUNTRIES: A META-ANALYSIS OF INDIVIDUAL-PARTICIPANT DATA

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Malaria is among the most important causes of death in adolescent girls (10-19 years) globally. However, information on the actual burden of the infection in this population is limited. The objective of this study was to estimate the prevalence of malaria in pregnancy among adolescent girls compared to that of adult women from five sub-Saharan African countries. An individual participant-level meta-analyses was conducted including data from 5804 pregnant women (1069 HIV-infected and 4735 HIV-uninfected) participating in two malaria prevention clinical trials in Benin, Gabon, Kenya, Mozambique, and Tanzania. Clinical episodes during pregnancy, peripheral parasitaemia at delivery and placental malaria were the main study outcomes. A two-stage meta-analysis approach was followed by pooling single multivariable regression results into standard DerSimonian-Laird random-effects models. Adolescent girls were more likely than adult women to present clinical malaria episodes during gestation (IRR 1·94, 95%CI 1·21;3·09, p-value<0·01), parasitaemia at delivery (OR 2·53, 95% CI 1·77;3·61, p-value<0·0001), and placental infection (OR 2·42, 95%CI 1·75;3·35, p-value<0·0001). Differences in malaria burden indicators between adolescent and adult women were not modified by HIV status. Subgroup analysis by gravidity showed similar associations between adolescence and study outcomes both for primigravid and multigravid women. The burden of malaria in pregnancy was higher in adolescent girls compared to adult women, making them a highly susceptible population regarding this infection. Provision of malaria control strategies should prioritise adolescent girls in malaria endemic settings.

0440

IMPACT OF PROACTIVE COMMUNITY CASE MANAGEMENT ON COMMUNITY MALARIA PREVALENCE IN BANKASS, MALI

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Despite ongoing efforts to improve access to malaria diagnosis and treatment at health facility and community levels, the proportion of children with fever who receive a diagnostic test and appropriate treatment remains low. The proactive community case management (ProCCM) model deploys community health workers (CHWs) to conduct proactive case finding, via door-to-door home visits for at least two hours daily, six days a week, with the goal of visiting each household at least twice monthly. For residents of all ages with febrile illness, CHWs perform rapid diagnostic tests (RDT) and offer antimalarial treatment for those with positive tests. Previous studies suggest that this strategy may decrease malaria burden. From 2017-2020, a three-year cluster randomized controlled trial (137 clusters) of ProCCM was conducted in rural Mali. All clusters had a CHW who received training, supervision, and supply chain support; only the proactive visit component was unique to the intervention arm. The endline household survey measured all-cause mortality, care seeking behavior, and case management, as well as RDTs for participants of all ages. Of 4,636 children < 5 years, 15.9% (95%CI 14.8-17.1) reported having a fever in the last two weeks, 68.3% (95% CI 64.4-72.1) sought care, and 48.0% (43.7-52.2) received care from a CHW. Of those who sought care, 70.3% (95% CI 65.5-75.0) received a diagnostic test. In total, 14,402 randomly selected participants consented to receive an RDT at endline across both arms. Overall, 31.0% had a positive RDT (95% CI 29.0-33.1); 25.7% (95% CI 22.9-28.4) among children < 5 years, 50.9% (95% CI 48.2-53.6) among children 5-14 years, 19.3% (95% CI 17.4-21.1) among non-pregnant adults >15 years, and 16.8% (95% CI 14.9-18.7) among pregnant women. The study will be unblinded in June 2021; results will be presented by arm to determine whether three years of ProCCM improved all-cause mortality and malaria prevalence by RDT compared to the traditional model of passive community case management. These results will inform malaria policy makers as to the impact of ProCCM on malaria prevalence.

0441

INCREASING COVERAGE OF COMMUNITY CASE MANAGEMENT OF MALARIA IS ASSOCIATED WITH REDUCTION IN SEVERE MALARIA AND MALARIA INPATIENT DEATHS IN ZAMBIA

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Community case management of malaria (CCM) has been intensively expanded across Zambia since 2013, with the goal that all Zambians can access quality basic health services within 5km or one hour travel of their home by 2021. However, data describing CCM impact in routine implementation settings are limited. This retrospective and observational study evaluates the impact of CCM expansion on inpatient malaria cases and deaths from 2015-2020 in 7 of Zambia's 10 provinces. We hypothesize that increasing the number of malaria providers per population (through CCM expansion) reduces delays in treatment seeking, consequently reducing the proportion of individuals progressing from uncomplicated to severe malaria or malaria-related death. We used a dose-response approach to explore associations between malaria service provider density and inpatient malaria cases and deaths by district and month. All data describing exposure, outcome, and covariates (vector control, rainfall) were extracted from existing routine and programmatic sources. Negative binomial mixed-effect models were generated using continuous and categorised exposure (providers per 1000) for outcomes of inpatient confirmed malaria cases and inpatient malaria deaths among all-ages and among under-fives (U5s). Data from 83 districts over 65 months were compiled, encompassing 310,855 inpatient malaria cases and 7158 malaria deaths. After controlling for vector control access and rainfall, final models found an increase of 1 provider per 1000 population was associated with a 20% reduction in malaria admissions in U5s (incidence rate ratio 0.80, $p<0.001$) and 17% reduction in malaria admissions among all ages (IRR 0.83, $p<0.001$). Similar effect sizes were observed for malaria inpatient deaths (IRR 0.79 $p=0.005$ for U5s, IRR 0.80 $p=0.001$ for all-ages). Models using a categorised exposure variable consistently found a significant reduction in malaria admissions and deaths only when malaria service provider density reached saturation: a least one provider per 750 people. These findings highlight the gains that can be achieved from expanding CCM in high transmission settings.

0442

GEOGRAPHICAL ACCESS TO HEALTH FACILITIES AND SEVERE MALARIA AMONG MALARIA POSITIVE CHILDREN IN RURAL AREAS: ANALYSIS OF HOUSEHOLD SURVEYS FROM 18 COUNTRIES IN SUB-SAHARAN AFRICA

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Access to a health facility is a key determinant of health seeking behavior in malaria endemic countries, particularly in rural settings. In this analysis, we examined the association between severe malaria and a rural household's geographical access (defined as walking time) to formal health facilities. We hypothesized that children from households located far from health facilities are more likely to have severe malaria symptoms compared to those from households close to health facilities, because of a delay in treatment seeking. This analysis used data from 25 Demographic and Health Surveys (DHS) and Malaria Indicator Surveys (MIS) across 18 countries in sub-Saharan Africa collected between 2015 and 2019. Severe malaria was defined as children age 6-59 months with positive rapid diagnostic test for *P.falciparum* malaria and at least one guardian-reported symptom of severe malaria, including loss of consciousness, rapid breathing, seizures, or severe anemia. The analysis includes a weighted descriptive pooled statistic and a multilevel mixed-effects logistic regression model adjusting for gender, age, wealth quintile, mother's education, and malaria endemicity. Children were excluded from the analysis if they recently sought care from a formal health facility or took ACTs in the past two weeks. Among malaria positive rural children who have not sought care or took an ACT in the past two weeks, 2.8% (95% CI 2.4-3.2) had at least one severe malaria symptom. Severe malaria was significantly associated with the child's age and the walking time to health care facility. Children who reside over an hour walking distance away from a health facility were 1.4 times more likely to have severe malaria as compared to children who reside less than 1 hour walking distance from a health facility ($p<0.05$). This analysis highlights distance to health facilities is a strong predictor of severe malaria. Improving access to prompt treatment could potentially minimize the progression from uncomplicated to severe malaria.

0443

RECTAL ARTESUNATE ADMINISTRATION IS ASSOCIATED WITH LOWER REFERRAL COMPLETION OF CHILDREN WITH SUSPECTED SEVERE MALARIA IN NIGERIA AND THE DEMOCRATIC REPUBLIC OF THE CONGO

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Rectal artesunate is a potentially life-saving pre-referral treatment for children with suspected severe malaria. However, the rapid improvement of a child's condition after the administration of rectal artesunate may result in children not being taken to a potentially distant referral facility where appropriate treatment for severe malaria is available. Within the framework of the Community Access to Rectal Artesunate for Malaria (CARAMAL) project, we enrolled 3,964 children with suspected severe malaria attending a community health worker and 2,202 children attending a primary health facility in Nigeria, Uganda and the Democratic Republic of the Congo (DRC) between June 2018 and July 2020. Data collection started 10 months before and ended 15 months after the roll-out of rectal artesunate. All children were followed up 28 days after enrollment and their treatment seeking history was recorded. Referral completion was 68% in DRC, 49% in Nigeria, and 58% in Uganda. In country-specific multivariable logistic regression models, rectal artesunate administration was significantly associated with lower referral completion in DRC (adjusted odds ratio [aOR] = 0.46, 95% CI 0.35-0.59) and among children attending primary health facilities in Nigeria (aOR = 0.16, 95% CI 0.06-0.46). In Uganda, referral completion decreased after the roll-out of rectal artesunate (aOR = 0.74, 95% CI 0.64-0.86) but there was no direct association between rectal artesunate administration and referral completion after the roll-out. In the three countries, referral completion was higher among children presenting at primary health facilities compared to children presenting to community health workers. The roll-out of rectal artesunate seems to have an impact on referral rates and hence should be complemented by measures to ensure referral completion. Children who do not complete referral after rectal artesunate administration may be less likely to receive appropriate severe malaria treatment, with potentially negative consequences for their health outcome.

0443

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0444

THE RELATIONSHIP BETWEEN SEVERE MALARIA PHENOTYPES AND AGE AND ESTIMATES OF BURDEN ACROSS SUB-SAHARAN AFRICA.

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An estimated 409,000 deaths occurred due to severe malaria in 2019, with the WHO African Region disproportionately accounting for approximately 95% of global malaria deaths. Quantifying the burden of severe malaria and identifying which areas are affected most has important implications for resource allocation. Here, we quantify the relationship between age and the probability of severe malaria cases presenting with specific phenotypes using a large dataset from hospital admissions across different geographical locations, using a Bayesian framework. Further, we apply this relationship and an existing and extensively validated model of malaria transmission to quantify the number of severe malaria cases with a specific phenotype, as well as the number of deaths expected for all upper-level administrative divisions in countries of Sub-Saharan Africa. Severe malarial anaemia (SMA) was more common in infants and younger children whereas presentation with cerebral malaria (CM) increased in older children. In areas with lower transmission intensity, the distribution of severe malaria is skewed towards older ages and consequently, a larger proportion of severe cases presenting with CM. In children under 5, we estimate an incidence of 15.9 (events per 1000 persons per year) for severe malaria (SMA=8.5, CM=2.0, RDS=4.3, hyperlactatemia=6.0, other=3.0). Preliminary findings suggest that the estimated proportion of malaria deaths in under-fives that had SMA, CM, respiratory distress syndrome, and hyperlactatemia was 0.46, 0.39, 0.57, 0.62, respectively, with a large number of deaths characterised by the presence of multiple phenotypes. Further work aims to identify whether there is an interaction in the effects on mortality between different phenotypes or whether case fatality rates of individual phenotypes are independent. Understanding spatial variations in the burden of severe malaria and its phenotypes is important in informing distribution of community case management programmes in areas with higher burden or interventions targeting specific phenotypes (e.g. allocation of post-discharge prophylaxis interventions for SMA).

0445

EARLY TRANSCRIPTIONAL PROFILES INDUCED BY LMCEN-/-PARASITES IN EX VIVO INFECTION OF HUMAN MACROPHAGES

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Live attenuated *Leishmania* parasites are being tested as potential vaccines against the blood borne parasitic pathogen *Leishmania*. We have developed centrin deleted live attenuated *Leishmania major* parasites using CRISP-Cas9 (LmCen-/-) as potential vaccine. Preclinical studies showed excellent safety and protective efficacy against wild type *Leishmania major* induced cutaneous infection following immunization with LmCen-/- parasites. To identify immune correlates of protection induced by infection with LmCen-/- parasites for advancing to clinical studies we performed ex-vivo infection of human macrophages obtained from blood donors (n=10) with wild type (LmWT) and attenuated (LmCen-/-) parasites. RNA sequencing followed by bioinformatic analysis were performed. Results revealed that distinct transcription profiles were induced in human macrophages infected with LmCen-/- parasites compared to uninfected controls or LmWT infections. Ingenuity pathway analysis revealed distinct networks of transcripts induced in *LmCen*$^{-/-}$ infection compared to LmWT infection. Notably, STAT-5B mediated pro-inflammatory cytokines, INSIG1 mediated cholesterol biosynthesis, and TLR-9/CCR-7 mediated programming of M1 phenotype were all observed uniquely in LmCen-/- infection compared to LmWT infection. In contrast, pathogenicity associated IL-10 response was mainly observed in LmWT infection, consistent with previous studies. Our studies revealed novel immune correlates of protection in LmCen-/- infections of macrophages from non-endemic population and help validate the immunogenicity characteristics obtained from the pre-clinical studies in rodent models. These results would enable evaluation of the efficacy of LmCen-/- parasites as vaccine candidates by advancing our understanding of the immune mechanisms of protection induced by live attenuated LmCen-/- parasitic vaccines.

0446

INFLAMMATORY MONOCYTES HAVE A PROMINENT ROLE IN LEISHMANIA DONOVANI DISSEMINATION AFTER SKIN INFECTION IN MALNOURISHED MICE

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Malnutrition plays an important role in dissemination of the visceralizing *Leishmania*. Our previous results showed that inflammatory monocytes (Imo) from malnourished MN mice have increased CCR7 expression, intrinsic migratory capacity, and trafficking from skin to spleen. For that reason, we hypothesized that Imo mediate *Leishmania donovani* dissemination in MN mice from skin to visceral organs. We used CCR7-specific siRNAi to knock-down CCR7 in monocytes isolated from bone marrow. A non-targeting siRNA was used as control. Transfer of CCR7-deficient monocytes into MN reduced parasite visceralization to the spleen compared to control monocytes (p=0.0015). *Ex vivo* migration assay showed spleen soluble factors from MN mice induced greater monocyte migration compared with WN mice (p<0.001). The migration was blocked by antibody neutralization of CCL19 and CCL21 (CCR7 ligands) and by inhibition of prostaglandin E_2 (PGE_2) synthase inhibitor, which also reduced PGE_2 production and CCR7 expression. These results were corroborated using CCR7-KO mice where skin-derived infected Imo were decreased in spleen and draining lymph node (dLN) (p=0.01). Since IMo egress from bone marrow is CCR2-dependent, we investigated *L. donovani* dissemination in CCR2-KO mice. CCR2 positive IMo were absent in the knockout mice, however, IMo CCR2 negative Ly6C$^{hi/int}$ were present in spleen. The proportion of monocytes and infected monocytes was decreased in the spleen and dLN of CCR2-KO mice. Finally, we use an inducible Cre-diphtheria toxin transgenic system (CSFR1-DTR mice) to deplete IMo using diphtheria toxin (DT). IMo were effectively depleted from peripheral blood in CSFR1-DTR mice after DT treatment, reducing parasite dissemination to the dLN and liver, but not spleen. Cell population analysis in spleen showed that the DT did not deplete all monocytic populations. These results indicate that IMo have an important role in *L. donovani* dissemination, but other cell populations, such as neutrophils, may also contribute.

0447

IDENTIFICATION OF KLRC1$^+$ Vδ2$^+$ γδ T CELLS AS A CORRELATE OF PROTECTION IN PFSPZ VACCINATION USING A NOVEL PLATFORM FOR THE TRANSCRIPTOMICS META-ANALYSIS

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Sanaria PfSPZ® Vaccine has demonstrated significant efficacy against *Plasmodium falciparum* (Pf) infections in both malaria naïve and exposed populations, where expansion of the Vδ2$^+$ γδ T cells have been associated with acquisition of sterile immunity. Similarly, in preclinical models, sterile immunity requires γδ T cells during the vaccination period, but these cells are not required as effectors during sporozoite infections. We performed RNA sequencing of peripheral blood samples on 56 subjects from three independent PfSPZ vaccine trials (two Mali, one US) and applied novel tools to report gene expression level and impute immune system differences at the cellular level. Specifically, we gathered 174 published RNAseq datasets spanning 28 sorted human immune cell subsets, including innate, B, and T cell subpopulations and differentiation status. A 28-way differential expression analysis identified associations of each gene to its cell type of highest differential expression, and created canonical cell type transcriptomes. Deconvolution was used to mathematically describe each PfSPZ participant sample as a blend of all 28 cell types, allowing quantification of changing cell type proportions directly from RNAseq data. KLRC1 was the most differentially expressed gene (along with Vδ2 γδ T cells markers such as TRDV2, TRGV9) between protected and unprotected vaccinees across all three trials. Cell subtypes Vδ2+ and Vδ2- γδ T cells as well as NK cells, which have overlapping transcriptomes, associated with protection. Using ex vivo flow cytometry, we show that a subset of the Vδ2 γδ T cells express high levels of KLRC1 and are expanded in protected vaccinees after PfSPZ vaccinations. In a murine model of whole organism vaccination, we show that KLRC1 is highly expressed on the Vδ6.3 γδ T cell subset in the liver, and sterile immunity is diminished when these cells are depleted prior to vaccinations. In summary, we report a novel and robust approach for the analysis of transcriptomics data that identified KLRC1 as a key molecule during PfSPZ vaccinations and further validates the requirement of Vδ2 γδ T cells expansion in inducing sterile immunity.

0448

AFUCOSYLATED PLASMODIUM FALCIPARUM-SPECIFIC IGG IS INDUCED BY INFECTION BUT NOT BY SUBUNIT VACCINATION

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IgG specific for members of the *Plasmodium falciparum* erythrocyte membrane protein 1(PfEMP1) family, which mediates receptor- and tissue-specific sequestration of infected erythrocytes (IEs), is a central component of naturally acquired malaria immunity. PfEMP1-specific IgG is thought to protect via inhibition of IE sequestration, and through IgG-Fc Receptor (FcγR)-mediated phagocytosis and killing of antibody-opsonized IEs. The affinity of afucosylated IgG to FcγRIIIa is elevated up to 40-fold compared to fucosylated IgG, resulting in enhanced antibody-dependent cellular cytotoxicity. Most IgG in plasma is fully fucosylated, but afucosylated IgG is elicited in response to enveloped viruses and to paternal alloantigens during pregnancy. Here we show for the first time ever that naturally acquired PfEMP1-specific IgG is likewise markedly afucosylated in a stable and exposure-dependent manner. In contrast, immunization with a soluble subunit vaccine based on VAR2CSA-type PfEMP1 resulted in fully fucosylated specific IgG. Finally, we demonstrate that afucosylation markedly impacts the ability of PfEMP1-specific IgG to induce NK-cell antibody-dependent cellular cytotoxicity (ADCC), as fucosylated IgG is unable to induce this response. These results have profound implications for understanding natural and vaccine-induced antibody-mediated protective immunity to malaria, and identifies a completely new aspect of antibody-mediated immunity to this disease.

0449

MATERNAL SCHISTOSOMIASIS REDUCES HUMORAL IMMUNITY OF OFFSPRING VIA DYSFUNCTIONAL B CELL PROGENITOR MATURATION AND RECEPTOR EDITING

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Maternal helminth infections are a global public health concern that correlates with altered infant immune responses to childhood immunizations and infection. A mechanistic understanding of how maternal infection and inflammation alters the immune responses of offspring is lacking but is critical to decrease childhood morbidity and to understand the consequences of specific long-lived immunity defects. Using our model of maternal *Schistosoma mansoni* infection, we have shown that murine pups born to mothers chronically infected with *Schistosoma mansoni* have reduced responses to vaccinations, similar to what has been reported in humans. Additionally, these pups have reduced humoral immunity cell frequencies in the draining lymph node following Tetanus/Diphtheria immunization. To determine the origin of this humoral immunity defect, we began investigating the plasticity and functionality of progenitors of lymphoid cells critical for a protective humoral response. We found an increase in the common lymphoid progenitors (CLPs) in the bone marrow of pups from Schistosome-infected mothers. Additionally, there is an increase in B cell skewing in pups from infected mothers, but a decrease in transitional B cells and mature B cells in the periphery. When immunized with a Tetanus/Diphtheria vaccination, there is a significant reduction in expansion of these progenitors in comparison to controls from uninfected mothers coupled with a decrease in bone marrow B cells after positive selection, suggesting a more exclusive selection process or differential selective pressure than in pups from uninfected mothers that is confirmed by single-cell RNAseq. We hypothesize that altered transcriptional regulation at the progenitor level caused by maternal Schistosomiasis is the mechanistic root of long-lived defects in humoral immunity to foreign antigens.

0450

ESTIMATING TIMELINES TO ELIMINATION OF TRANSMISSION FOR SLEEPING SICKNESS AND MEASUREMENT OF THIS GOAL IN THE PRESENCE OF POSSIBLE CRYPTIC HUMAN AND ANIMAL RESERVOIRS

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Sleeping sickness (*gambiense* human African trypanosomiasis, gHAT) is a vector-borne disease and one of the neglected tropical diseases. The World Health Organization has targeted gHAT for global elimination of transmission (EOT) by 2030. Despite this ambitious target, there are still unknowns surrounding the transmission cycle and natural history of disease in humans, which have the potential to impact the path to EOT and measurement of this goal. Firstly, there is evidence of asymptomatic infection of gHAT - with potential to self-cure or to harbour skin-only infection - and secondly there is also evidence of gHAT infection in animals and a debate around the possibility of animals maintaining transmission and the resultant impact on the goal. In this work, we compare predictions from two extensions of a baseline model to explore the potential role of asymptomatic human infection and animal reservoirs on achieving the elimination goals. We calibrated each model independently using the available historical human incidence data (from the WHO HAT Atlas) in different health zones in DRC and investigated how gHAT transmission changes in the two modified frameworks compared to the baseline model. This study provides a careful estimate of the impact of asymptomatic infections and animal reservoirs on reaching EOT and how this would alter monitoring of progress towards or achievement of EOT using routine surveillance data from active and passive screening. Our results suggest minor influences of animal transmission and asymptomatic infection on overall gHAT dynamics in many regions when models are fitted to the available data; in these regions the successful reduction in case burden to date could not have been achieved with large contributions from these infection reservoirs. However, neither animal reservoirs nor asymptomatic skin-only human cases contribute directly to the incidence data and so even low-level infection in these groups could impact how we should interpret case reporting data and our estimates of the likelihood that a health zone has already met EOT.

0451

COST EFFECTIVENESS OF A COMMUNITY-BASED STRATEGY FOR THE DIAGNOSIS AND FOLLOW-UP OF TREATMENT IN PATIENTS WITH CUTANEOUS LEISHMANIASIS IN COLOMBIA

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Cutaneous leishmaniasis is a global public health problem. Over 40,000 cases per year occur in the Americas and Colombia has the second highest incidence. Most cases (82%) occur in rural areas and affect people living in poverty. Access to diagnosis and treatment is a challenge due to geographic, economic and armed conflict barriers. First line medication (intramuscular meglumine antimony for 20-28 days) is highly toxic; approximately 50% of patients lack access to treatment, and effectiveness of treatment is largely undocumented. We developed a community-based strategy to facilitate access to diagnosis, treatment and follow-up. We conducted a prospective cohort study between 2016 and 2019 in the Colombian Pacific coast to evaluate the cost-effectiveness of

this community-based strategy compared to the standard of care, from the patient, healthcare and societal perspectives. For diagnosis, a novel molecular test using filter paper samples taken by community leaders in rural areas and processed in a primary health facility was adapted, and Directly Observed Treatment using oral miltefosine, supervised by community leaders, was implemented. In the diagnostic phase 118 patients were enrolled. From the society and healthcare perspectives, microscopy of direct smear of lesions was less costly and more effective than the new molecular test, with an incremental cost-effectiveness ratio of $ -7.46 dollars [mean US dollar 2017-2018 (1 USD = 2,956.43 Colombian pesos)] per patient correctly diagnosed. In the treatment phase (n=70), the costs of the intervention with miltefosine were lower than antimonials ($3.99 ± $1.12 vs $87.99 ± $8.13) from patients' perspective. Implementation of the new treatment strategy has a societal cost of $4,071.24 / Quality Adjusted Life Years. Using the gross domestic product of Colombia as a reference, the new treatment intervention was cost-effective. Our study shows that a community based Directly Observed Treatment follow-up strategy is cost-effective and has potential to overcome the treatment access barriers for patients with leishmaniasis from isolated rural areas.

0452

MATHEMATICAL MODELLING OF SLEEPING SICKNESS IN NORTHERN UGANDA

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Sleeping sickness (*gambiense* human African trypanosomiasis, gHAT) is a vector-borne disease targeted by the World Health Organisation for elimination of transmission (EOT) in humans by 2030. Uganda's gHAT cases have been reported in seven districts in the north of the country during the last two decades. Through a combination of medical interventions and, since 2011, vector control, the case burden has now been reduced to very low numbers. Between 2018 and 2020 only four Ugandan cases were reported across the districts as well as two additional reports of isolated importations from South Sudan diagnosed in Uganda. The goal of this study is to ascertain whether the current interventions are sufficient to maintain the gains and ensure permanent interruption of transmission while considering the resource implications. In this study we utilise a mathematical transmission model to assess the infection dynamics and case reporting in each district between 2000-2020 using data from the WHO HAT Atlas and the Ugandan National Programme. The model factors in improvements to passive screening, the introduction of vector control and variable active screening coverage over time. The fits suggest that local EOT has already been met in all districts, and that subsequent case reporting in the Ugandan population is likely to arise only from people infected several years ago, even if active screening and vector control are stopped. We expect there to be no indigenous case reporting beyond the next few years. These results support a plan to scale back vector control deployment, however this should be done with careful monitoring in place, especially with the potential of imported cases from South Sudan and the DRC to cause resurgence.

0453

ENVIRONMENTAL AND CLIMATIC DETERMINANTS OF LEISHMANIASIS RISK IN SRI LANKA: HISTORICAL VERSUS CONTEMPORARY CLIMATIC FACTORS AND LOCALIZED SEASONALITY

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Leishmaniasis is a neglected tropical vector-borne infectious disease that poses an increasing public health threat in Sri Lanka. Disease risk assessment will help to identify high-risk areas to aid targeted intervention planning. We collected division-level leishmaniasis incidence data 2015-2019, historical average climatic data 1970-2000 and contemporary climatic and environmental data 2014-2019, and Sri Lanka-specific geoclimatic zones, climatic seasons, population and landscape data. We built three models using a gradient boosting machine (GBM) with multinomial logistic regression (MLR) analysis to examine the importance of historical versus contemporary climatic/environmental factors in predicting the high, moderate and low risks of leishmaniasis in Sri Lanka. Based on the observed clinical incidence, about half of the divisions could be classified as low risk and about a quarter of divisions with high risk. GBM-MLR results indicated that contemporary precipitation seasonality (relative influence RI = 1), log-transformed population density (RI = 0.90) and 2014-2018 average October-November minimum temperature (RI = 0.62) were the top three risk factors. Higher variation in 2014-2018 precipitation and lower population density were associated with higher leishmaniasis risk. Fourteen of the contemporary climatic/bioclimatic/climatic-season variables were among the top 20 risk factors. Land use land cover, geoclimatic zones, and vegetation indices were not among the top 20 risk factors. Model-predicted areas with high probability of low risk were along the coast, except in the south, and in the southwest of the country. Model predictions identified two high-risk areas, the north central and south coast, with high probability (>0.8). All models showed high uncertainty in predicting moderate-risk divisions and low uncertainty in predicting high- and low-risk divisions. Contemporary localized climatic/bioclimatic/climatic-season data were more powerful than historical data for predicting leishmaniasis transmission risks in Sri Lanka. Targeted interventions should focus on the high-risk areas.

0454

DEEP LEARNING APPROACHES FOR CUTANEOUS LEISHMANIASES DIAGNOSIS BASED ON SKIN LESION IMAGES

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Cutaneous Leishmaniases (CL) are a major public health issue worldwide. Although not lethal, these neglected diseases inflict major social impediment and morbidity. They affect the quality of life and self-esteem through life-lasting stigma, which leads to social isolation of the patients. Early diagnosis efficiently enhances patient management and treatment outcomes, thus mitigating the disfiguring aspects of CL. In this context, digital tools using artificial intelligence can be applied during the screening and decision-making steps, as they may easily be deployed in remote, peripheral and low-resources settings. We herein present a skin lesion processing system based on deep learning architectures,

able to recognize CL based on a simple photograph of a skin lesion. We gathered 636 images of CL lesions, collected from various sources including the Atlas of Leishmaniases in the Americas. Besides, we used the ISIC dataset containing 25331 images of 8 types of skin cancer. The workflow is articulated in three components: Region Of Interest (ROI) detection, segmentation and classification. The first step is mandatory in preprocessing CL images as they present noise, different levels of contrast and are not lesion-focused. We trained YOLOv3 to detect lesions using 600 ISIC images and tested its performances on 150 images. A mean Average Precision mAP=89% was obtained. We then applied it to extract the lesions as ROI from the CL dataset and cropped lesions were subject to data augmentation. Second, we used the ISIC segmentation set to train the U-Net algorithm. This consists in making the algorithm able to differentiate normal skin from a lesion. With 95% accuracy, U-Net was used to segment the CL dataset prior to classification. Finally, we trained 4 architectures (VGG-16, ResNet50, InceptionV3 and NasNetLarge) on the ISIC (cancer) and the CL datasets. VGG-16 performed as the best classifier with 96% of accuracy. In next steps, we will evaluate the VGG-16 performances in classifying multiple skin conditions presenting a differential diagnosis with CL. This tool will be then deployed as a mobile application and validated in clinical settings.

0455

ONE-YEAR EFFICACY OF BENZNIDAZOLE OR NIFURTIMOX AMONG ASYMPTOMATIC TRYPANOSOMA CRUZI SEROPOSITIVE IN COLOMBIA: A PLACEBO-CONTROLLED RANDOMIZED TRIAL

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Despite apparent geographical variations in their effects, Benznidazole (BZN), and Nifurtimox (NFX, with less supporting data) are widely recommended treatments for *Trypanosoma cruzi* infection. Whether NFX is an effective trypanocidal therapy, and equivalent to BNZ, among Colombian patients, remains uncertain. We conducted a randomized trial (CHICAMOCHA 3, NCT02369978), allocating *T. cruzi*-seropositive (TCS+) adults with no apparent clinical disease to a 120-day blinded treatment BID with BZN, NFX or matching placebo (2:2:1 ratio). Active medication arms included a) 60-day conventional-dose (60CD) regimes with either BZN 300mg/day or NFX 480mg/day, (ratio 1:1), followed or preceded by a 60-day treatment with placebo in a randomly allocated sequence, or b) 120-day half-dose (120HD) regimes with either BZN 150mg/day or NFX 240mg/day (ratio 1:1). The primary outcome was the (intention-to-treat) proportion of participants with a positive PCR 12 months after randomization, after up to 3 tests (repeated if negative in the months 13 or 14). Out of 358 eligible individuals completing a 10-day run-in phase with placebo, 307 participants were randomized (mean age 50.6, SD 8.1 years, 42.7% females, 60% with normal EKG). Eighteen of 246 participants receiving active agents (7.3%, 4.0% on CD vs. 10.7% on HD, p=0.042) abandoned their treatment, as opposed to 3/61 on placebo. After the first run of PCR (n=288, 93.8%, 2-5 lost for analysis in each group), 45/59 (76.3%) placebo-treated participants tested positive. This compares with 84/116 (72.4%) for NFX-treated, p=0.584 (40/58 [69%] and 44/58 [75.9%] in the 60CD and 120HD groups), and 74/113 (65.5%), p=0.146 for BZN-treated (38/57 [66.7%] and 36/56 [64.3%] for 60CD and 120HD groups). We found no evidence that treatment regime impacted on the effect of the agents used (p value for interaction=0.100). In conclusion, neither a treatment with NFX, nor BZN significantly reduced the parasitic load in this sample of Colombian TCS+ individuals. Using 120HD vs. 60CD regimes did not change efficacy, but implied lower adherence. Testing new agents for this population is warranted.

0456

IN HOUSE ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) AS A SENSITIVE DIAGNOSTIC TOOL OF CUTANEOUS LEISHMANIASIS CAUSED BY LEISHMANIA DONOVANI

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Leishmaniasis includes several clinical forms. Serological tests with high sensitivity and specificity are currently available diagnosis of visceral leishmaniasis. While routine diagnosis of cutaneous leishmaniasis (CL) is by microscopy an antibody response to CL has been reported in several recent studies. The objective of this study was to evaluate the detection of anti-leishmanial IgG antibodies as a diagnostic tool in a setting endemic for CL caused by *Leishmania donovani.* Study was carried out with sera collected from patients (50 untreated patients and 140 patients under treatment) and 280 healthy controls from endemic areas as part of an epidemiological survey. All patients had cutaneous lesions positive for *Leishmania* parasites by microscopy and or culture. Clinical features of the patients were recorded using a standardized questionnaire. Sera from patients, endemic healthy controls and 14 healthy controls from non-endemic areas were tested by an in-house indirect enzyme linked immunosorbent assay (ELISA) established using crude antigens of *L. donovani*. Receiver operating characteristics curve (ROC) was used to determine the cut-off. Cut-off was 0.147 at 98.0% test sensitivity and 91.4 % test specificity. The mean absorbance level of anti-leishmanial antibodies was significantly higher (p = 0.000) in untreated patients (0.392 ± 0.152, Range: 0.112 - 0.928) in comparison to patients under treatment (0.210 ± 0.154, range: 0.030 - 0.882), endemic healthy controls (0.091 ± 0.046, range: 0.012 - 0.290) and non-endemic healthy controls (0.071 ± 0.019, range: 0.040 - 0.104). Positive ELISA results were observed in 96/140 (68.6%) patients under treatment, 49/50 (98.0%) of untreated CL patients and 22/280 (7.85 %) of endemic healthy controls and were found to be seropositive for anti-leishmanial IgG antibodies whereas the non-endemic healthy controls were 100% seronegative. The present study demonstrates that the developed indirect ELISA can reliably diagnose a person with active disease in an endemic setting.

0457

FUNCTIONAL GENETICS TO CHARACTERIZE THE PLASMODIUM FALCIPARUM ACETYL-COA SYNTHETASE AND DISCOVER TARGET-SPECIFIC INHIBITORS

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Acetyl-CoA is an essential metabolite at key junctions of cellular and metabolic processes including gene regulation through protein acetylation. One route to producing this cofactor in the human malaria pathogen, *Plasmodium falciparum*, is via direct synthesis from acetate catalyzed by the parasite's acetyl-CoA synthetase, *Pf*AcAS (PF3D7_0627800). To study *Pf*AcAS, we used CRISPR-Cas9 to introduce a C-terminal epitope tag and a TetR-DOZI- RNA aptamer module to conditionally regulate *Pf*AcAS expression levels. We determined that *Pf*AcAS is essential for parasite growth, and its knockdown results in multiple defects across the intra-erythrocytic developmental cycle including: stalling of development at

trophozoite stage; compromised daughter cell segmentation; and failure to egress from the host red blood cell. *Pf*AcAS predominantly localizes to the nucleus, but redistributes to nuclear-associated punctate structures in late stages, suggesting a potential role in acetylation of nuclear proteins such as histones involved in gene regulation. Indeed, functional studies demonstrated inhibition of histone acetylation in *Pf*AcAS knockdown but not in wild-type parasites. Amongst other systems-level approaches, we are using developmental stage-dependent transcriptomic analyses to determine whether *Pf*AcAS knockdown impacts global gene expression. Interestingly, evolution of resistance and whole-genome sequencing identified several compounds as potential inhibitors of *Pf*AcAS. A phenotypic screening platform, based on our conditional gene regulation technology, provided further verification of these compounds as specific *Pf*AcAS inhibitors. Altogether, these findings demonstrate that *Pf*AcAS is critical for proper blood stage parasite development and constitutes a promising antimalarial target.

0458

GETTING IN SHAPE: A NEW PROTEIN COMPLEX REQUIRED FOR MALARIA BLOOD-STAGE PARASITE FORMATION

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The human malaria parasite *Plasmodium falciparum* is a major cause of death around the world, and resistance to current treatments is a threat to malaria control. Clinical symptoms of malaria are caused by the parasite replication inside of host red blood cells and exploring the mechanisms of this replication can identify novel therapeutic targets. The inner membrane complex (IMC) is a membranous organelle that coordinates that shape of parasites and invasion into host cells. Despite our knowledge of IMC function and composition, we do not know the molecular mechanisms that underly IMC biogenesis. Here we describe a novel *Plasmodium* specific protein that is required for IMC biogenesis, which we have named PfAnchor. We utilized a CRISPR/Cas9-based system to endogenously tag PfAnchor and introduce the Tet-repressor knockdown system. Using immunofluorescence assays, we localized PfAnchor near the IMC during blood stage replication. PfAnchor deficient parasites halted IMC biogenesis at early stages of formation, leading to parasite death. Co-immunoprecipitation experiments showed PfAnchor interacting with a GTPase-containing protein named Dynamin-like protein 2 (PfDyn2). We also generated parasites with tagged PfDyn2 under the control of the Tet-repressor knockdown system. Like PfAnchor, PfDyn2 localized near the growing IMC, and IMC biogenesis halted in PfDyn2-deficient parasites leading to parasite death. All together, we have identified two novel *Plasmodium* specific proteins that are required for IMC formation and parasite blood stage replication. Currently, we are investigating potential PfAnchor interacting proteins using proximal labeling combined with co-immunoprecipitation to gain an understanding of the mechanism of the PfAnchor-PfDyn2 complex, as well as investigating the role of the GTPase domain of PfDyn2 in IMC biogenesis.

0459

GENETIC ABLATION OF SEX-SPECIFIC TRANSCRIPTION FACTORS IN CRYPTOSPORIDIUM: DISSECTING THE REGULATORY PATHWAYS OF SEXUAL DEVELOPMENT AND REPRODUCTION

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The parasite *Cryptosporidium* is a leading cause of diarrheal disease and infects millions of people worldwide each year, with a disproportionally high burden on children in south Asia and sub-Saharan Africa. There is no vaccine and only limited treatment. A greater understanding of *Cryptosporidium* biology is necessary to identify the stages that can be targeted by therapeutics or vaccines to block infection and transmission. The parasite life cycle begins upon ingestion of the oocyst, which releases invasive sporozoites into the gut. These invade intestinal epithelial cells and initially propagate asexually before transitioning to male and female gametes. Sex then leads to the production of new oocysts. While these stages are clearly distinguished morphologically, the genes which define them remain largely unknown. Recently, we developed a *Cryptosporidium* single-cell atlas which documents the transcriptional changes that occur throughout the life cycle and reveals an abrupt switch to male or female development. We discovered AP2 and Myb transcription factors associated with this conversion to sexual development and initiated functional characterization. Three factors were deemed essential *in vivo*: Myb-M, AP2-M, and AP2-F, with Myb-M expressed in the earliest males and AP2-M and AP2-F expressed during later stages of male and female development, respectively. To enable genetic studies of these essential genes, we developed a conditional gene regulation system to either ablate factors or to force their expression at different phases of the life cycle. We used this approach to conditionally ablate AP2-F and observed downregulation of meiotic genes *in vitro* and a significant loss of shed oocysts *in vivo*, implicating that this gene is involved in the post-fertilization stage and may be used as a transmission-blocking target. Current work is focused on dissecting the roles of the two essential male transcription factors and their capacity to drive male fate.

0460

PLASMODIUM FALCIPARUM UTILIZES TWO CRITICAL BUT DISTINCT MEMBRANE FUSOGENS FOR GAMETE FERTILIZATION

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The fertilization of the female *Plasmodium* gamete by the male gamete occurs in the mosquito midgut and is the central event in sexual reproduction. Fertilization requires gamete fusion and represents a major bottleneck in the malaria life cycle. In many plant species and lower eukaryotes, the Hapless 2 (HAP2)/Generative Cell Specific 1 (GCS1) family of proteins are conserved and mediate gamete fusion. Although HAP2 is encoded by a single gene in most of these species, *Plasmodium falciparum* expresses two distinct paralogs: *PfHAP2* and *PfHAP2p*. While PfHAP2 is expressed exclusively by the male gamete, PfHAP2p is expressed by both sexes. Gene deletion analyses shows that both *PfHAP2* and *PfHAP2p* are essential for zygote formation and thus for parasite infection of the mosquito. Importantly, using a mammalian cell fusion assay, we demonstrate that *Pf*HAP2 is a *bona fide* membrane fusogen. Furthermore, antibodies targeting a critical fusion peptide (the *cd*-loop) of PfHAP2p blocks parasite transmission to mosquitoes. Our results demonstrate essential roles for *PfHAP2* and *PfHAP2p* in mediating gamete fusion in *Plasmodium falciparum* and their potential as a transmission blocking target.

0461

CAUSES OF STILLBIRTHS IN SUB-SAHARAN AFRICA AND SOUTH ASIA FINDINGS FROM THE CHAMPS STUDY

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Globally 2.6 million stillbirths occur annually, yet there are major knowledge gaps about their causes and the circumstances associated with these events. Investigating causes of stillbirth is critical to proposing effective preventive measures. The Child Health and Mortality Prevention Surveillance (CHAMPS) Network was established to understand and track causes of stillbirths and childhood deaths in South Asia and Sub-Saharan Africa by routinely conducting postmortem minimally invasive tissue sampling (MITS). We aimed to describe the main conditions of stillbirths and their epidemiologic characteristics across 7 CHAMPS sites. Between December of 2016 and 2019, 453 stillbirths were enrolled and underwent MITS. The determination of cause of death process was completed for 433 of these stillbirths by March 2021 and an expert panel determined the main condition based on histopathology of MITS, microbiological diagnostics, clinical data abstraction, and verbal autopsies. Of these 433 cases, 415 (96%) stillbirths were delivered at a healthcare facility and 184 (42%) were macerated stillbirths. The most frequent main condition was intrauterine hypoxia (326/433; 75%), ranging from 58% in South Africa to 91% in Kenya followed by congenital infections (48/433; 11%), with Streptococcus species, E. coli/Shigella species and cytomegalovirus being the most common etiologies. The majority of congenital infections were documented in macerated stillbirths (31/48, 65%). In 21% (90/433) of cases, the main maternal condition was placental complications, mostly separation of placenta. Maternal hypertension was the main maternal condition leading to stillbirth in 17% (74/433) of cases. The expert panels in each country concluded that 70% (303/433) of all stillbirths were preventable through increased access to antenatal care, intrapartum fetal monitoring, earlier C-section and improved obstetric care. Systematic monitoring and inclusion of stillbirth rate as a marker of quality maternal care is critical to generate robust evidence that can aid stakeholders in formulating feasible strategies to reduce the burden of stillbirths globally.

0462

ETIOLOGY OF DEATH AMONG INFANTS AND CHILDREN ENROLLED THROUGH THE MULTISITE CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE (CHAMPS) NETWORK

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In spite of the increasing importance of the neonatal period to overall child mortality, 2.8M deaths still occur globally each year in children aged 1-59 months (53% of all U5 deaths). Characterizing post-neonatal deaths may help to improve child survival. The Child Health and Mortality Prevention Surveillance (CHAMPS) Network conducts childhood mortality surveillance in Sub-Saharan Africa and South Asia, innovatively using postmortem minimally invasive tissue sampling (MITS). We describe the causes of post-neonatal mortality across CHAMPS sites in 7 countries. 502 MITS on child deaths (263 in infants; 239 in children 12-59 months) were completed between 2016 and 2019. Expert panels at each site determined the underlying condition and chain of events leading to death, based on histopathology, microbiological diagnostics, clinical data, and verbal autopsies. The 5 most common underlying causes of death were malnutrition (80/502; 15.9%); HIV (59, 11.8%); malaria (53, 10.6%); congenital birth defects (52, 10.4%) and lower respiratory tract infections (LRTI; 42, 8.4%). However, when considering any position (underlying, antecedent, immediate) occupied within the chain of events leading to death, LRTI (232, 46.2%) and sepsis (192; 38.3%) contributed to nearly half and one third of all deaths, respectively. Pathogens most frequently

contributing to death included *Klebsiella pneumoniae* (133; 26.5%), *Streptococcus pneumoniae* (89; 17.7%) and *Plasmodium falciparum* (88; 17.5%). Among the 5 top underlying causes of death, the median number of other conditions in the chain of events leading to death was 3 for malnutrition, 3 for HIV, 1 for malaria, 3 for congenital birth defects and 1.5 for LRTI. 76% (382/502) of all post-neonatal deaths were considered preventable by the expert panels. CHAMPS results highlight gaps and opportunities for action to effectively prevent death during infancy and childhood and reveals the common interaction of various etiologies in the path towards death. The significance of *Klebsiella Pneumoniae*, hitherto insufficiently recognized, calls for a closer focus on this particular pathogen to improve child survival.

0463

COST-BENEFIT ANALYSIS OF FOUR-PANEL RAPID SCREENING IN ANTENATAL CARE IN THREE COUNTRIES IN SUB-SAHARAN AFRICA

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We performed an economic analysis of a new technology used in antenatal care (ANC), the ANC panel. Introduced in 2019-2020 in 5 Rwandan districts, the ANC panel screens for 4 infections (hepatitis B, HIV, malaria, and syphilis) using a blood sample from a single fingerstick. It increases the scope and sensitivity of screening. We developed an Excel-based mathematical and economic model to perform cost-effectiveness and cost-benefit analyses of this technology. We used it first for these Rwandan districts and then applied it to 2 neighboring sub-Saharan African countries (Uganda, and Kenya). Key parameters included the infant mortality rate, life expectancy, and rates of positivity (with 95% confidence intervals, CI) and treatment success of each infection. Rwanda had actual current positivity rates, compared to literature estimates for Uganda and Kenya. We calculated costs by combining the cost of the ANC panel, its administration, and follow-up treatment. We conducted a literature review of the impacts of the four infections on maternal and infant health. We used data from in-country and international sources, and expert opinion. Benefit-cost ratios (with positivity CIs) for each country were 21.9 (21.3-22.4) in Rwanda, 8.4 (8.2-8.9) in Uganda, and 10.0 (9.2-10.6) in Kenya. The ANC panel averted 76 (71-81) disability-adjusted life years (DALYs) per 1,000 women in ANC in Rwanda, 204 (181-234) in Uganda, and 121 (98-144) in Kenya. Net economic benefits per woman ranged from $105 ($100-$111) in Rwanda, $165 ($149-$190) in Uganda, and $213 ($171-$255) in Kenya. Net healthcare costs per woman ranged from -$40.52 to -$43.68 in Rwanda, -$6.01 to -$7.71 in Uganda, and -$0.30 to -$2.82 in Kenya. With negative net costs, the ANC panel saved healthcare costs in all three countries. Each $1 in ANC screening generated societal economic benefits worth $8 to $22. By combining literature and field data, the ANC model is applicable to other countries and technologies in sub-Saharan Africa.

0464

DISTANCE MATTERS: BARRIERS TO ANTENATAL CARE AND SAFE CHILDBIRTH IN A MIGRANT POPULATION ON THE THAILAND-MYANMAR BORDER FROM 2007-2015, A PREGNANCY COHORT STUDY

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Background: Antenatal care and professional childbirth services are important interventions to improve maternal health and lower the risk of poor pregnancy outcomes and mortality. Recent literature has shown that distance to clinics can be a disincentive towards seeking care during pregnancy. On the Thailand-Myanmar border antenatal clinics serving migrant workers have found high rates of loss to follow-up of 17.4%, but decades of civil conflict have made this difficult to study. Here we perform a comprehensive study examining the geographic, demographic, and health-related factors contributing to loss to follow-up.Methods: Using patient records we conducted an epidemiological analysis looking for predictors of loss to follow-up and pregnancy outcomes between 2007-2015. We used multivariable regressions to assess for predictors of loss to follow-up, pregnancy complications, and time of first presentation for antenatal care.Results: We found distance travelled to clinic strongly predicts loss to follow-up, miscarriage, malaria infections in pregnancy, and presentation for antenatal care after the first trimester. Women lost to follow-up travelled 45% farther than women who had a normal singleton childbirth (a ratio of distances (DR) 1.45; 95% confidence interval (CI): 1.40 – 1.51). Women with pregnancies complicated by miscarriage travelled 23% farther than those who did not have miscarriages (DR: 1.23; CI 1.14 - 1.31), and those with *Plasmodium falciparum* malaria in pregnancy travelled 62% farther than those without *P. falciparum* (DR: 1.62; CI: 1.44 – 1.82). Women who delayed antenatal care until the third trimester travelled 46% farther compared to women who attended in the first trimester (DR: 1.46; CI: 1.39 – 1.53). Conclusions: This analysis provides the first evidence of the impact of distance on access to antenatal services and pregnancy outcomes in the rural, remote, and politically complex Thailand-Myanmar border region. Our findings can be applied to other similar environments, in which increased patient support services may be required to improve continuity of care and provide for a more positive pregnancy experience.

0465

TWIN PREGNANCIES: HIGH RISK OF PERINATAL DEATH, PRETERM BIRTHS, AND MATERNAL COMPLICATIONS IN RURAL BANGLADESH

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Twin pregnancy rates vary by geographic region, ranging from 8-19 per 1000 deliveries. Compared to singletons, twin pregnancies involve higher risk for adverse outcomes for babies and mothers, including premature birth, neonatal and maternal death, and severe maternal complications. In this study, we describe the risk of adverse outcomes among twin pregnancies in Baliakandi, a rural sub-district of Bangladesh. We used a health and demographic surveillance system to identify births and deaths between Sep 2017-Dec 2019. In Dec 2018, we began detailed pregnancy surveillance to collect data on pregnancy and delivery, including complications and antenatal care (ANC). Excluding miscarriages/abortions,

we identified 11547 deliveries; 102 were twin deliveries (8.8 per 1000 deliveries): 90 live birth, 4 stillbirth, and 8 discordant pairs. Compared to singletons, twins were more likely to be delivered at a health facility (79% vs 66%, 79/102 v 7608/11434; χ^2 p<0.018), preterm (57% v 20%, 58/102 v 2301/11445; χ^2 p<0.001), and stillborn (78 v 45 per 1000 births, 16/204 v 268/11445; Fisher p<0.001). Twin live births were more likely to end in early neonatal death (175 v 22 per 1000 live births, 28/160 v 245/10932; Fisher p<0.001). Compared to singletons, women with twin pregnancies in 2019 were more likely to report complications during pregnancy (62% v 42%, 28/45 v 2306/5468; χ^2 p=0.010) and delivery (51% v 31%, 23/45 v 1709/5481; χ^2 p=0.007). Statistically significant complications included hemorrhagic (16% v 7%, 7/45 v 378/5520, χ^2 p=0.032) and hypertensive disorders (14% v 5%, 6/43 v 268/5135, χ^2 p=0.023). There were no significant differences in attending ≥4 ANC visits (39% v 32%, 17/44 v 1676/5159; χ^2 p<0.481) and receiving key procedures such as blood pressure checks, urine tests, blood tests, and ultrasounds (range 48-71%, all χ^2 p>0.05). Twin pregnancies are at higher risk of adverse outcomes compared to singletons and require added preventative measures including more frequent ANC visits and maternal/fetal testing. Our findings suggest this may not be practiced in Baliakandi and emphasize a need for early detection and improved local care.

0466

HIGH BURDEN OF NEURAL TUBE DEFECTS IN EASTERN HARAGHE, ETHIOPIA: INITIAL FINDINGS FROM CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE

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Neural tube defects (NTDs) can result in fetal loss and severe disability in children and, most of them are related to folate deficiency in the mother. In Ethiopia, the estimate rate of neural tube birth defects is 2 cases per 1,000 live births although published data from northern Ethiopia showed up to 13 cases per 1,000 births. In spite of a lack of data from Eastern Ethiopia, a recent nutritional survey in reproductive-aged women of Ethiopia in the region found that 81% were folate deficient, almost two times the national prevalence (46%); The public health recommendation to reduce burden of NTDs is mandatory folic acid fortification, but this policy is yet to be legislated in Ethiopia. Child Health and Mortality Prevention Surveillance (CHAMPS) aims to determine causes of death (CoD) among <5 children (U5) using Minimally Invasive Tissue Sampling (MITS) and advanced diagnostic methods. Mortality surveillance was established in a demographic surveillance system (DSS) area in Eastern Ethiopia in February 2019. A notifications system was implemented to detect MITS-eligible deaths (stillbirths and U5 children who died within the last 24 hours and were DSS members). An expert panel assigned the final CoD after analysing clinical information, pictures, microbiological and histopathological findings, and verbal autopsy. From 4th February 2019 to 3rd February 2021, families of 196 (63.8%) among 307 MITS-eligible deaths were approached and consented for MITS. Of these, CoD was assigned for 129; 67 stillbirths, 42 neonates and 20 U5 infants/children. Among them, nine had a neural tube defect (7%), all identified as the underlying cause of death, 8/67 stillbirths (12%) and one neonate/42 (2%) who died within 24h of birth. The most common type of NTD was anencephaly (5/9, 60%), of which three had craniorachischisis. Mortality surveillance identified that NTDs are common among stillbirths in Eastern Ethiopia, a region with high prevalence of folate deficiency among reproductive-aged women. Due to the lethality and disability of NTDs, the role of folic acid fortification as public health intervention to prevent NTDs in Ethiopia urgently needs to be explored.

0467

SUSTAINABLE PALM WEEVIL FARMING TO ADDRESS FOOD INSECURITY AND INCOME GENERATION AT MATERNITY WAITING HOMES IN LIBERIA: A PROOF-OF-CONCEPT PROJECT EVALUATION

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Food insecurity is widespread in Liberia, with every fifth household considered food insecure. Nearly two-thirds of pregnant women in Liberia are anemic. In 2010, the University of Michigan and the Bong County Health Team established the first six maternity waiting homes (MWHs) in post-conflict Liberia where women in the late stages of pregnancy stay to await safe childbirth. In 2018, a country-wide assessment of 118 MWHs across Liberia was conducted to understand successes and barriers to scale-up. Food security at MWHs was identified as a barrier to their use. Women and community members residing at these MWHs identified indigenous insects are an acceptable food source consumed by pregnant women in rural areas. The goals of the current intervention were to: 1) educate local Liberians on the concept of rearing edible insects; 2) train participants residing at MWHs to complete two cycles of insect production; 3) evaluate insect farming as a potential income generating activity for MWHs. Training was successfully conducted for 60 participants at four MWHs in Bong County, Liberia. Participants were provided palm larvae rearing kits, starter culture of adults, larvae and a wooden structure. Three of the four sites were able to produced larvae for consumption, while two were also able to produce additional product for income generation. The three most common methods of palm weevil preparation were grilled (50%), boiled (25%), and fried (25%). In terms of production patterns, 50% of the participants indicated that the production of palm weevil larva was successful whilst 25% reported their production was limited due to challenges such as sourcing of palm yolk and cassava feed for larvae production. The intervention was able to reduce food insecurity for pregnant women awaiting delivery and served as an income generating activity at one MWH through the sale of palm weevils. Rearing palm larvae has the potential to provide a sustainable source of high protein food supplement at rural MWHs in Liberia while also serving as an income generating activity.

0468

HUMAN-IMMUNE-SYSTEM HUMANIZED DRAGA MICE AS SURROGATE IN VIVO HUMAN MODEL FOR COVID-19 RESEARCH AND GENERATION OF THERAPEUTIC HUMAN MONOCLONAL ANTIBODIES

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There is an urgent need for generation of animal models for SARS-CoV-2 to accelerate development of vaccines and therapeutics for COVID-19. Herein, we report the first human-immune-system humanized mouse model ("DRAGA": HLA-A2.HLA-DR4.Rag1KO.IL-2RgcKO.NOD) for COVID-19 research. This mouse reconstitutes a functional human-immune-system upon infusion of HLA-matched human hematopoietic stem cells from umbilical cord blood. The DRAGA mouse also reconstitutes human epithelial and endothelial cells expressing the human ACE2 receptor for SARS-CoV-2, and TMPRSS2 serine protease co-localized on human lung epithelial cells, as demonstrated by immunofluorescence microscopy (IFA). All DRAGA mice (n=17) challenged by the intranasal route with SARS-CoV-2 (10^3 pfu/mouse) became infected, showing deteriorated clinical condition (5%-15% loss body weight, ruffed fur, hunched back,

reduced mobility), replicating virus in the lungs as measured by RT-qPCR, and human-like lung immunopathology including human T-cell infiltrates, microthrombi and pulmonary sequelae. Among T-cell infiltrates, lung-resident ($CD103^+$) $CD8^+$ T cells were sequestered in epithelial ($CD326^+$) lung niches and secreted granzyme B and perforin as measured by IFA, indicating cytotoxic potential. All SARS-CoV-2 infected DRAGA mice also developed human antibodies against the SARS-CoV-2 viral proteins at day 25 post-infection. Using human B cells from immunized DRAGA mice we have generated a panel of human monoclonal antibodies (hmAbs, n=9) against the SARS-CoV-2 spike receptor-binding-domain (RBD). The hmAbs have been re-engineered on different Ig-Fc scaffolds (IgG1 and IgG2) to investigate the role of Ig-Fc biological functions on their prophylactic and therapeutic efficacy against SARS-CoV-2. In summary, DRAGA mice proved to be a surrogate *in vivo* human model for SARS-CoV-2/COVID-19 research, and a unique research resource for generation of therapeutic hmAbs against emerging infectious diseases.

0469

THE SARS-COV-2 SPIKE GLYCOPROTEIN INDUCES EPITHELIAL AND ENDOTHELIAL BARRIER DYSFUNCTION AND VASCULAR LEAK INDEPENDENTLY OF ACE2

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SARS-CoV-2 is the causative agent of COVID-19, characterized by acute respiratory distress syndrome (ARDS) in severe cases; however, the triggers of pathogenesis are unclear. As a clinical feature of ARDS is epithelial and endothelial barrier dysfunction, we monitored endothelial (human pulmonary microvascular endothelial cells; HPMECs) and epithelial (Calu-3) hyperpermeability and endothelial glycocalyx layer (EGL) disruption induced by recombinant soluble SARS-CoV-2 trimeric Spike (S) and its receptor-binding domain (RBD), as well as vesicular stomatitis virus (VSV) particles pseudotyped with SARS-CoV-2 S (VSV-S) or VSV-glycoprotein (VSV-G) as a control. We found that SARS-CoV-2 S, RBD, and VSV-S, but not VSV-G, triggered both endothelial and epithelial hyperpermeability, measured by Transendothelial Electrical Resistance (TEER), and EGL disruption, measured by cell surface levels of sialic acid and heparan sulfate (HS). Intriguingly, SARS-CoV-1 and MERS-CoV S did not induce hyperpermeability in HPMECs comparably to SARS-CoV-2 S. Surprisingly, we observed SARS-CoV-2 S-mediated endothelial dysfunction using cells that do not express ACE2 (HPMECs), suggesting a distinct pathogenic mechanism from the ACE2-dependent inflammatory responses previously reported. Using murine models of dermal and systemic vascular leak in C57BL/6 mice, we showed that SARS-CoV-2 S was sufficient to mediate vascular leak *in vivo*, supporting that this activity is independent of human ACE2. To further characterize this pathway, we conducted RNA-Seq on HPMECs treated with SARS-CoV-2 S, revealing transcriptional signatures indicative of endothelial dysfunction and proinflammatory responses. Further, a point mutation in the SARS-CoV-2 S HS binding site abrogated the capacity of S to induce EGL disruption, indicating that S interaction with HS could be a trigger of pulmonary COVID-19 pathogenesis. Overall, our results indicate that SARS-CoV-2 S glycoprotein alone can directly mediate endothelial hyperpermeability independently of both ACE2 interaction and viral infection, serving as an additional feature of SARS-CoV-2 pathogenesis.

0470

GENOME-WIDE, BIDIRECTIONAL CRISPR SCREENS IDENTIFY MUCINS AS CRITICAL MODULATORS OF SARS-COV-2 INFECTION

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The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, has resulted in an unprecedented public health catastrophe for which new therapeutic strategies are desperately needed. Gaining a basic understanding of host-pathogen interactions mediating viral infection or viral control in relevant human cells is critical for identifying new host targets for therapeutic intervention. To this end, we conducted genome-wide CRISPR inhibition and activation screens in human lung epithelial (Calu-3) cells, identifying numerous factors that implicate diverse and druggable host pathways important for mediating or restricting SARS-CoV-2 infection. These include cell-cycle factors and inflammatory responses, as well as components of intercellular junctional complexes. Intriguingly, numerous mucins, a family of high-molecular weight highly-glycosylated proteoglycans and the main constituent of mucus, emerged as critical modulators of SARS-CoV-2 infection, with the membrane-anchored MUC1, 4, 13, and 21 playing an antiviral role and the gel-forming MUC5AC exhibiting proviral activity when overexpressed. Using a SARS-CoV-2 spike pseudotype entry assay, we determined that overexpression of these diverse mucins modulated viral entry in a manner consistent with their proviral or antiviral role. Our data suggest that mucins encountered by SARS-CoV-2 in the human airway may serve both to prevent virus attachment and entry into permissive lung cells, or conversely, to promote virus attachment to cells. In summary, these data provide a valuable resource for the field and generate insight into the host-pathogen interactions of SARS-CoV-2 in relevant human epithelial cells, highlighting the potential of ubiquitous mucins to serve both as predictors of patient disease progression as well as druggable host targets.

0471

COMPARATIVE PHYLODYNAMICS OF SARS-COV-2 VARIANTS IN THE NORTHEAST UNITED STATES

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In the midst of a national vaccination campaign, COVID-19 cases are rising in the United States (US). A contributing factor to this trend may be the emergence of SARS-CoV-2 variants. B.1.1.7, the variant first identified in the United Kingdom, was detected in the US in January 2021, and is classified as a Variant of Concern (VOC). This variant has rapidly risen in prevalence among globally circulating SARS-CoV-2 likely due to its increased transmissibility. To monitor the frequency of this and

other variants, we are conducting genomic surveillance in Connecticut (CT). Since November 2020, we have generated over 2,300 SARS-CoV-2 whole genomes collected across the state. We found that the prevalence of B.1.1.7 rose to 60% by April 5 in CT. Interestingly, the majority of the remaining viruses belong to the lineage B.1.526, which was first identified in New York (NY) and is not a VOC. To investigate, we combined our dataset with publicly available genomes on GISAID, selecting all B.1.526 genomes (n ≈ 7,000). Due to the large number of B.1.1.7 genomes, we included all genomes from CT and neighboring states and a random sample of genomes collected globally (n ≈ 2,500). We tabulated the number of sustained introductions of each variant into CT by constructing separate time-resolved trees, which served as a fixed topology for discrete phylogeographic trait reconstruction in BEAST. We assigned three geographic trait states: CT, NY, or other. We defined a sustained introduction as a CT clade with a non-CT common ancestor containing at least three tips. Of the 23 sustained B.1.1.7 introductions, 74% came from New York, while 68% of the 19 sustained B.1.526 introductions originated there. To ensure that these introductions have strong phylogenetic support, we will build bootstrapped maximum likelihood trees. We will next investigate the spread of these lineages within and between CT and NY using a continuous phylogeographic approach. We hypothesize that NY disproportionately influences SARS-CoV-2 dynamics in CT, which would provide a strong motivation to cultivate inter-state surveillance and control efforts as the US looks towards the end of the pandemic.

0472

COMPREHENSIVE MUTAGENESIS OF SARS-COV-2 S PROTEIN TO MAP ANTIBODY EPITOPES AND IDENTIFY RESIDES ESSENTIAL FOR FUNCTION

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To characterize the immune response to SARS-CoV-2 infection, we have created a comprehensive Ala-scan mutation library (>1240 mutations) of the SARS-CoV-2 S protein. We are using this library to epitope map human anti-SARS-CoV-2 monoclonal antibodies (MAbs), by high-throughput, rapid screens of MAb binding to each mutant S protein. Individual mutant expression plasmids are transfected into human cells to achieve native protein expression and folding. Immunoreactivity of MAbs to each mutant S protein is then quantified by high-throughput flow cytometry, allowing us to identify the S protein epitope residues with the highest energetic contribution to MAb binding. We have mapped over 50 MAbs that target different regions of the S protein, identifying conformational epitopes in the S1 receptor binding domain (RBD) and N-terminal domain (NTD), and in S2, helping characterize MAbs that neutralize and protect in animal models of disease. To provide critical reagents for analyses of MAb or serum immune responses to SARS-CoV-2 infection, we developed a pseudotyped lentiviral system to generate SARS-CoV-2 reporter virus particles (RVPs) displaying S protein. The replication-incompetent RVPs perform one round of infectivity and enable safe (BSL-2) and reproducible virus neutralization assays with luminescent or fluorescent readout. We have produced over 30 different SARS-CoV-2 RVPs incorporating known S protein variants. We also used RVP technology to screen the S protein mutant library for infectivity, to identify residues whose mutation eliminates virus infectivity but does not impact S protein expression, antigenicity, or reporter virus budding, indicating that these residues perform critical functions for infectivity. To identify uncharacterized SARS-CoV-2 cellular binding factors we are assaying wild-type SARS-CoV-2 RVP infectivity in non-permissive cells expressing our membrane proteome array (MPA) of 6,000 unique human membrane proteins. Similar experiments with other viruses have yielded previously unidentified candidate membrane proteins that enable virus infectivity.

0473

ORACOV, AN ORAL, SELF-ADMINISTERED, ROOM TEMPERATURE STABLE, TRIPLE ANTIGEN SARS-COV-2 VACCINE AND AN APPROACH TO VACCINES AGAINST OTHER EMERGING INFECTIOUS DISEASES

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Vaccines are the optimal intervention to prevent COVID-19 and emerging infectious diseases. The goal is billions of effective immunizations. Our recombinant, attenuated *Salmonella Typhi* (Ty21a=Vivotif) vaccine platform expresses multiple SARS-CoV-2 proteins. The vaccine, OraCOV, is manufactured using methods amenable to inexpensive scale-up, is self-administered orally in 3-4 doses over 4-6 days, has an excellent safety record (&lt150 million Ty21a doses administered), and induces protective immunity, in large part through cellular immune responses, protective for 3-7 years against typhoid fever. Our recombinant Ty21a expresses SARS-CoV-2 proteins and, 1) is stable at room temperature, 2) is administered orally and taken up via the oral and intestinal mucosa and 3) intended to induce secretory IgA antibodies and protective T cell responses to eliminate SARS-CoV-2 at the respiratory mucosa. Our innovations depart from approaches taken by most COVID-19 vaccines: A) *Vaccine delivery and administration*: We envision immunizing in a short period huge numbers of people without congregating for inoculation, because vaccine will be shipped in envelopes at ambient temperature for oral self-administration at home over one week. B) *Induction of protective immunity*: Immunization via the oral mucosa is intended to lead to durable immunity mediated by secretory IgA antibodies and protective T cells targeting not only the spike (S), but also the membrane (M) and nucleocapsid (N) proteins. Our live vaccine candidate(s) is stabilized by foam drying and is room temperature stable. We have integrated the coding regions for the ecto-domain of S, M, and N proteins into the chromosome of Ty21a, as individual and all-in-one constructs. These multi-subunit, bi-valent vaccine candidates express SARS-CoV-2 proteins and protective O-antigen of *S. Typhi*. We will present results on induction of viral neutralizing antibodies and cellular responses against SARS-CoV-2. We anticipate success with Ty21a-SARS-CoV-2 will establish our recombinant Ty21a platform as universal for responding to many emerging, re-emerging, and current infectious diseases.

0474

EPIDEMICS OF CHIKUNGUNYA, ZIKA, AND COVID-19 IN NICARAGUAN COHORT STUDIES REVEAL BIAS IN CASE-BASED MAPPING

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Chikungunya virus, Zika virus, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) recently caused large epidemics across the Americas, resulting in many symptomatic and inapparent infections. Spatial analyses usually measure cases (symptomatic infections) and estimate incidence rates (cases/total population). Using data from two prospective cohort studies of children and households in Managua, Nicaragua, we separately analyzed infection risk (infections/total),

disease risk (cases/infections), and the incidence rate (cases/total), which are commonly conflated in the literature. We assessed ~3,000 initially uninfected children over two chikungunya (2014, 2015) and one Zika (2016) epidemic, as well as 1811 household members who experienced a COVID-19 epidemic (2020). We confirmed infections by ELISAs and cases by rRT-PCR and serological assays; we used spatial/spatiotemporal models, the intracluster correlation coefficient, and SaTScan to analyze the data. Across the four epidemics and all analyses, incidence rates substantially underestimated infection and disease risks. The spatial infection risk differed from the spatial disease risk, showing that areas with many infections did not necessarily have many cases and that among infections, the risk of disease varied spatially. We identified large clusters of infection outcomes, but not clusters of disease outcomes. Thus, infections but not cases were spatially modulated. Infection outcomes were poorly correlated within households. Spatiotemporal modeling of infection dynamics showed extensive month-to-month changes in infection risk across the study area. In sum, robust results across alphavirus, flavivirus, and coronavirus epidemics show when and how interpreting the incidence rate as the risk of infection or disease leads to considerable bias (e.g., missing clusters, incorrect inferences), a general finding applicable to a wide variety of pathogens that cause many inapparent infections. We argue for the expanded use of serosurveillance to overcome the inherent limitations of relying solely on case-based incidence rates during epidemics.

0475

CAPPABLE-SEQ REVEALS PROLIFIC NON-CANONICAL TRANSCRIPTION START SITES IN WOLBACHIA STRAINS WMELPOP-CLA AND WALBB UNDER STRESS CONDITIONS

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Symbiont-mediated control of arbovirus transmission entails the transfection of the alpha-proteobacterium *Wolbachia* into mosquito vectors, which greatly reduces vector competence. The choice of optimal *Wolbachia* strain for mosquito population replacement programmes needs to consider several factors, such as potential negative fitness effects on the mosquito host, the speed of replacement induced by cytoplasmic incompatibility, and the stability of the transinfection in the face of stressors such as heat. Mounting evidence indicates that different *Wolbachia* strains exhibit profound differences in resilience to the high temperatures that are regularly experienced in dengue-endemic countries. For instance, *w*MelPop-CLA (originally isolated from *Drosophila melanogaster*) is more easily lost from transinfected *Aedes aegypti* at elevated temperatures than is *w*AlbB (native to *Aedes albopictus*). However, very little is understood about the regulatory response to stress in *Wolbachia* at the transcriptional level. Here, we apply Cappable-Seq to unveil the primary transcriptome of these *Wolbachia* strains under low, optimal and high temperatures during mosquito cell culture. Stark differences in temperature tolerance between *w*MelPop-CLA and *w*AlbB were confirmed *in vitro.* Transcription start sites (TSS) were mapped at single-nucleotide resolution for the first time in an endosymbiotic bacterium responding to stress, demonstrating that both *w*MelPop-CLA and *w*AlbB have the capacity to utilise all four known TSS types, including a high proportion of antisense TSS. Global TSS discovery also enabled the identification of TSS promoter motifs associated with the global regulator sigma-70. Interestingly, wMelPop-CLA exhibited very high levels of expression for prophage-related TSS, whereas insertion sequence-associated TSS were more dominant in *w*AlbB. This study represents the first comparative transcriptomic analysis between *Wolbachia* strains in use for transinfected mosquito strategies and will facilitate experimentally guided strain choice in future.

0476

MATING STIMULI CAUSE DRAMATIC CHANGES IN THE FEMALE TSETSE FLY (GLOSSINA MORSITANS) REPRODUCTIVE TRACT AT THE LEVELS OF GENE EXPRESSION, BIOCHEMISTRY AND MORPHOLOGY

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Tsetse flies (*Glossina sp*.) transmit the human and animal forms of African Trypanosomiasis, neglected diseases, which affect marginalized populations in sub-Saharan Africa. Tsetse flies have low reproductive capacity and low population numbers relative to other disease vectors as they reproduce by obligate viviparity (intrauterine larval development/nutrition and live birth). This work investigates the physiological responses by female tsetse flies to mating stimuli. Physical mating stimuli and male seminal fluid proteins, induce physiological and behavioral changes observed in mated female insects. In *Glossina*, these include reduced receptivity to remating, increased blood meal volume and initiation of ovulation. Time course analysis of the post mating response by tsetse fly females by gene expression, metabolomic and morphological analyses of female reproductive tissues reveal significant changes in gene expression patterns, metabolite abundances, and morphological shifts over a 72-hour time course after mating. The transcriptional response peaks at 24 hours post mating and is distinguished by the dramatic increase in transcripts encoding a female reproductive specific OBP, orthologs of royal jelly protein in bees (responsible for initiation of developmental programs resulting in queen development from workers), and upregulation of structural, transporter, calcium signaling and stretch reception genes. Metabolomic analyses reveal increases in phospholipid moieties likely derived from the male seminal secretions which may function as a nuptial gift. Byproducts of glycosaminoglycan degradation are also enriched and may represent the degradation of glycoprotein constituents of the spermatophore by the female. MicroCT scans reveal changes in the constitution of fat body cells between virgin and mated flies indicating that the post mating effect also induces changes in nutrient storage. These analyses reveal an array of new insights into the physiology of the post-mating response in *Glossina* which will be the focus of targeted research to identify processes with potential as targets for reproductive inhibition.

0477

Β-TRIKETONES, A NEXT GENERATION SCABICIDE?

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Scabies is a contagious human skin disease caused by the obligate parasitic mite *Sarcoptes scabiei*. It affects approximately 300 million people per year globally and is a major problem in Australian Aboriginal communities. Scabies is linked to secondary bacterial infections with life threatening sequelae. Available drugs target mainly the motile parasite stages and fail to kill eggs, hence they are suboptimal. Manuka oil (MO) has extensive bioactive properties but its scabicidal efficacy has not been explored. Six commercially available MOs were tested *in vitro*. The MO fractionation was followed by gas chromatography and Spearman's Correlations analysis revealed the acaricidal compounds are β-triketones. β-triketones were tested on mites and eggs at different concentrations *in vitro* and LC50 values were generated. Mites and eggs were also exposed to 150mM of β-triketones for different durations and LT50 values were generated. Probit analysis was used to generate LC50 and LT50 values. The synergistic effects of β-triketones were checked using different combinations on mites and eggs and the data were analysed using Compusyn Software. Proteo-transcriptomic analyses of MO treated mites and eggs for 30 mints

versus untreated were done to assist target elucidation. A 1.2-fold cut-off value was used to identify up or down regulated proteins (p-value<0.05). KiwiHerb® showed highest ovicidal (>95%) and miticidal activity (100%). KiwiHerb® was fractionated into 17 fractions and 28 compounds. The β-triketones flavesone, isoleptospermone and leptospermone were identified as the active compounds against *S. scabiei*. Flavesone showed highest miticidal activity with LC50 of 57.81 mM (95% CI 53.97-61.68) at 4 h and leptospermone showed the highest ovicidal activity with LC50 of 33.59 mM (95% CI 29.23-38.04) at day 3. The lowest LT50 values for mites and eggs, 1.34 (1.24-1.45) h and 8.03 (6.30-9.70) h respectively were measured from 150mM Flavesone. There was a little or no synergism in all combination β-triketones tested. β-triketones show the potential to be a novel scabicide and broader implications on other arthropod-associated diseases.

0478

ABAMETAPIR - A POTENTIAL SINGLE DOSE SCABICIDE WITH OVICIDAL ACTIVITY.

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Scabies is a contagious skin disease in humans caused by the obligatory parasitic, burrowing mite *Sarcoptes scabiei* var *hominis*. Intense pruritus facilitates secondary bacterial infections associated with potentially life threatening sequelae. Scabies is common in overcrowded and disadvantaged communities. There is no vaccine, molecular diagnostic methods and only limited treatment options are available. Suboptimal efficacies of the existing drugs, and patient incompliance to repeat treatments, as well as emerging resistance are major concerns. Therefore, our objective is to develop a single-dose regimen targeting the entire lifecycle. Xeglyze® lotion is a FDA approved ovicidal lousicide containing 0.74% abametapir as active ingredient. Abametapir has metal chelating properties and therefore acts on multiple targets in an organism. It is capable of inhibiting multiple enzymes (including metalloproteases) and pathways that requires metal ions as co-factors. Abametapir has been shown to inhibit Meprin, a metalloprotease important in arthropod embryonic development. Considering the above facts, we hypothesized that Abametapir inhibits a range of metalloproteases in *S. scabiei* mites and eggs involved in development (moulting), metabolism and egg hatching. Proposed research will validate Abametapir as a new scabicide with novel modes of action(s) and efficacy against all developmental stages of *S. scabiei*. *In vitro* drug assays were conducted using pure abametapir. Experiments were conducted using *S. scabiei* var *suis* mites and eggs. Mites and eggs were exposed to abametapir at different exposure times. Mite mortality and egg hatchability inhibition were recorded over time compared to an untreated control. Lethal exposure time (LT) was determined by probit analysis using IBM® SPSS® Statistics 23.0. Software. LT_{50} and LT_{95} for 0.74% pure abametapir were 15.30 (14.00-16.75) h and 49.50 (41.20-63.00) h respectively for mites, and 1.23 (0.98-1.52) h, 9.32 (6.75-14.65) h respectively for eggs. Our results show that abametapir affects all developmental stages of *S. scabiei* and has potential as a single dose treatment for scabies.

0479

COMPARTMENTALIZATION AND EFFECT OF TRYPANOSOMA CRUZI INFECTION ON THE GUT MICROBIOTA OF TRIATOMA DIMIDIATA

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Chagas disease is an endemic zoonosis in Latin America, and it is mostly concentrated in rural and poor areas. It is considered a neglected tropical disease, and the main vector in Central America is *Triatoma dimidiata*. Given the diversity of biological factors involved in the host-parasite association, we studied the effect of *Trypanosoma cruzi* infection on the microbiota and the localization of important microbial taxa in the gut of *T. dimidiata*, which would facilitate identifying key factors associated with the interaction of endemic microbiota and *T. cruzi.* In this study, 8 insects (4 infected and 4 uninfected with *T. cruzi*) from a single site in Costa Rica were dissected, and the anterior midgut, posterior midgut, and rectum were separated. The bacterial metabarcode of the sections was sequenced and compared between infected and uninfected insects. In addition, aerobic bacteria and yeasts were isolated in BHI medium and identified by sequencing of the genes 16S or ITS, in order to evaluate a possible effect on the replication of *T. cruzi* epimastigotes *in vitro.* We identified 3218 microbial amplicon sequence variants (ASVs) based on the 16S rRNA gene analysis. Overall, Actinobacteria was the most abundant phylum, with *Corynebacterium* and *Tsukamurella* as the most abundant genera. We determined significant changes in the microbial composition and diversity among gut sections. A higher diversity was observed in the posterior midgut, where epimastigote replication occurs. In general, insects infected with *T. cruzi* showed a higher gut bacterial diversity than insects without the parasite. Moreover, we obtained 34 different microbial isolates, including Firmicutes and Actinobacteria. Interaction assays against epimastigotes of *T. cruzi* are being conducted. Our study gives relevant ecological information on microbiota compartmentalization and microbial interactions in the gut of *T. dimidiata*. Further investigations on interactions and localization of important microbial taxa in the gut of *T. dimidiata* may help to develop new strategies and/or improve current control efforts.

0480

EVALUATION OF ENDEMIC AND EPIDEMIC STRAINS OF VESICULAR STOMATITIS VIRUS INFECTION IN CULICOIDES SONORENSIS MIDGES

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Vesicular stomatitis (VS) is a viral disease primarily affecting cattle, horses, and swine. Endemic to Central and South America, VS outbreaks in the US occur sporadically every five to ten years, lasting for a single year (incursion year) and often re-emerging for a second year (expansion year). Virus spreads from animal-to-animal by direct contact but is also vector-borne. During outbreaks, especially in expansion years, *Culicoides* biting midge vectors likely play a key role in inter-herd viral spread. Earlier phylogeograhic studies showed that in 2012, a VS virus (VSV) (epidemic strain) successfully spread northward out of an endemic area in southern Mexico and into the US, causing disease in horses in New Mexico and Colorado. In contrast, a genetically related strain from the same area in southern Mexico (endemic strain), did not move out of the endemic zone and into the US. It is not clear why some viral lineages escape the endemic areas and move northward into the US, and some do not. Whole-genome sequencing revealed the two strains differed in seven significant

amino acid changes. Comparative infection studies in pigs showed that the epidemic strain was more virulent than the endemic strain, possibly contributing to its overall ability to escape the endemic area and spread northward into the US. Here, we evaluated the efficiency of these two viral strains to infect *Culicoides* biting midges and to disseminate to the salivary glands for subsequent bite transmission. Our results showed that the epidemic strain had a significantly higher infection rate and a higher dissemination rate in midges, compared to the endemic strain. Thus, in addition to affecting virulence, small genetic changes also affect virus-vector interactions, which may contribute to the ability of a specific viral lineage to move out of an endemic area in Mexico and into the US.

0481

EMERGING ENTOMOLOGICAL EVIDENCE OF INCREASED CUTANEOUS LEISHMANIASIS BURDEN IN SRI LANKA

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There is a sustainable rise in the global health burden of cutaneous leishmaniasis (CL), as a truly neglected tropical disease. CL has transmitted to all 25 districts in Sri Lanka with persistent hotspots in selected areas, without specific control or prevention programs. This is the first island-wide molecular-based evaluation of entomological factors contributing to the escalating burden of CL in Sri Lanka. DNA was extracted from 1520, morphologically identified, female *Phlebotomus argentipes glaucus* (*Ph. glaucus*), probable vector in Sri Lanka. Sandflies were collected through an island-wide survey and molecularly confirmed with COI and Cyt b markers. DNA was tested for the presence of *Leishmania* parasite in sandflies using a conventional PCR and confirmation as *Leishmania donovani* using specific nested PCR. Blood meal source identification was done using a light trap collected 270 blood-engorged female sandflies targeting the universal vertebrate Cyt b gene. Positive amplicons of all PCR experiments were sequenced and analyzed. Thirteen blood-fed and 2 non-fed sandflies were infected with *L.donovani* indicating an infection rate of 1% (15/1520). Eleven infected sandflies were engorged with human blood implicating humans as a possible parasitic reservoir. The presence of infected non-engorged sandflies reinforces the view of *Ph.glaucus* as *L.donovani* vector in Sri Lanka. Out of 216 positive PCR amplicons of blood-meal analysis, 153 (70%) were *Ph.glaucus* and 63 (30%) were *Sergentomyia* species. Further analysis revealed the presence of human blood in 133 (87%) out of 153 vertebrate blood-engorged *Ph.glaucus*, favoring the probable anthropophilic nature of *Ph.glaucus*. The presence of 70% *Ph.glaucus* in sandfly collection, rising infection rate (a threefold rise compared to the previous rate of 0.3%), likely anthropophilic nature of *Ph.glaucus* with human as a possible parasitic reservoir, may partly explain the rising case incidence with escalating health threat. The evidence uncovered will aid future planning and implementation of vector control strategies, required to contain the rapid spread of leishmaniasis in Sri Lanka.

0482

INFRARED THERMAL IMAGING AS A NOVEL NON-INVASIVE POINT-OF-CARE TOOL TO ASSESS FILARIAL LYMPHOEDEMA AND THE IMPACT OF SELF-CARE INTERVENTIONS IN ENDEMIC AREAS OF BANGLADESH, ETHIOPIA, AND MALAWI

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Lymphatic filariasis causes disfiguring and disabling lymphoedema, which is commonly and frequently exacerbated by acute dermato-lymphangioadenitis (ADLA). Affected people require long-term care and monitoring but health workers lack objective assessment tools. We assess the use of an infrared thermal imaging camera as a novel non-invasive point-of-care tool for filarial lower limb lymphoedema before and after a self-care intervention in five highly endemic areas of Bangladesh, Ethiopia and Malawi. A prospective cohort study of ~150 people in each of the five study areas was conducted over six months. Participants were surveyed at three-time points; baseline before being training in an enhanced self-care protocol (pre-intervention) and at 3- and 6 months (post-intervention). Limb temperatures were captured using the FLIR C3 Compact Thermal Imaging Camera and differences by lymphoedema stage (mild, moderate, severe) and ADLA status were visualised and quantified using descriptive statistics and regression models. Baseline results from Bangladesh found temperatures to increase by severity and highlighted subclinical differences between no lymphoedema and mild lymphoedema, and differences between moderate and severe stages. Toes and ankle temperatures detected significant differences between all stages other than between mild and moderate stages. Significantly higher temperatures, best captured by heel and calf measures, were found in participants with a history of ADLA, compared to participants who never had ADLA, regardless of the lymphoedema stage. Baseline surveys are completed with data analyses underway in Ethiopia and Malawi. All post-intervention surveys are in progress and due to be completed by August 2021. The results from three countries will be presented. This novel tool has great potential to be used by health workers in LF endemic areas to detect subclinical cases, predict progression of disease and ADLA status, and monitor pathological tissue changes and stage severity following enhanced care packages or other interventions in people affected by lymphoedema.

0483

POPULATION PHARMACOKINETICS OF IVERMECTIN IN LYMPHATIC FILARIASIS PATIENTS AND HEALTHY INDIVIDUALS

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Ivermectin (IVM) combined with diethylcarbamazine (DEC) and albendazole (ALB) is part of triple-drug therapy (IDA) recommended for mass drug administration to eradicate lymphatic filariasis in certain populations. IDA had reduced efficacy for sustained suppression of microfilaremia in residents of West Africa compared to Papua New Guinea. Some of this variable efficacy may arise from differences in

population pharmacokinetics (PK) of IVM and the presence and intensity of LF infection. We have previously shown that IVM has substantial pharmacokinetic variability with systemic drug exposures (AUC) ranging over 10 fold when administered as a fixed dose. Population PK modeling of IVM was conducted to evaluate dose rationale and evaluate the impact of factors associated with observed PK variability. This study evaluated the PK of IVM following single-dose (0.2 mg/kg) oral administration in lymphatic filariasis infected subjects compared to healthy individuals. A total of 56 participants were enrolled in the study and 724 samples were collected from treatment naïve *Wuchereria bancrofti*-infected (n=32), and uninfected (n=24) adults who also received IVM as a part of triple drug therapy. Plasma samples were collected from 0-168 hours post administration and analyzed using liquid chromatography-mass spectroscopy. PK analysis was conducted with Phoenix NLME 8.0 software. A two-compartment model with mixed first and zero order absorption and linear elimination with time-lag function best described IVM disposition. IVM PK was not altered by lymphatic filariasis infection. However, gender was found to be a significant covariate on the peripheral volume of distribution (higher in women than men). The IVM PK model was suitable for predicting drug exposure in patients with lymphatic filariasis and identification of important covariates associated with pharmacokinetic variability. Additional studies evaluating the influence of body size are warranted for IVM PK. The studies will allow sparse sampling of populations to determine if PK variability of IVM may account for variability in IDA efficacy across populations and to optimize dosing.

0484

ILC2 CELLS MEDIATE RESISTANCE TO LITOMOSOIDES SIGMODONTIS INFECTION

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The *Litomosoides sigmodontis* mouse model is a well-established model to study immune responses during filarial infection. BALB/c mice develop chronic *L. sigmodontis* infection and microfilaremia, whereas C57BL/6 mice clear the majority of filariae shortly after the molt into adult worms ~30 days after infection (dpi) and do not develop microfilaremia. In the present study we investigated group 2 innate lymphoid cells (ILC2s) during *L. sigmodontis* infection in BALB/c and C57BL/6 mice. Mice were naturally infected via the mite vector and ILC2s ($CD45^+$ $Lineage^-$ $TCRb^-$ $CD90.2^+$ $Sca\text{-}1^+$ $IL\text{-}33R^+$ $GATA\text{-}3^+$) were analysed in the thoracic cavity, the site of *L. sigmodontis* residence, on 9, 30 and 70 dpi. C57BL/6 mice had a significant increase in ILC2 numbers compared to BALB/c mice at 30 dpi. At this time point the ILC2 numbers positively correlated with the worm burden in both mouse strains (Spearman $r = 0.7$; $p = 0.02$). Additionally, C57BL/6 mice had significantly higher levels of IL-5 and IL-13 at 30 dpi in comparison to BALB/c mice, indicating a stronger type 2 immune response in C57BL/6 mice during the onset of adult worm clearance. Pleural cavity ILC2s and $CD4^+$ T cells were the dominant source of IL-5 in 30 day-*L. sigmodontis*-infected C57BL/6 mice with ILC2s showing a significantly higher IL-5 expression than $CD4^+$ T cells. In order to investigate the role of ILC2s on *L. sigmodontis* infection, we depleted ILC2s using anti-CD90.2 antibodies before the molt into adult worms (26-28 dpi) from B and T cell deficient $RAG2^{-/-}$ C57BL/6 mice and compared the outcome to isotype-treated $RAG2^{-/-}$ controls. $RAG2^{-/-}$ mice had lower ILC2 numbers in comparison to wildtype C57BL/6 controls and were susceptible to *L. sigmodontis* infection with the development of microfilaremia. Depletion of ILC2s in $RAG2^{-/-}$ C57BL/6 mice did not alter the adult worm recovery at 63 dpi, but significantly increased microfilariae numbers in comparison to isotype-treated $RAG2^{-/-}$ mice. In conclusion, our data reveal differences in the ILC2 subset during *L. sigmodontis* infection in susceptible and semi-susceptible mouse strains and demonstrate that ILC2s are involved in the control of microfilaremia.

0485

ADJUVANTED RECOMBINANT FUSION PROTEIN VACCINE INDUCES DURABLE AND PROTECTIVE IMMUNITY TO LARVAL ONCHOCERCA VOLVULUS IN MICE

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Control mechanisms for *Onchocerca volvulus* include vector control and mass drug administration. Given challenges in current control programs, further exacerbated by the COVID-19 pandemic, an effective prophylactic vaccine against *O. volvulus* is urgently needed. Previous studies demonstrated that vaccination with recombinant *O. volvulus* antigens *Ov*-103 and *Ov*-RAL-2, with the adjuvant Advax-2, induced protective immunity in mice. The current study aimed to determine: (1) whether a fusion of the two antigens (FUS-1) could replace the single antigens in the vaccine, (2) will other adjuvants optimize the induction of protective immunity and (3) how durable is the protective immunity induced by the adjuvanted FUS-1 vaccines? Mice were immunized with the fusion protein FUS-1 formulated with three different adjuvants: alum (which induces Th2 bias), Advax-2 (delta inulin with CpG oligonucleotide that induces combined Th1 and Th2 immunity) and AlT4 (alum plus a TLR-4 agonist that induces a Th1 bias). Mice were immunized 3 times and then challenged with third-stage larvae implanted subcutaneously within diffusion chambers. At 3-weeks post-final-booster immunization, mice vaccinated with either FUS-1/alum or FUS-1/Advax-2 had significantly reduced larval survival when compared to adjuvant controls, whereas mice vaccinated with FUS-1/AlT4 did not. At the 12-week time point, only mice vaccinated with FUS-1/Advax-2 exhibited significantly reduced larval survival. Flow cytometry analysis of infiltrating cells in the parasite microenvironment did not reveal differences between mice immunized with FUS-1/AlT4 versus the other adjuvants nor between cell populations at 12-weeks between FUS-1/alum and FUS-1/Advax-2. This suggests that the lack of protective immunity at 3-weeks with FUS-1/AlT4 and at 12-weeks with FUS-1/alum was not due to a deficiency in infiltrating effector cells but another immune mechanism or factor yet to be determined. The present study demonstrates that FUS-1 in combination with the adjuvant Advax-2 consistently induces a durable protective immune response against infection with *O. volvulus* in mice.

0486

IMMUNIZATION WITH CO-ADMINISTERED ONCHOCERCA VOLVULUS 103 AND RAL-2 VACCINE ANTIGENS REDUCES INFECTION IN A NATURAL BOVINE ONCHOCERCA OCHENGI INFECTION MODEL

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Human onchocerciasis is a neglected tropical disease affecting 21 million sub-Saharan Africans. 30 years of elimination efforts have resulted in only

a 31% reduction in prevalence and is complicated by the restricted use of ivermectin in *Loa loa* co-endemic areas. Emerging ivermectin-resistant *Onchocerca volvulus* is also a major concern. Novel interventions, such as vaccines, are needed to complement ongoing elimination efforts. In small animal models, we've shown that *O. volvulus* 103 and RAL-2 antigens (Ov-103 and Ov-RAL-2) are the most promising vaccine candidates for clinical development for human use. Here, 24 immunologically naïve calves were split into two equal groups. One group was co-immunized (via intramuscular injection), with 103 and RAL2 antigens individually formulated with Montanide ISA 206VG adjuvant; 500µg/antigen for primary immunisation, plus two boosters at 250µg/antigen, at 4 and 8 weeks. The control group received adjuvant only. Animals were naturally exposed to infection with *O. ochengi* for 24 months. Throughout the study, calves were sampled to evaluate serum antibody titres, peripheral blood leucocyte counts and *in vitro* cellular assays. Post exposure, calves were also examined monthly for evidence of *O. ochengi* infection by palpation for female nodules and skin snips to detect microfilariae. All vaccinated calves showed strong serum IgG responses composed of IgG1 and IgG2 isotypes following primary and boost immunizations to both Ov-103 and Ov-RAL-2. These waned progressively following final immunization but were still detectable at 6 months post-exposure. All calves acquired *O. ochengi* infections following exposure. Parasitological assessments during, and at the completion of, the exposure period indicated reduction in female worm burdens and microfilarial densities in vaccinated calves, although these findings are pending statistical analysis. Immunization of *O. volvulus* 103 and RAL-2 antigens formulated with Montanide ISA 206VG in cattle elicits a strong antibody response that was associated with the protective and anti-fecundity effects to natural exposure to infection with *O. ochengi*.

0487

A PROMISING NEW SEROLOGIC TEST FOR EVALUATING THE SUCCESS OF ELIMINATION PROGRAMS FOR BANCROFTIAN FILARIASIS

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The Global Programme to Eliminate Lymphatic Filariasis (LF) uses mass drug administration (MDA) of anti-filarial drugs to treat existing infections and interrupt transmission. New diagnostic options are needed to verify the success of MDA and identify areas at risk for ongoing transmission. The prevalence of circulating filarial antigen (CFA) and antibodies to the recombinant filarial antigens Bm14 and Wb123 decline slowly after treatments that clear microfilariae (Mf) from the blood. The Brugia Rapid Test (BRT) detects antibodies to BmR1, a *B. malayi* antigen that is expressed by Mf and gravid female worms. The BRT is a useful tool for MDA end-point decisions in areas with brugian filariasis, because antibodies to BmR1 clear fairly rapidly after successful treatment. However, the BRT has low sensitivity for *W. bancrofti* infections. We have explored the utility of a *W. bancrofti* orthologue of BmR1 (Wb-R1) for filariasis serology. An IgG4 ELISA with the Wb-R1 antigen has high sensitivity (~85%) using sera from persons with *W. bancrofti* Mf from Egypt, Sri Lanka, India and Papua New Guinea. Low amino acid sequence conservation of the Wb-R1 orthologues in *L. loa* and *O. volvulus* may account for the superior specificity of this assay relative to an IgG4 assay for antibodies to Bm14. A study of archived samples from Egypt showed that antibodies to Wb-R1 were significantly more common in treatment-naïve persons with Mf than in amicrofilaremic persons with positive CFA tests (70% vs 53%). In addition, a study of sera from a clinical trial in Papua New Guinea showed that antibodies to Wb-R1 decreased in persons with long-term Mf clearance after treatment with the triple drug combination of ivermectin-DEC-albendazole (IDA) despite persistence of CFA. Additional studies are needed to verify these preliminary findings. However, if antibodies to Wb-R1 are better predictors of Mf persistence, this new test may prove to be a very useful surveillance tool for LF elimination programs that need to assess the potential for ongoing transmission of LF following MDA.

0488

POTENTIAL USE OF ANTIBODIES TO PROVIDE AN EARLIER INDICATION OF LYMPHATIC FILARIASIS RESURGENCE IN POST-MASS DRUG ADMINISTRATION SURVEILLANCE, AMERICAN SAMOA

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American Samoa conducted seven rounds of mass drug administration for lymphatic filariasis (LF) between 2000 and 2006. Subsequently, the territory passed transmission assessment surveys in February 2011 (TAS-1) and April 2015 (TAS-2) with two and one antigen (Ag)-positive children respectively, below the critical cut-offs. However, the territory failed TAS-3 in September 2016 with nine positive children and a crude prevalence of 0.8% (95% confidence interval [CI] 0.4-1.5%); a positivity rate higher than the cut-off and the recommended upper confidence limit of 1%. Anti-filarial antibodies (Ab) are detected earlier in infection than parasite antigen and thus could potentially provide an initial indication of resurgence. Earlier identification of resurgence would enable timelier control measures and optimise long-term success of elimination programs. We examined the school-level Ag status (presence/absence of Ag-positive children) and prevalence of single and combined IgG responses to Wb123, Bm14, Bm33 Ags in the three TAS to assess if Ab testing could have provided earlier signals of transmission than Ag. Pearson's chi-squared tests and univariate logistic regression were used to examine associations between school-level Ab prevalence in TAS-1 and TAS-2 and school Ag status in TAS-3. School-level Wb123 Ab prevalence in TAS-2 was significantly associated with Ag-positive status in TAS-3 (p 0.02). This association also provided the best balance between sensitivity (100%) and specificity (70%) of test results. Our analyses also found that schools with higher prevalence of Wb123 Ab in TAS-2 had higher odds of being Ag-positive in TAS-3 (odds ratio [OR] 24.5, 95% CI:1.2-512.7). Similarly, schools that were positive for Ab responses in the combinations Wb123∩Bm14, Wb123∩Bm33 and Wb123∩Bm14∩Bm33 in TAS-2 also had higher odds of being Ag-positive in TAS-3 (OR 16.0-24.5). Results suggest that anti-filarial Ab provided earlier signals of LF resurgence than Ag alone. Further studies are required to determine the role of Ab testing in LF elimination programs, and how results can be used to inform programmatic decision making.

0489

DETECTION OF CLINICALLY RELEVANT CHANGES IN COUGH PATTERNS USING ARTIFICIAL INTELLIGENCE

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Cough is a landmark symptom of respiratory disease. However, its objective monitoring is challenging. Adoption of technology-enabled alternatives to clinical questionnaires is hindered by issues of privacy and portability, limiting their use in clinical practice. As part of an ongoing acoustic surveillance study, we instructed a group of participants at the University of Navarra (Spain) to monitor their night-time cough for thirty days. A smartphone application using a machine learning convolutional neural network was used to automatically detect cough events among ambient sounds. Population cough data was aggregated to construct cough time series. Cough incidence in the cohort was compared to incidence of medical consultations due to respiratory symptoms. We identified three archetype cases for which changes in cough patterns identified by the surveillance app were clear and linked to specific explanatory events. We describe (1) an evident, yet unnoticed increase in cough in a participant newly diagnosed with COVID-19; (2) a sharp reduction in cough frequency in a participant with idiopathic chronic cough newly treated with gabapentin; and (3) similar cough reductions following smoking cessation in a long-time smoker. Our results highlight the potential usefulness of artificial intelligence in the detection and monitoring of cough patterns associated with acute and chronic respiratory diseases. These systems can accurately capture the influence of clinically relevant changes in cough, representing a promising alternative to standard self-reporting methods.

0490

DEVELOPMENT AND EVALUATION OF MOTOMEDS: A PEDIATRIC TELEMEDICINE AND MEDICATION DELIVERY SERVICE FOR RESOURCE-LIMITED SETTINGS

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Acute respiratory infection and diarrheal disease are the two leading causes of pediatric mortality between 1 month and 5 years of age globally. These, and other common childhood illnesses have well-established low-cost treatments. However, treatment is most effective when administered early, which is a challenge in resource-limited settings. MotoMeds, a pediatric telemedicine and medication delivery service, was launched in Gressier Haiti as a pre-pilot feasibility study. When a child becomes sick at night a parent calls MotoMeds to connect with a nurse who uses clinical decision-support tools that our team adapted from WHO in-person guidelines. The nurse synthesizes information relayed over the phone to triage, assess and develop a treatment plan, which may include medication delivery. To evaluate the approach, a clinical congruence study was conducted where a nurse accompanied each delivery to perform a parallel in-person exam. The primary outcome measure was congruence of illness severity between call center and household. Cohen's Kappa statistic and sensitivity and specificity measurements were calculated. From September 2019 to January 2021 394 cases were enrolled. The most common chief complaints were fever (42%), respiratory illness (17%), and skin infection/irritation (14%). At the call center, 6.8% (27) of patients were categorized as severe and sent to the hospital and 88% (348) received a nurse visit. At the household, danger signs were identified in 1.5% (6) of patients. Agreement between mild cases was substantial Kappa=0.63(95%CI 0.50-0.77) with 95.0%(95%CI 92.0%-97.1%) sensitivity and 76.7%(95%CI 57.7%-90.1%) specificity and agreement between moderate cases was moderate Kappa=0.60(95%CI 0.45-0.75) with 83.3%(95%CI 62.6%-95.3%) sensitivity and 93.8%(95%CI 91.0%-96.4%) specificity. These data indicate aspects of the information collected at the call center hold clinical value. The service was robust and remained operational despite an unstable environment. Future analysis will evaluate congruence of all assessment components and explore rates of conversion to severe illness and seeking in-person care.

0491

IS E-LEARNING EFFECTIVE AT IMPROVING KNOWLEDGE OF MALARIA AMONG HEALTH WORKERS COMPARED TO TRADITIONAL TRAINING? RESULTS FROM A PRAGMATIC ASSESSMENT IN ANGOLA, 2020-2021

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Malaria causes 60% of hospital admissions in children in Angola, with poor quality-of-care being a driver of in-patient mortality. The Malaria Strategic Plan 2021-2025 prioritizes traditional training as an important quality of care improvement strategy; however, its effectiveness is limited by unstandardized training quality. The Ministry of Health (MOH) developed an e-learning platform (KASSAI) to enhance quality of training on Malaria Case Management (MCM). KASSAI's content is aligned with national guidelines and includes 4 modules (8 hours each): MCM in children, pregnancy, adults, and epidemiology. Each module is organized by learning objectives and interactive exercises, with a pre-test and post-test to assess knowledge improvement. Between July 2020-March 2021 KASSAI was rolled out in 4 high malaria transmission provinces. It was implemented in 45 public health units with high malaria caseloads, prioritizing health workers (HWs) from the pediatric, obstetric, and emergency departments. HWs enrolled into KASSAI using tablets, receiving support on course navigation, and troubleshooting by an MOH trainer. By mid-March 2021, 751 HWs had enrolled onto KASSAI, with a completion rate of 88.4% (n=664). Of these, the percentage who scored ≥75% points increased from 28.6% in the pre-test to 65.5% in the post-test (37 points increase). In contrast, 678 HWs enrolled in traditional training (July-December 2020), with a completion rate of 100.0%. The percentage who scored ≥75% points increased from 4.9% in the pre-test to 40.3% in the post-test (35 points increase). Importantly, KASSAI was able to identify in real-time areas with persistent knowledge gaps in the post-test: severe MCM in children (scored 47.0%) and comorbidity diagnosis (scored 51.0%). Inadequate digital literacy might be a barrier to completion of KASSAI. However, KASSAI offers the advantage of standardizing the quality of trainings across provinces. It can be used for self-learning and as part of continued medical education program in health units. It provides similar learning improvements as traditional training, becoming ideal to implement in the COVID-19 context.

0492

IMPACTS OF MASS NUTRITIONAL SUPPLEMENTATION ON DYNAMICS OF MEASLES: A SIMULATION STUDY

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Undernutrition and infection interact in a bidirectional manner. Nutrient deficiencies in children stunt growth, impair immunity, and increase susceptibility to infection. Simultaneously, infections compound undernutrition by increasing metabolic demand, reducing food intake

and impairing nutrient absorption. Nutrition-specific approaches like mass supplementation of children with specially designed foods has been shown to improve growth, reduce wasting and all-cause mortality. Combined with a package of health support, including immunization, it could lead to overall improved health of children. To understand how mass nutritional supplementation and vaccination collectively affect the dynamics of a common vaccine-preventable infection, beyond providing therapeutic feeding for wasted children, we developed a population-level, dynamic model of measles transmission stratified by undernutrition status. We simulated a range of scenarios to assess the benefits of these interventions on measles morbidity and mortality. Mass nutritional supplementation was assumed to increase engagement with the health sector leading to increased proportions of children being vaccinated. We found a strong effect of mass supplementation in reducing measles infection and death, as well as measles-induced kwashiorkor mortality. In addition to treatment of wasted children, 60% mass supplementation coverage of children aged 6-23 months followed by an increase in vaccination coverage of non-wasted children from baseline of 65% to 80%, leads to a ~40-55% reduction in all three outcomes, regardless of the wasting treatment coverage. This synergy of mass nutritional supplementation and increases in vaccine coverage provides sizeable reductions in measles morbidity and mortality well above treatment of acute malnutrition or increased vaccine uptake alone. Our work highlights the need to account for nutrition in infectious disease models to prevent overestimation of impact interventions, as well as the need to collect quantitative information about how undernutrition may be associated with higher susceptibility to infection.

0493

IMPACT OF VACCINATION AND NON-PHARMACEUTICAL INTERVENTIONS ON SARS-COV-2 DYNAMICS IN SWITZERLAND

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Having started as a zoonotic tropical infectious disease, COVID-19 quickly spread into a global pandemic. Capturing SARS-CoV-2 transmission dynamics, and more importantly preventing cases numbers, ICU admissions, and deaths, has become of utmost importance to scientists and policymakers. With this study we present a new individual-based transmission model, OpenCOVID, which has been used to support policymakers in formulating public health control strategies against COVID-19 in Switzerland. As vaccination coverage continues to increase, amidst the emergence and spread of more infectious and potentially more deadly viral variants, decisions on timing and extent of relaxing effective, but unsustainable, non-pharmaceutical interventions (NPIs) continue to need to be made. Using OpenCOVID, we estimated that even if NPIs were not relaxed and continued at high levels from March 2021 onwards, a 'third wave' would still arise with more than 1250 ICU admissions over a 6-month period even with the assumption of high vaccine coverage (100,000 vaccines being administered daily). Would NPIs be lifted quickly, combined with 50,000 vaccines administered daily, we estimated nearly 5-times more ICU admissions (>7000), over the maximum ICU capacity limit for Switzerland. However, a cautious, phased relaxation of NPIs that reaches a similarly low level but 9 weeks later, combined with 100,000 vaccines being administered daily, could offset the size of such a wave and could substantially reduce population-level morbidity and mortality leading to fewer ICU admissions (~2500), from 400,000 confirmed cases, and 3000 deaths in a population of 8.5 million. Showing that the right balance between vaccine uptake and ongoing NPIs can alleviate the size of any future wave and speed up the timing of its peak. Presenting the latest insights around SARS-CoV-2 transmission dynamics and the impact and interplay of interventions that lead to the latest COVID-19 projections for Switzerland, will highlight the importance of future tropical infectious disease outbreak preparedness.

0494

COVID-19 TREATMENTS SOLD ONLINE WITHOUT PRESCRIPTION REQUIREMENTS: A GLOBAL CONCERN

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The COVID-19 pandemic has increased online purchases and heightened interests in treatments. Dexamethasone, hydroxychloroquine, and lopinavir-ritonavir have been touted as potential treatments for COVID-19. We assessed the availability of these potential COVID-19 treatment options online and evaluated the safety and marketing characteristics of websites selling these products. A cross-sectional study was conducted in June-August 2020 searching the first 100 results on Google, Yahoo, and Bing. Unique websites were included if they sold targeted medicines, were in English, offered to ship to the US, and were free to access. Identified online pharmacies were categorized as rogue, unclassified or legitimate based on LegitScript classifications. We found 117 websites: 30 selling dexamethasone, 39 selling hydroxychloroquine, and 48 selling lopinavir-ritonavir. This included 89 unique online pharmacies: 62 (70%) rogue, 20 (22%) unapproved, and 7 (8%) legitimate. All rogue pharmacies selling dexamethasone (n=19) did not require a prescription. Half of the rogue online pharmacies selling hydroxychloroquine (50%, n= 11) and lopinavir-ritonavir (61%, n= 20) did not require a prescription. Rogue sites rarely offered pharmacist counseling (3-9%). Drug warnings were unavailable on 86% of unapproved dexamethasone sites. Illegitimate pharmacies were more likely to offer bulk discounts and claim price discounts; yet dexamethasone and hydroxychloroquine were more expensive online. Some websites claimed hydroxychloroquine and lopinavir-ritonavir were effective COVID-19 treatments despite lack of evidence. Only 8.5% of IP addresses matched their claimed locations, with servers located worldwide. The lack of safety measures taken by illegitimate online pharmacies endanger patients, facilitating access to medications without appropriate monitoring by healthcare providers. Health care professionals must urgently educate the public of the dangers of purchasing drugs from illegitimate websites and highlight the importance of seeking treatment through authentic avenues of care.

0495

EFFECTIVENESS OF SOCIAL MEDIA-BASED INTERVENTIONS FOR HEALTH BEHAVIOR CHANGE IN LOW- AND MIDDLE-INCOME COUNTRIES: A SYSTEMATIC REVIEW

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Use of social media interventions to effect health behavior change has been well-documented to be effective in the context of high-income countries (HICs). However, there is limited information on the effectiveness when such interventions target residents of lower- and middle-income countries (LMICs). We performed a systematic review of the available literature (following the 2009 Preferred Reporting Items for Systematic Reviews and Meta-Analyses [PRISMA] guidelines) to identify interventions that have been implemented using social media in LMICs, and to characterize the evidence of their effectiveness. We searched PubMed, Embase, Elsevier, CINAHL, PsycInfo, and Global Index Medicus for studies published between January 1, 2000 and April 5, 2021 (PROSPERO # CRD42020223572). We restricted to articles available in English and required that studies evaluated interventions that were at least partly conducted on a social media platform and used the social components of the medium. Among 1,832 identified studies, 107 passed title-abstract review, and 34 passed full-text review to final data abstraction. Among these, 22 studies concluded that the social media intervention was effective, but only 13 of 22 quantified the level of social media engagement. The desired behavior change varied widely, but studies

frequently aimed to increase knowledge and prevention behaviors for viral infections, including HIV (n=5) and Dengue, Zika, and Nipah virus (one study each). Few studies identified a theoretical (n=8) or conceptual (n=5) model of behavior change. Our review of these 34 studies identified methodological gaps in settings and types of interventions, length of follow-up, evaluation techniques, and discussions of the privacy implications of the use of social media in LMICs. As social media becomes a more powerful and omnipresent factor in people's lives, its potential as a platform for public health work grows rapidly. This review highlights the need for improved methodological rigor in the planning, implementation and evaluation of health interventions for LMIC in order to increase the current, relatively small, evidence base.

0496

SUPPLY CHAIN DATA-TO-ACTION FOR IMPROVED MALARIA COMMODITY AVAILABILITY IN LAO PDR

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Lao PDR seeks to eliminate all forms of malaria by 2030. To achieve elimination, the Center for Malaria, Parasitology and Entomology (CMPE) Logistics Management Unit aims to ensure that quality malaria commodities, such as rapid diagnostic tests (RDTs) and artemisinin-based combination therapies (ACTs), are continually available. Despite the wealth of data in the national health management information system (HMIS), CMPE lacked a way to quickly synthesize, interpret, and act on data to improve supply availability. Stockouts remained a challenge in five high burden provinces in southern Lao PDR, with an average of 4% health facilities (HFs) stocked out of RDTs in 2019. While only 0.1% of HFs stocked out of adult ACTs in this time, 10% of HFs were below the required minimum stock. To address these challenges, CMPE Logistics, with support from partners CHAI and WHO, designed stock dashboards in the HMIS to facilitate interpretation of stock data at provincial, district, and HF levels. The team created color-coded maps and tables to provide user-friendly outputs indicating when a HF is over-stocked, under-stocked or stocked-out of key commodities. They also built a distribution planning tool that specifies the quantities required to meet forecasted product needs at each level. By recommending these quantities per recent trends, seasonality, and stock minimums, the dashboards allow staff to identify facilities at risk of stock-out and proactively resupply to prevent service interruption. CMPE launched the stock dashboards nationally in September 2020, with focused implementation in the five high burden provinces. The dashboards have been incorporated into routine program management planning and review meetings, leading to resupply before HFs stock out of products. By March 2021, program staff succeeded in reducing the HF average monthly stock-out rates in the five high burden provinces from 4% to 2% RDTs. Below minimum ACT inventory in HFs dropped from 17% to 12%, with further improvements in the coming months. With these improved tools, CMPE expects to eliminate stock outs in 2021 and maximize availability of malaria testing and treatment.

0497

DIGITALLY ENABLED CLINICAL TRIALS IS SUB SAHARAN AFRICA READY TO GO

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Innovations in drug development approaches are needed to address its increasing complexity andthe observed trends of efficiency loss. Drugs and vaccines development during the COVID-19pandemic has proved that there is room for improvement. Novel digital health technologiesapplication is gaining attention in drug development in the North. These new models foster trialsparticipants' engagement through self-reporting, remote samplings to accelerate and improveclinical trials quality. The growing contribution of Sub-Saharan Africa (SSA) to clinical research callsfor local investigators to become involved in this transition to digitally enabled clinical trials (DECT) inSSA.Our study aims to assess the opportunities, gaps and pitfalls for clinical trials digitalization. Aqualitative study including semi-structured interviews was conducted in two clinical research centersof excellence in Burkina Faso and Mali. In addition, clinical trial procedures, site organization andlevel of efforts were assessed through work shadowing. Data were analyzed using a thematicapproach. Overall, nineteen interviews were conducted from December 2020 to February 2021 with siteleaders, principal investigators, study coordinators and Contract Research Organizations based inSSA. The investigators had on average seven years of experience in clinical research. None of the sitehas been involved in a fully digitally enabled trial. Investigators consider DECT as feasible and as anopportunity for participant adherence improvement and adverse events reporting. However, theadaptation of these technologies to the sociocultural specificities is crucial. Acceptability challengesof remote participants' management, e.g. with follow-up or data collection through wearable devicesare multifactorial. *"Doctor to patient relationship is very important – we should not dehumanizedclinical research,"* stressed a senior researcher.Substantial communication and investigators involvement will be important for a successful rollout ofDECT in SSA. Patient to investigator relationship and mutual trust in clinical trials should beconsidered.

0498

COMPARING THE ROI OF TECHNOLOGIES FOR DETECTING POOR QUALITY AMOXICILLIN: A KENYA CASE STUDY

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Substandard and falsified medicines harm patients and have economic implications for health systems. An effective post-market surveillance system involves the identification and removal of poor-quality medicines in circulation, averting them from use by patients. Cost is a barrier to conducting post-market surveillance in some low- and middle-income countries, but screening technologies exist that both speed up the identification of poor-quality samples and lower the investment by relying less on the costlier high performance liquid chromatography (HPLC). However, there is little guidance on which screening technology to implement. We calculated the return on investment of using five screening methods: Minilab, Near Infrared (NIR), Paper Analytical Devices (PADs), Raman, and HPLC alone to conduct post-market surveillance of amoxicillin in Kenya. The Examining Screening Technologies using Economic Evaluations for Medicines (ESTEEM) agent-based model was employed to simulate sampling, testing, and removal of substandard and falsified medicines from the Kenyan market. Assuming that identified poor-quality batches were removed from circulation, we analyzed the population impact of amoxicillin quality used for pediatric pneumonia. Compared to doing no post-market surveillance, screening technologies prevented 2,230 - 10,137 poor-quality treatments from reaching patients annually, averting 132 - 60 pediatric deaths. Using NIR, we estimate it costs $12 per death averted, and $17, $21, $59, and $238 using PADs,

Minilab, Raman, and HPLC, respectively. The cost per substandard and falsified treatment averted ranged from $0.70 (NIR) to $14.11 (HPLC). The NIR and PADs yielded the highest ROI of $62 and $43 per dollar invested, respectively. PADs, Minilab, NIR, and the Raman are similar in their abilities to quickly identify substandard and falsified amoxicillin but differences in costs, usability, and country-specific capabilities affect the implementation. National medicine regulatory authorities should consider cost evidence among other factors when deciding which of these screening technologies to implement.

0499

EXCESSIVE SEEPAGE DURING MITS PROCEDURE: AN EXPLORATIVE STUDY TO IDENTIFY THE UNDERLYING CAUSES

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Minimally invasive tissue sampling (MITS) is a postmortem sample collection approach used to identify the cause of death where full diagnostic autopsy is not feasible. Under the Child Health and Mortality Prevention Surveillance (CHAMPS) Network, findings from MITS are helping to identify and analyze specific causes of stillbirth and under-5 deaths. Families, who are largely unfamiliar with this procedure, may have socio-cultural and religious concerns about consenting. Tissue sample collection, using specialized needles, sometimes causes excessive seepage from the puncture sites that may cause family dissatisfaction or give rise to community rumors leading to reduced acceptance for MITS. The objective of the study was to identify characteristics that increase the chance of excessive seepage so that additional counseling can be provided to the family before the procedure. From September 2017 to December 2020, we conducted 213 MITS in the CHAMPS Bangladesh site where 11% cases (23/213) had excessive seepage. There was no correlation with the time interval between death and MITS procedure (p=0.94). Among the 23 with excessive seepage, 74% (17/23) were early neonatal deaths and 17% (4/23) stillbirths. We compared them with 180 early neonatal deaths (46%, 83/180) and stillbirths (54%, 97/180) undergoing MITS but without seepage to investigate characteristics associated with excessive seepage using binary logistic regression. Of the total 203 cases, 21% had spontaneous bleeding without any trauma from either the nose, mouth, or ear prior to MITS, but the incidence of bleeding was higher (48%) among the children with excessive seepage; they had 3.7 higher odds of bleeding from the nose (95% CI: 1.1-12.0, p<0.05). Odds of seepage in stillborn children were lower (OR: 0.1, 95% CI: 0.1-0.6) compared to early neonatal deaths. None of the macerated stillbirths had excessive seepage. Preliminary findings suggest that family members of children who died within the first 7 days of birth, already having signs of bleeding prior to the MITS, should be counseled about the possibility of seepage to minimize post-MITS dissatisfaction or rumors.

0500

MACHINE LEARNING-BASED PREDICTION OF CLINICAL OUTCOMES FOR CHILDREN USING LINKED ELECTRONIC HEALTH RECORDS IN MANHIÇA DISTRICT, MOZAMBIQUE

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The increasing availability of individual-level electronic health records (EHR) presents a major opportunity to understand the progression and outcome of childhood illnesses in clinical settings. Recently, predictive machine learning (ML) algorithms have been used with EHRs to identify the vital clinical and laboratory information to assess individual risk and aid prognosis. ML methods result in algorithms that can identify key characteristics or patterns in large complex datasets, which may otherwise be hard to detect by traditional regression methods or by health care workers in clinical settings. However, many ML studies predicting outcomes have been conducted in high-income countries and lack detailed pre-admission health and demographic data which has constrained the development of clinical prediction algorithms suitable for these settings. We applied ML algorithms to predict child outcomes using data from; a longitudinal child morbidity surveillance study, demographic surveillance system (DSS) and a fever aetiology study (FIEBRE), for all child (under 15 years) visits to Manhiça District Hospital, Mozambique. Child out- and inpatient data were collected for 1,384,354 hospital visits between January 1997 and October 2020. These data included; medical history, physical examination, laboratory samples, diagnosis and health outcomes. These data were linked with a unique ID to a health and a demographic surveillance site dataset that monitors a population of 201,845 bi-annually. These data include socio-economic position, immunisation history, pregnancies and use of malaria control interventions. We applied a standard set of ML classification algorithms to predict; a) admissions, b) length of stay (<72 or ≥72 hours) and c) mortality. For all visits, the median age was 3 years (IQR 1-7) and 49% were female, 1,315,628 were outpatients and 68,726 were inpatients, with a median length of stay of 3 days (IQR 2-5) and 4% inpatient mortality. We will present the critical evaluation of ML algorithms to identify vital clinical, demographic and health information to predict child outcomes in resource-limited settings.

0501

KAP COVID CASE STUDY: MASKS, BELIEFS, AND VACCINE ACCEPTANCE - GLOBAL DISSEMINATION OF COVID BEHAVIORS

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The COVID-19 pandemic challenges global health responders to continuously motivate populations to uptake and adhere to COVID-19 prevention and control behaviors. Such monumental effort requires rapid, nuanced data on populations' COVID-19 knowledge, attitudes, risk perceptions, and behaviors. Without such data, those on the COVID-19 frontlines lack the information needed to roll out COVID-19 policies, research, risk communication, health education, social mobilization, and community engagement. To fill the gap, the World Health Organization (WHO), Global Outbreak Alert and Response Network (GOARN), Massachusetts Institute of Technology (MIT), Johns Hopkins University Center for Communications Program (CCP), and Facebook teamed up to develop the KAP COVID Dashboard. Data for the dashboard were collected via a survey promoted on the Facebook platform. Thus far, 19 waves of data have been collected, yielding five dashboard visualizations: (1) Global Vaccine Acceptance, (2) Trend Analysis of 23 Countries, (3) Global and Regional Views, (4) Individual Country Views, and (5) US and India Subnational Views. These interactive dashboards display data from 67 countries, representing over 1.7 million participants. The user-centered approach is aimed at supporting the efforts of COVID-19 practitioners, decision-makers, and responders around the globe to make informed decisions related to COVID-19 policies, communication, and resource allocation. This presentation will discuss the design process as well as the benefits of such collaborative approaches to data collection, visualization, and partner engagement. Case studies will be presented to demonstrate

the range of how this dashboard has been utilized by in-country teams. With numerous outbreaks, epidemics, and pandemics on the horizon, it is imperative that global researchers and responders aim to uptake collaborative, innovative, rapid, and agile approaches to data collection, visualization, and operational research.

0502

A PROCESS OF SETTING UP TUBERCULOSIS RESEARCH PRIORITIES AT A NATIONAL SCALE IN ZIMBABWE THROUGH THE NATIONAL TUBERCULOSIS PROGRAM

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Zimbabwe is a high-burden country for Tuberculosis (TB), Multi-drug resistant tuberculosis (MDR-TB) as well as Tuberculosis/ Human Immunodeficiency Virus (TB/HIV) co-infection. The Government of Zimbabwe is committed to ending TB by 2030. By 2025, the National Tuberculosis Program (NTP) aims to reduce TB incidence and mortality by 80%. To achieve these targets, one of the pillars set was the research and innovation pillar of the End TB Strategy, which promotes research along a continuum that links upstream fundamental research to discovery, new tool development and to operationalize and implement research. Thus, allowing innovative strategic approaches to be adapted to specific country needs. The process of setting up research priorities started with identifying a Tuberculosis Research Technical Working Group (TBR TWG). The TBR TWG aimed to capacitate the NTP's relevant operational research plan in conducting operations research and this followed a research prioritisation process. The research prioritisation process was conducted at a national scale through a multi-stakeholder workshop. The various stakeholders were put into 4 groups to identify research questions in the context of Zimbabwe-specific needs. A tool by Varkevisser et al (1991), was used basing on a template to categorise research questions under selected thematic areas. The template was presented as a public consultation to stakeholders. The template was presented on a Likert scale, thus prioritisation made use of a scoring system. Research questions were ranked into high, medium & low priority research questions. The research questions were then compiled together by order of priority to produce a prioritised list which was presented to stakeholders in a series of 3 stakeholder engagement meetings to facilitate review. A literature review was conducted by graduate students basing on the set-up TBR database, allowing for research questions which need further research to be identified and to refine the rationale of the prioritised list. The final TB research plan was then consolidated at the TBR TWG level to strategically include the prioritised list of research areas.

0503

MAJOR DEPRESSIVE DISORDER SCREENING AMONG LATIN AMERICAN IMMIGRANTS AND REFUGEES IN FLORIDA DURING COVID-19

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Major depressive disorder (MDD) among immigrants and refugees has significant implications for mental health wellness and integration into society after migration. We screened Latin American immigrants and refugees (N=258) originating from Mexico, and other countries found in Central and South America who had emigrated to Florida for evidence of MDD at participating medical clinics located in north Florida. Using the Patient Health Questionnaire-9 (PHQ-9) we screened participants for MDD during the COVID-19 pandemic from 11/10/20 until 04/16/21 at our medical clinics for those who were largely uninsured, and Spanish was their primary language. A total of 31% (N=80/258) scored 5 or greater using the PHQ-9, indicating risk for MDD. Median age was 40 years with 64% female, and median arrival to the United States was 2015. Participants migrating from 14 Latin American countries (Mexico, Central, and South America) were included, with greater than one-third of those screening positive migrating from Venezuela (N=28/80; 35%). Mexico (N=16/80; 20%), Colombia (N=9/80; 11%), Honduras (N=8/80; 10%), and Guatemala (N=7/80; 9%) were other regions with increased rates of MDD within this cohort. Average PHQ-9 scores among these five countries (Venezuela, Mexico, Colombia, Honduras, and Guatemala) were 11. Feeling down, depressed, or hopeless was substantial in the entire group screened, with 18% (N=46/258) expressing this occurs several days a week, 11% (N=29/258) more than half the days of week, and 4% (N=9/258) expressing these feelings occur nearly every day. Among those who screened positive for MDD, 51% (N=41/80) felt these problems were somewhat difficult to manage work, things around the home, and get along with other people, with 28% (N=22/80) stating this was very difficult. Each participant who screened positive for MDD was provided resources for further support and management in our clinic network. As demonstrated among this ongoing study, the current COVID-19 pandemic and other factors, such as political unrest among home country, are likely having a major impact on some who are immigrating to the United States from Latin America.

0504

THE ASSOCIATION THAT GENDER ROLES HAVE WITH HEALTH SEEKING BEHAVIOR FOR WOMEN IN KENYA, TANZANIA, AND UGANDA

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There are negative effects on maternal and child health outcomes in areas with greater gender inequalities, but it is not clear that there is an association between education status, control of earnings, and who makes health care decisions. Our research investigates the association between gender equality and health seeking behavior for women in Kenya, Tanzania, and Uganda. Using the 2014 Demographic Health Surveys (DHS), we examined who decides how to spend a woman's earnings, who decides on women's healthcare, and women's highest level of education. The outcomes of interest were health care seeking behaviors, including antenatal care and the place of care. We used R to conduct multivariate logistic regression to examine this association adjusting for age of head of household, age of respondent, urban or rural residence, wealth index, and age of respondent's first birth. There were 83,591, 57,906, and 37,169 women in Kenya, Uganda, and Tanzania, respectively included. We found that women were less likely to go to a government hospital if their husband controlled their earnings (Kenya OR=0.88, 95% CI:0.84 - 0.93; Uganda OR=0.77, 95% CI:0.73 - 0.81). When the husband controlled health care decisions, the women were less likely to visit a health care facility in the last 12 months (Kenya OR=0.71, 95% CI:0.70-0.72; Uganda OR= 0.87, 95% CI:0.86 - 0.88; Tanzania OR= 0.84, 95% CI:0.82 - 0.85). College educated women were more likely than those without a formal

education to seek antenatal care at government-run hospitals (Kenya OR= 1.56, 95% CI:1.50 - 1.62; Uganda OR= 2.94, 95% CI:2.81 - 3.07; Tanzania OR= 2.12, 95% CI:1.96 -4.96). For those with a primary education, the strength of the association was typically smaller and only statistically significant in Uganda and Tanzania (Kenya OR= 1.01, 95% CI:0.97 -1.03; Uganda OR= 1.16, 95% CI:1.12 -1.13; Tanzania OR= 3.14, 95% CI:2.91-7.34). This study, the first to use a large sample across the three countries, showed that with more access to education and control of earnings and health care decision making, women are more likely to go to their local hospital and seek antenatal care

0505

ANTIBIOTIC ENTANGLEMENTS. HEALTH, LABOUR AND EVERYDAY LIFE IN AN URBAN INFORMAL SETTLEMENT IN KAMPALA, UGANDA

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Antimicrobial resistance (AMR) is considered a global health crisis, threatening the effectiveness of all classes of antibiotics, and putting people at risk of untreatable infections. Current literature on AMR suggests low- and middle-income countries (LMICs) are at high risk for the emergence of AMR, which is depicted as a potentially catastrophic burden to already constrained health care systems. In Uganda, AMR is a growing area of concern for public health actors. A 2015 situational analysis revealed increasing trends in antimicrobial resistance to commonly used antimicrobials. To further explore the everyday use of antibiotics in Uganda, we engaged in ethnographic research, including a survey of 350 households, 16 semi-structured interviews and 2 participatory focus group discussions. Participant observation was conducted over 18 weeks among selected day-wage urban workers. We found that antibiotics were used frequently; their use intertwined with ongoing struggles with infection and a need for quick recovery to return to work. We found that significant and chronic infrastructural challenges were linked with antibiotic use – through three interconnected examples: flooding, limited toilet facilities, and water contamination. Antibiotics, we argue, have become a way to negotiate the inequalities written into the landscape; their use entangled with ongoing relations with labour, environment and bodily suffering; certain environments are overburdened with infection due to inequality and marginalisation. Attending to the structural reasons for antibiotic use in urban informal settings beset by inequality is necessary. Antibiotic use interventions often depend on theories and models of individual behaviour change, placing responsibility for action at the individual level which requires a behaviour change intervention. We propose that attention must be paid to the structural conditions that make avoiding antibiotic use nearly impossible.

0506

PRELIMINARY FINDINGS FROM A POINT PREVALENCE SURVEY OF ANTIMICROBIAL USE AT THREE RURAL DISTRICT HOSPITALS IN RWANDA

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Antimicrobials significantly mitigate the burden of communicable diseases, specifically infections. However, their misuse and overuse have resulted in antimicrobial resistance (AMR). The World Health Organization (WHO) has declared AMR one of the top ten global public health threats across the globe. In Rwanda, AMR is not well documented due to the lack of adequate surveillance systems. Here, we assessed antimicrobial prescribing patterns among health care providers at three rural district hospitals (DHs) in Rwanda. We conducted a point prevalence survey (PPS) using a standardized study tool adapted from the WHO Methodology for PPS tool at three Rwandan district hospitals (DHs) - Butaro (BDH), Kirehe (KDH) and Rwinkwavu (RDH). Patients' and prescribers' level data was collected between March - April 2021. Data of all admitted patients and prescribed antimicrobials was recorded on the same day and analyzed using Stata v.15.1. Data collection was completed at KDH and BDH. 242 admitted patients were included in the survey as they were prescribed antimicrobials -with 147 patients from KDH and 95 patients from BDH. Antibiotics from the "Access and Watch" WHO categories were the most prescribed, with three antibiotics ranking higher -- ampicillin (30%), gentamicin (20%), and third-generation cephalosporin such as cefotaxime (12.0%). From the prescribers' survey, 30.6% of the prescribed antibiotics were based on microbiology reports. Common indications for antibiotics prescriptions in this sample were; neonatal risk of infection, C-section risk of infection, and pneumonia Prevalence of antibiotics use per facility was 57.2% for BDH and 72.8% for KDH. Study findings show significant broad-spectrum antibiotic prescribing rates against a reported low rate of laboratory results. Prospective surveillance of antibiotic use over time and between different health facilities, promoting laboratory tests guided prescription habits, and develop site-specific data-driven antibiotic guidelines may improve rational prescribing practices which, in consequence, will support rational AMS in Rwanda.

0507

PRENATAL ALCOHOL USE KNOWLEDGE AND OUTCOMES IN LEYTE, THE PHILIPPINES

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Women in resource-limited settings may be at higher risk of giving birth to babies with fetal alcohol spectrum disorder due to consumption of unregulated brews without warning labels and lack of systemic preventive education. This is a major unaddressed public health issue in Leyte, The Philippines, where tuba, a locally made palm wine, is consumed. In this study, 100 postpartum women, established subjects in a longitudinal birth cohort in Leyte, were administered surveys in the local language of Waray via interview at their scheduled visits in June-Sep. 2019. The survey examined participants' knowledge, attitudes, practices, and beliefs regarding healthy pregnancies and prenatal alcohol use. The Phosphatidylethanol (PEth) dried blood test, which measures an abnormal phospholipid formed in red blood cells 2-4 weeks post alcohol exposure, was collected at enrollment, 32 weeks gestation, and birth. Chi-square and Fisher's exact tests were used to analyze bivariate relationships. 75% of subjects answered yes to drinking tuba and/or other alcoholic beverages during their most recent pregnancy. Participants who endorsed drinking tuba while pregnant were more likely to have positive PEth results at 32 weeks gestation than participants who denied drinking tuba prenatally (18% vs. 0%; $p < 0.05$). Mothers who said tuba is healthy during pregnancy were more likely to have positive PEth results at 32 weeks gestation (25.00% vs. 2.27%; $p < 0.05$). Some participants who reported not drinking tuba or alcohol during pregnancy had positive PEth results at enrollment (5/21, 23.81%). Almost all surveyed (98%) indicated that they would cut back on drinking alcoholic beverages if they were told that this negatively impacted them and their unborn child. Lack of information about tuba and its risks seem to contribute to its continued

use by pregnant women in Leyte. The PEth results provide a quantitative assessment of participants' prenatal alcohol consumption. Based on our results, educating women of reproductive age in Leyte regarding prenatal tuba use would likely lead to a reduction in tuba use among this population and FASD risk among their children.

0508

EXPERIENCE OF THAI RESIDENTS DURING THE FIRST WAVE OF THE COVID-19 PANDEMIC: A QUALITATIVE STUDY

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The impact of COVID-19 is being mitigated using non-pharmaceutical interventions (NPIs). Here we report a qualitative study in Thailand, which was part of a mixed-methods study, 'Social, ethical and behavioural aspects of COVID-19' (SEBCOV) in Thailand, Malaysia, UK, Slovenia and Italy. A total of 28 online in-depth interviews (18-74 years old) were conducted between 8th May and 21st July 2020 in Thailand. This represents the period in which the number of cases in the country was steadily decreasing and the government was relaxing its NPIs. At the time of the interviews, all participants had already experienced the strictest NPIs in Thailand, which was from 26th March to 3rd May 2020. Participants were selected using purposive sampling to gain a maximum variation sample, based on six characteristics: age, gender, educational level, household size, location, and risk of contracting COVID-19 due to their occupation and living conditions. Our results describe how Thai residents conceptualised and made sense of COVID-19. Some participants attributed the cause of COVID-19 to nature's revenge, eating "exotic" meat or acts of God. Most participants viewed COVID-19 as a serious disease, appeared to understand the rationale for strict public health measures, and reported complying with them. The challenges participants faced during the COVID-19 pandemic included economic, social and emotional challenges. Among participants most negatively impacted were those who worked in or with the tourism industry. Despite these challenges, some saw silver linings of COVID and NPIs, such as an opportunity to rethink their future life plans and not taking things for granted. This study also highlighed some coping strategies that individuals adopted, which included practicing self-care, limiting exposure to news, practicing acceptance, and turning to religion. Those who were financially able said they helped others by "making merit" (*tum bun*) such as organising "sharing pantries" (*tu pan suk*). The findings of this study are important to inform future policies in order to minimize their negative impacts on people's lives in the current pandemic and in the future.

0509

EFFECTS OF CORONAVIRUS PANDEMIC ON HEALTH SEEKING BEHAVIORS OF YOUNG ADULTS IN UGANDA

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Background: The widespread social and economic effects of the COVID-19 pandemic in Uganda has likely affected access to, and perceptions of, health care services at large, yet there is still limited attention on young adults as key contributors to its spread. We sought to understand the effects of COVID-19 on health seeking behaviors of young adults aged 18-29 in Uganda. Methods: A national population-based mobile phone survey of men and women was conducted in December 2020. Multivariable regression analyses were used to explore the associated effect of COVID-19 pandemic on access to health care services among young adults aged 18-29. Control variables included region, education level, parity, and source of health information. Results: The majority (98%) of the participants perceived COVID-19 as a serious threat to Ugandans. Although the majority reported taking action on handwashing (96%) and masking (91%), fewer participants avoided shaking hands (38%), ensured physical distancing (56%), avoided groups of more than 4 people (43%), and stayed home most of the days (30%), avoided touching eyes, nose and mouth(13%), and sneezing/coughing in the inside of the elbow (7%). Participants noted that the COVID-19 pandemic affected their access to family planning (40%), HIV (49%), maternal health (55%), child health (56%), and Malaria (63%) services. The perceived effect of the COVID-19 pandemic on services was higher among participants in the Northern region (OR= 2.00, 95% CI 1.00-4.02), higher education OR= 2.26, 95% CI 1.28-3.99), having five or more children (OR= 2.05, 95% CI 0.92-4.56) or trust in radio as a source of COVID-19 information (OR= 1.65, 95% CI 1.01-2.67). Conclusion: Young adults are a priority target audience and there is a need to intensify social and behavior change interventions that promote COVID-19 preventive measures and address challenges to accessing health services.

0510

KNOWLEDGE, ATTITUDE AND PRACTICE OF COMMUNITY-ORIENTED RESOURCE PERSONS ON COVID 19 IN NIGER AND KEBBI STATES, NIGERIA: A RAPID ASSESSMENT

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Integrated Community Case Management (iCCM) is an equity-focused strategy that extends the reach of public health services by providing timely and effective treatment of malaria, pneumonia and diarrhoea to children under 5 years in hard-to-reach populations. During the COVID-19 pandemic, global technical guidance on infection prevention and control protocols among community health workers was issued by WHO and UNICEF and then adapted by countries. We assessed the effect of the COVID-19 pandemic on service provision and evaluated the knowledge, attitudes, and practices (KAP) of community oriented resource persons (CORPs) in an existing iCCM program in two states (Niger and Kebbi) in Nigeria from August 2021 to October 2021. 341 CORPs were randomly selected from the two states and a rapid assessment of commodities, clients' patronage, community awareness and KAP of CORPs regarding COVID-19 was done. Telephone interviews were carried out by trained data collectors using structured questionnaires and appropriate KAP regarding COVID-19 among CORPs as well as possible influencing factors were analyzed. Response rate for the study was 100% and all CORPs had received information about COVID-19, majority through the radio (82.9%). Majority of CORPs (95.9%) reported they observed no significant changes in number of caregivers seeking iCCM services compared to the pre-COVID-19 era. However, 10.3% and 13.2% of the CORPs reported stock-out of malaria rapid diagnostic tests and artemisinin-based combination therapies respectively, lasting more than 3 months. Most CORPs interviewed knew signs and symptoms of COVID-19 (83.6%) and 95.9% knew how to prevent the disease. Also, 80.1% of CORPs reported they did not avoid patients with symptoms indicative of COVID-19 in previous week before the study and 96.2% wore face masks while attending to patients. In conclusion, the pandemic did not adversely affect iCCM service provision and CORPs demonstrated satisfactory knowledge, attitudes and practices in delivering safe and effective iCCM services in the context of COVID-19.

0511

HANDS-ONLY CPR TRAINING PROGRAM OF SECONDARY SCHOOL STUDENTS IN IBADAN, NIGERIA: PILOTING COMBINATION VIRTUAL AND IN-PERSON LEARNING

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Out-of-hospital cardiac arrest (OHCA) has an estimated average global incidence of 55 adults per 100,000 person-years. Despite advances in medical care and technology, survival to hospital discharge remains abysmally low at 8-10%. In low to middle income countries like Nigeria, where cardiovascular disease is rising but emergency response systems are poor, the rate of survival after OHCA is lower. Bystander cardiopulmonary resuscitation (CPR) training is effective, scalable, and low-cost interventions that can reduce the OHCA mortality. Training schoolchildren to perform Hands-only CPR (HOCPR) has been shown to increase bystander CPR. CPR Peer Educator's Program (CPEP) is a community-based program in Ibadan, Nigeria, designed to enhance understanding of OHCA and CPR in secondary school students. The American Heart Association's CPR in Schools module was adapted. The training was run jointly by the Revolving Hearts Foundation, Atlanta, and Issachar Generation, Nigeria. The training had in-person and virtual components due to COVID-19 travel restrictions. We present results of training on knowledge, comfort level, and perceived barriers of performing HOCPR. Convenience sampling was used to recruit participants. Pre- and post-training surveys were conducted in February 2021. Participant responses were matched to assess changes and analyzed using Stata 16.1 software. A paired t-test analysis was conducted. The primary outcome was percentage change in mean knowledge scores and secondary outcome was change in comfort level and perceived barriers pre- and post- training. A total of 45 secondary school students from 4 schools completed the survey. Females comprised 31.1% of students and the average age was 15.02 + 0.18 years. Paired t-test showed a significant change of 44.6% ($p < 0.0001$) in the mean knowledge score. There was also an increase in comfort level in performing HOCPR, and a decrease in perceived barriers. Trainees then disseminated their knowledge in schools via skits. Follow-up evaluation and longitudinal expansion to other schools and states is planned.

0512

MAKING THE CONNECTIONS: ORGANIZATIONAL NETWORK MAPPING TO SUPPORT IMPLEMENTATION OF AN EVIDENCE-BASED CONTROL INTERVENTION FOR CYSTICERCOSIS IN NORTHERN PERU

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We are working with community members and the health system in Northern Peru to implement ring treatment - an evidence-based focal control strategy for cysticercosis - as a government-administered program. Successful implementation requires health personnel to work across teams and alongside animal health agencies to deliver the intervention. As part of the formative evaluation phase of this 5-year project, we are creating a map to describe the organizational network of the health system involved in implementation of ring treatment. Using questionnaires based on the Consolidated Framework for Implementation Research (CFIR), we have completed 258 interviews, of an expected 423, with key stakeholders and staff at 18 health posts and centers, in four rural districts, about their intra- and inter-organizational networks and communications. Stakeholders also identify which people or organizations should be responsible for implementing specific steps in ring treatment. We are using R and UCINET software to visualize supervision networks, team networks, and inter-organizational networks, and calculate network centrality and cohesion. We will present the current intra- and inter-organizational networks of health staff who have an essential role to play in implementing ring treatment. We will also share a comparison of the current networks to the networks described by participants as ideal for successful implementation, and recommendations for network modifications and linkages to support implementation and scaling ring treatment. The study of organizational and community networks provides important information to support implementation and scaling ring treatment to regional and national levels, across heterogeneous settings.

0513

TRENDS IN MORTALITY AND PERCEIVED CAUSE OF DEATH IN KERSA HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM, 2008-2019, EASTERN ETHIOPIA

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Demographic transition and population aging are health issues this days. Attached to this, epidemiological transition is concomitantly changing disease and mortality patterns. These changes in patterns have been observed in some urban areas of Ethiopia. We examine health and demographic trends in rural Ethiopia using data from a demographic surveillance (Kersa HDSS), established in 2007. We used life table to calculate population death rates (CDR), age specific death rates, and life expectancy at birth. Neonatal, infant, and under-five mortality have also been calculated. The perceived causes of death as reported by a close relative of the deceased were analyzed using the global burden of disease classification as Group I (communicable, perinatal, maternal and nutritional deficiency diseases), Group II (non-communicable diseases), Group III (injures and accidents). Jaundice and Sudden Death were treated separately as they don't fall in any of the groups. The overall CDR dropped from 11.19 per 1000 population in 2008 to 5.53 in 2019. The consistent reduction in mortality yielded a 13.3-year average gain in life expectancy between 2008 and 2019 increased from 4.6 in 2008 to 17.4 in 2019 for age 85+ years. There was a drop in under-five mortality from 147.4 per 1000 births in 2008 to 85.4 in 2019. Thought overall the primary causes of death were Group I causes, over the twelve-year period in this rural population, there is a slight shift from Group I causes to Group II causes.. The CDR has dropped yielding a significant gain in life expectancy, although the under-five mortality remains above the national average (78 per 1000 live birth). Interventions targeting communicable diseases, maternal and child deaths should be intensified to avert the tall of burden of mortality from these causes.

0514

NEW CONFIRMED EBOLA OUTBREAK IN NORD KIVU, DEMOCRATIC REPUBLIC OF CONGO, FEBRUARY 2021

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INRB received a new Ebola-positive sample from Nord Kivu on February 9, 2021. The individual was a 42-year-old patient and the wife of a recovered EVD survivor from the previous Ebola virus disease (EVD) outbreak 2018-2020. She resided in the Biena health zone and her symptoms began on January 25, 2021. She first visited the Ngubi health post where she remained under observation for 4 days. Then, she consulted the Masoya reference health center where she remained in hospital until February 03, 2021. A blood sample was taken the same day for suspicion of EVD due to the onset of hallucinations, diarrhea, melaena, epistaxis. She was transferred the same day to Matanda Hospital in Butembo where she died on February 04, 2021.The blood sample, along with historical samples from her husband, were subjected to sequencing. Four genomes with high coverage were obtained and compared to other genomes from the 2018-2020 Nord Kivu/Ituri EVD outbreak. The new case could have been caused by 1) a new spillover event, meaning a new separate EVD outbreak; 2) a transmission chain from the Nord Kivu EVD outbreak that continued unnoticed since the end of the outbreak, or 3) an infection from a persistent source, either from a survivor of the Nord Kivu outbreak or a relapse of her own prior infection (although she was not known to have had EVD).The new case is descended from the Ituri variant and therefore does not represent a new spillover event, but indicates this variant is from the 2018-2020 EVD outbreak. The new case clusters with samples from Mandima and Mabalako collected around October 2019, including the samples from her husband. It has 5 more mutations than the nearest genome. This is indicative of a slowed substitution rate which is a hallmark of infection from a persistent source.

0515

CAUSE-SPECIFIC UNDER-FIVE MORTALITY IN SUB-SAHARAN AFRICA AND SOUTH ASIA: ADJUSTMENT FOR SELECTIVE NON-RESPONSE BIAS IN THE CHAMPS NETWORK

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Despite reductions in the global rate of stillbirths and under-five (U5) mortality, geographic disparities persist. Child Health and Mortality Prevention Surveillance (CHAMPS) collects standardized, population-based data on stillbirths and U5 deaths in Bangladesh, Ethiopia, Kenya, Mali, Mozambique, Sierra Leone, and South Africa. Laboratory analyses are performed on post-mortem biopsies from key organs and body fluids obtained through minimally invasive tissue sampling (MITS). However, not all deaths are eligible or consent for MITS; some families only consent to collection of antemortem clinical and verbal autopsy (VA) data. In-country expert panels use all available data to deduce the most plausible causes of death (CoD). Crude calculations of mortality fractions using only deaths consented for MITS may be biased due to selective non-response. Using the Horvitz-Thompson strategy, this analysis estimated unadjusted and adjusted mortality fractions for the top five CoD anywhere in the causal chain (immediate, underlying, other comorbid conditions) in each age group (stillbirths, neonates, infants and children) and country from 2017 to 2019; selection probabilities were estimated based on subgroup membership of all CHAMPS cases. Subgrouping variables identified *a priori* included season and location of death, maternal education, caretaker's religion, sex of child, and VA CoD; those statistically significantly associated with MITS conducted were selected for adjustment. Across all sites, CHAMPS enrolled 1,346 stillbirths, 1,585 neonatal and 1,337 infant and child deaths; on average (SD), 57% (19%), 50% (23%) and 59% (31%) declined to consent for MITS, respectively. Of 103 cause-specific mortality fractions estimated, 27 were meaningfully different (>10%) from the crude after adjustment; the mean (SD) change was 13% (15%). For example, the mortality fraction (95% CI) due to HIV among infants and children in Mozambique, increased 57% from 0.15 (0.07, 0.23) to 0.24 (0.15, 0.33). Adjusted mortality fractions more accurately represent the true distribution of CoD in the population, which may help target prevention efforts.

0516

YEAST-LIKE FUNGI ASSOCIATED WITH CHILD DEATH AND ILLNESS IN BANGLADESH: FINDINGS FROM CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE STUDY

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In the United States, yeast like fungi accounts for about 1-4% of nosocomial infections in neonatal intensive care units, which result in 20-30% mortality. Data on these infections in Bangladesh are rare. The aim of this analysis is to understand the contribution of yeast like fungal infections in child death and illness from the Child Health and Mortality Prevention Surveillance (CHAMPS) site in Bangladesh. Laboratory results (post-mortem specimens - blood, cerebrospinal fluid, nasopharyngeal swabs, rectal brush, liver, lungs and brain and tissue specimens), clinical, demographic and verbal autopsy data are presented to a panel of experts for determination of cause of death (DeCoDe). From August 2017 to March 2020, specimens were collected utilizing minimally invasive tissue sampling method from the 178 cases from stillbirths and under-5 deaths from Faridpur and Rajbari districts. In addition, blood specimens were collected for microbial culture from 110 under-5 children hospitalized with suspected sepsis. Among post-mortem cases and living suspected sepsis cases, 126/178 (70%) and 86/110 (78%) were positive for any microorganism in different testing platforms, respectively. Among these cases, *Candida* spp. were detected from 4/126 (3%) post-mortem cases and 5/86 (6%) suspected sepsis cases. All deceased children were early neonates aged 1 to 3.5 days from delivery who were born and died in hospital, and organisms were detected from blood. The DeCoDe panel

determined *Candida albicans* as the cause of death from 1 of 4 (25%) post-mortem cases. This case was a neonate born at 30 weeks gestation who also tested positive for *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus.* Our initial analysis showed that yeast-like fungi is important in hospital related neonatal deaths and illness in Bangladesh. More extensive investigation covering species level identification and species-specific antifungal susceptibility patterns will help to better understand the real situation of yeast like fungal infection and trigger necessary infection control measures to prevent its spread in health-care settings.

0517

COVID-19 SURVEILLANCE IN THE SOUTHERN PROVINCE OF SRI LANKA

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Since identification of the first case of COVID-19 in Sri Lanka in January 2020, surveillance PCR testing was implemented across the country. The Department of Microbiology, Faculty of Medicine, University of Ruhuna volunteered to survey the Southern Province of the country. The purpose of this study was to determine the prevalence of COVID-19 in the Southern Province during the early stages of the pandemic. A prospective observational study conducted among a sample of outpatients obtaining medical care at the three tertiary care hospitals in the Southern Province. Patients' demographic data, clinical information, and potential exposure to COVID-19 was documented. In all patients, a nasopharyngeal and an oropharyngeal swab was tested for COVID-19 using RT-PCR (CDC protocol). A total of 859 persons were tested from 19th June to 20th November 2020. The participants were from Galle (44.8%) Matara (28.1%) and Hambantota (27.1%) Districts. The majority were adults of 19-59 years (736/820, 89.8%) and male (474/833, 56.9%), and 12.8% (37/290) were frontline workers involve in combating COVID-19. Symptoms suggestive of COVID-19 at the time of testing included fever (14.5%, 99/685), cough (25.5%, 175/686), common cold (7.8%, 47/603), sore throat (24.3%, 165/680), shortness of breath (5.4%, 35/651) and diarrhea (0.8%, 5/602). Total of 253 persons (29.5%) had at least one feature suggestive of influenza like illness (ILI). None of the participants had been previously positive for COVID-19, admitted to a COVID-19 ward, or quarantined in isolation camps. Potential exposure to COVID-19 was identified in 9.1% (78/859) of participants. Only one person (0.1%, 1/859) tested positive for COVID-19 by RT-PCR. This patient had a sore throat and had been exposed to a COVID-19 positive patient at work. COVID-19 prevalence was 3.9/1000 in those having symptoms suggestive of ILI (1/253) and zero in the non-ILI group (0/606). During the latter half of 2020, the prevalence of COVID-19 in the community of the Southern Province of Sri Lanka was low (1.2/1000 population) with no evidence of infection among the asymptomatic population; but continued surveillance is needed.

0518

PHYSICAL ACCESSIBILITY TO COVID-19 RELATED HEALTH SERVICES ANALYSIS IN BANGLADESH

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Ensuring the population has equitable access to COVID-19 testing and treatment facilities is a key component of the response to the COVID-19 pandemic. This study aimed to identify the proportion of the population that can reach testing, treatment and intensive care unit (ICU) health services within a given travel time (1, 2, 3 hours) in Bangladesh and, as such, support planning and decision making. The analysis was done for wet and dry seasons using AccessMod 5.6.0 and used geospatial and statistical data that was compiled, checked, cleaned and, when necessary, improved before analysis. The estimates of travel time represent a best case scenario. Delays due to unavailability of a vehicle, disruptions to transport networks, waiting times for, or interruptions to health services at the destination were not considered due to wide variation and a lack of data on these factors. At the national level, 65.7% of the total population could reach a testing facility within 1 hour of travel time during the dry season, 85.7% for treatment facilities and 56% for ICU. There was substantial variability in these results between different administrative units. During the wet season, physical accessibility was reduced in the subdivisions experiencing flooding by up to 85% for testing facilities and 11% for treatment facilities and ICU facilities. While all results presented are dependent on the quality of the data and the validity of the travel scenarios that were considered, the findings to date allow for the identification of potential areas for which the government might require more in-depth analyses. These results should be regularly updated to account for the changes of the COVID-19 health services delivery network put in place by the government to respond to the evolution of the pandemic. Travel times to health services for COVID-19 testing, treatment and ICU care were estimated and found to vary widely across the country. The analyses presented can also be extended to examine access to other health services such as COVID-19 vaccination, provided the relevant data are available on the locations of service providers.

0519

DETECTION OF SARS-COV-2 CASES IN A PHILIPPINE MILITARY HOSPITAL

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The unique capabilities of the military such as expertise in command and control, coordination and collaboration are vital in the Philippine COVID-19 outbreak response where >900,000 cases and >15,000 deaths have been recorded from the initial detection of SARS-CoV-2 on 30 Jan 2020. The Philippine military serve as frontliners that support the implementation of SARS-CoV-2 strategic approaches in the Philippines through the transport of food, people, medical supplies and equipment that are needed in communities, clinics and hospitals. Implementation of community quarantine measures by the Philippine government also allows the deployment of Philippine military personnel to high density areas with high concentrations of COVID-19 such as checkpoints and local government areas. Such military movements can increase the risk of acquiring SARS-CoV-2 infection which can lead to morbidity and mortality among the soldiers. Therefore, early detection and management plays a vital role in controlling the spread of COVID-19 in the military and decreasing casualties among these frontliners. As part of a collaboration

between the Armed Forces of the Philippines (AFP) and the Armed Forces Research Institute of Medical Sciences (AFRIMS), the COVID molecular laboratory was established for the early detection and control of SARS-CoV-2 cases via RT-PCR testing of military personnel, dependents and authorized civilians. From 14 April 2020 to 31 March 2021, a total of 59,632 oropharyngeal and nasopharyngeal specimens were tested for SARS-CoV-2 with 3,668 (6%) positive and 55,964 (94%) negative cases. Cases were highest among the active military age group (21-50 years old) with 2,885 cases (78%) and males with 38,495 cases (65%). Typical symptoms were fever, cough and sore throat. Aside from early detection and rapid isolation of positive cases, COVID-19 transmission control strategies include: contact tracing; screening of hospital and non-hospital staff; control of entry and exit points, and information and education strategies. These assets were also leveraged for the Philippines COVID-19 vaccine rollout, which was initiated in early March of 2021.

0520

IMPACT OF THE COVID-19 PANDEMIC AND ASSOCIATED RESTRICTIONS DURING THE FIRST WAVE ON MATERNAL AND CHILD HEALTHCARE PROVIDED AT FACILITIES IN RURAL BALIAKANDI, BANGLADESH

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According to UNICEF, 2.4 million babies and mothers in Bangladesh had restricted access to health during enforced movement restrictions from 26th March to 31st May 2020 due to the COVID-19 pandemic. The Child Health and Mortality Prevention Surveillance (CHAMPS) project has maintained mortality surveillance in Baliakandi, a rural area in Bangladesh, allowing CHAMPS staff to conduct this study to identify healthcare facility closures and reductions maternal and childcare services received. We contacted the 16 facilities (6 hospitals, 4 clinics, 3 health centers, and 3 private chambers) that provided the greatest proportion of maternal and child healthcare to inquire about closures and the number of women and children receiving care during August 2020 compared to February 2020, before the pandemic. The public health facilities (8/16) provided limited scheduled and routine immunization, antenatal care, and patient admissions in April 2020, and 85% (6/7) of clinics and doctor's chambers were closed during the restrictions. The pediatric ward in the largest public tertiary care hospital was converted to a COVID-19 ward from the 1st of April 2020, resulting in the referral of children to other facilities through October 2020. In August 2020, there were 15-68% fewer outpatient visits, including wellness visits, ANC, and malnutrition follow-ups compared to February 2020, and 14-174% fewer inpatient admissions in hospitals (6/16). Eight out of 11 facilities that conduct deliveries had 11-37% fewer in August, but the three private facilities conducting deliveries experience an increase. Hospitals and health centers reported shortages in vaccines, PPE, lab equipment, reagents, and oxygen starting in March 2020, which was resolved by August 2020. COVID-19 among staff was a major concern, and at one hospital, 33% (28/84) of staff were infected during April and May 2020. The imposed restrictions in movement and closure of public facilities had a negative impact on healthcare access in Baliakandi. Future directives need to mitigate the rural health care delivery system.

0521

USING EMERGENCY OPERATIONS CENTERS TO SUPPORT THE COVID-19 RESPONSE: CREATION OF AN ONLINE RESOURCE FOR SELF-DIRECTED TRAINING AND TECHNICAL ASSISTANCE

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The rapid spread and severity of the novel coronavirus 2019 (COVID-19) pandemic prompted countries around the world to pursue strategies to manage transmission and protect their healthcare systems. The fundamental approaches for responding to the COVID-19 pandemic remain the tried and tested tools of public health response: identify affected individuals, monitor them (and isolate or quarantine as necessary, depending on the context), and trace and monitor their contacts. However, the scale of the pandemic has required most countries to adopt substantial additional measures to prevent community transmission, including stay-at-home orders, closing public spaces and recreation areas, reducing or closing public transport, and suspending schools and universities. With the advent of safe and effective vaccines, countries have also developed, and begun to deploy, strategies to deliver vaccines at large-scale, starting with highest priority groups such as the elderly and healthcare workers. These efforts require close operational coordination across multiple sectors, and operational response efforts also need to be coordinated with broader policy decision-making processes, managed by national and sub-national political leaders. In all these ways, COVID-19 response efforts can benefit from application of the principles of emergency management, including coordination through structures like emergency operations centers. To this end, we present here a newly developed website, developed with input from international subject matter experts, containing training materials, checklists, country case studies, and other resources for development and strengthening of public health emergency management capacities, with an emphasis on emergency operations centers. Since its launch in June 2020, the website has garnered substantial international interest, and positive feedback on its utility from end-users, highlighting the potential for online tools and resources to complement more traditional technical assistance for delivering public health emergency management capacity strengthening in the future.

0522

A NOVEL APPROACH TO PUBLIC HEALTH PREPAREDNESS FOR SARS-COV-2: A STATEWIDE REPRESENTATIVE SAMPLING

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The global SARS-CoV-2 pandemic has had devastating effects on public health systems worldwide, and emergency preparedness agencies require timely information on current representative prevalence, vaccination status and neutralizing antibody kinetics post-infection. An evidence based representative sample at state-wide level for SARS-CoV-2 is lacking in most of the US. For this reason, a collaborative effort between academic, medical, and public health partners created the SC Strong COVID-19 Project, a yearlong randomly selected surveillance initiative to attain this goal. The South Carolina Department of Health and Environmental Control together with the University of South Carolina contacted &gt 449,000 residents from November 2020 to May 2021. In conjunction with clinical community partners, participants were offered free viral and antibody testing, and asked to complete an online health survey to evaluate symptomatology, perception of the public health response, social distancing and other preventive measure compliance, and mental health status of participants during the pandemic. A project website,

email and hotline phone number were available to gain the public's trust, address concerns, and facilitate participation. This presentation will cover the design, implementation, and response to a large-scale surveillance project to enhance understanding of SARS-CoV-2 at the state level over 4 distinct time points. An overview of the infrastructure that allowed for this large prospective cohort, a time series analysis of participant's behaviors and attitudes, and results of this project will further be addressed. To our knowledge, South Carolina is the only state to implement a year-long statewide representative sampling as part of the state's public health response to the SARS-CoV-2. This program can be used as template for other states, attempting to meet their respective populations' needs and to strengthen public health preparedness for this and future pandemics. This talk will provide a timely pandemic response example and provide guidance for the development of similar infrastructures at other health departments.

0523

LESSONS LEARNED: OCCUPATIONAL HEALTH SUPPORT TO GLOBAL PROJECTS FOR SAFETY AND BUSINESS CONTINUITY DURING COVID-19 PANDEMIC

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The nature of global projects is that they utilize resources and workforce in multiple countries. This presents an unprecedented challenge during the COVID -19 pandemic due to the asynchronous nature of response not only at a national level but also regional and global scale. Global projects have adopted an agile strategy whilst balancing both economic and health risk to ensure continuity. We use an occupational health support lens to review lessons learned and share opportunities that when considered would improve efficiency in future pandemics or outbreaks with pandemic potential. The focus areas of occupational health support in global projects during a pandemic were to ensure 1) we protect people's health 2) pursue multi-lateral partnerships and 3) medical infrastructural assessment including care of workers not just for COVID-19 but other diseases in a stretched system. Due to the scale of the pandemic, vulnerabilities emerged. We outline the critical lessons of the pandemic's impact including the successes in managing health especially in remote set up while navigating new business environment, providing travel, occupational & mental health guidance that is scalable (one size does not fit all) and rapidly developing procedures with focus on protective preventive equipment and mitigation measures. Opportunities were realized by risk analysis and mitigated by developing strategies and tactical restructuring to minimize impact and likelihood of poor health outcomes. There is need for alignment in the international guidelines, and whilst this may take time, effort can be channeled internally to update pandemic plans, preposition supplies, and enhance design of outbreak response to be robust and outlast long health crisis. Global projects in oil and gas help provide critical products and work on a structured limited timeline and challenging environment. Providing support during a pandemic needs a comprehensive beyond traditional health plans. There is need to be intentional and exponentially flexible, to not only protect the health of workers but also to ensure business continuity not just for the energy we need today but also tomorrow.

0524

SEROPREVALENCE OF SARS-COV-2 AMONG MEDICAL UNDERGRADUATES IN A STATE UNIVERSITY OF SRI LANKA

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In Sri Lanka's state universities, medical undergraduates are recruited from throughout the country and can provide an estimate of COVID-19 seroprevalence across many geographic regions. A repeated cross-sectional study was conducted to estimate the prevalence of SARS-CoV-2 antibodies among medical undergraduates during the two waves of the COVID-19 pandemic in Sri Lanka. Demographic and clinical data and blood samples were collected during September-November 2020 and January-February 2021. COVID-19 antibodies were tested by ELISA. Of 279 collected samples, 175 (62.7%) were just after the students returned to the university following the first wave of COVID-19 and balance after the onset of second wave. Median age of both groups was 25 years (IQR 24-27). One-fifth of each group were residents of the local Galle district. Recent (within last 3 months) febrile illness was reported by 2.3% and 8.8% in the two groups respectively. One-third of the second group reported staying in a COVID-19 quarantine center for possible COVID-19 exposure, but none in the first group. Over 95% in both groups did not report close contact with overseas returnees or security personnel, but close contact with healthcare staff was seen in 32.6% and 63.5% in groups 1 and 2, respectively. Proportion of students who reported about undergoing PCR testing has increased from <1% to 94.2%. PCR positive cases were detected only in second group (n=5, 4.8%). Frequencies of contact with a confirmed or suspected patient or an identified contact were negligible in the first group while in second group 34.3%, 14.4% and 13.5% Prevalence of SARS-CoV-2 antibodies were detected only during second wave 6.7% (n=7) and asymptomatic infection in 1.9% (n=2) were detected during routine surveillance. Serological evidence of COVID-19 was not seen in November 2020. But two months later antibodies were detected. Surveillance at regular intervals, targeting individuals from diverse geographic regions is important for tracking the spread of SARS-CoV-2 in Sri Lanka.

0525

RAPID IDENTIFICATION OF INFECTIOUS AGENTS IN HUMAN PLASMA WITH LASER-BASED DIAGNOSTICS

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Rapid detection of pathogens within minutes on-site, with simple specimen preparation, no requirement for highly skilled personnel, and the ability to expand for detecting emerging pathogens is needed to reduce blood donor screening time and risk of transfusion-transmission disease. Laser-induced breakdown spectroscopy (LIBS), a detection method utilizing optical emissions from a sample interrogated by a laser beam, then resolved by spectrometry and processed by programmed analysis, has been effectively used with bloodborne pathogens spiked in blood. There is potential to build a device that is compact and affordable for use in low resource settings. To investigate laser-based technology for the rapid identification of infectious agents in plasma specimens, donor plasma spiked with HCV and HIV-1 and clinical specimens were tested. Virus stocks for spiking were FDA CBER reference vials with validated copies per ml. Pathogens were spiked as low as 100 copies/ml in healthy volunteer plasma. Clinical donor plasma was identified by approved tests

as negative, HCV positive, or HIV-1 positive. The pathogen-spiked plasma, negative controls, and clinical plasma were applied to paper filters, air dried, wrapped, baked, and analyzed with a benchtop assembled LIBS device. Detection algorithms were developed for two studies; study one had 65 samples including 20 clinical specimens and study two had 69 samples including 20 clinical specimens. The LIBS system with detection algorithms was 100% correct in differentiating spiked plasma from unspiked plasma and infected clinical plasma from uninfected clinical plasma with identification of HCV or HIV-1. There were no false positives nor false negatives. Our advances with LIBS analysis allow for effective and rapid differentiation of infected clinical plasma from uninfected and identifying the type of infection. Advantages are rapid and minimal specimen preparation, simultaneous analytical processing, and flexibility to add new algorithms for emerging pathogens. This laser-based study for multiplex pathogen detection suggests LIBS has potential for use as a blood safety diagnostic.

0526

DEPLOYMENT OF A REMOTE FIELD SEQUENCING LABORATORY IN NORD KIVU: A PREPAREDNESS STRATEGY FOR BETTER MANAGEMENT OF FUTURE OUTBREAKS IN THE DEMOCRATIC REPUBLIC OF CONGO

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Since the recent large Ebola Virus Disease, EVD, outbreaks in West Africa, 2013-2016 and Democratic Republic of Congo, DRC, 2018-2021, real-time pathogen sequencing has been an effective tool for assisting with field epidemiology. In DRC, sample shipment is challenging and delays the sequencing results, with the risk of sample degradation. The EVD outbreak in Equateur Province 11th outbreak and EVD re-emergence in North Kivu 12th, highlighted the need to deploy tools for rapid results and to enable adequate responses as seen during the 10th EVD outbreak. In April 2021, we used a blueprint developed with a collaboration of scientists from CDC, UNMC, GWU, and Gates Foundation to gather the minimal equipment and supplies required to perform viral sequencing in a resource-limited environment. With minimal power requirements, the equipment allows robust implementation of viral genomic sequencing in hours, rather than days. In order to field test the final iteration of the portable sequencing lab, a trained team of scientists from the National laboratory in Kinshasa, DRC, were deployed with the portable laboratory to Goma. After visiting the Goma INRB facility, it took 2 hours to set up the lab and get it ready to start sequencing. Local Goma staff, unfamiliar with MinION sequencing, were trained to use this laboratory and workflow over a 6 day period. During 48h, RNA from 23 EVD patients were processed, including 11 RNA extracts from the 12th EVD outbreak, and a library with 12samples was prepared and loaded onto the MinION. Local staff trainers generated 7 genomes with coverage above 70% and 5 above 90%. The local Goma staff also performed SARS-CoV2 sequencing and the laboratory is continuing to operate in Goma and provides SARS-CoV2 sequencing support for the pandemic surveillance. The equipment and workflow have proven to be robust, portable, and trainable, enabling effective implementation of MinION sequencing in resource-limited settings. This experience is included in the response preparedness of the country to face future outbreaks by improving and increasing detection capacity and genomic surveillance

0527

FINANCIAL ANALYSIS TO INFORM PROGRAMMATIC DECISION MAKING AND DOMESTIC RESOURCE MOBILIZATION FOR NEGLECTED TROPICS DISEASES

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With the elimination and control of certain Neglected Tropics Diseases (NTDs) increasingly within reach, effective, sustainable programs to prevent disease reemergence are of paramount importance. In fact, NTD programs have made substantial progress over the last decade leading to global declines in the burden of five NTDs responsive to Preventive Chemotherapy (PC) : lymphatic filariasis, trachoma, onchocerciasis, schistosomiasis, and soil-transmitted helminths. Many endemic countries have substantially reduced their prevalence, and subsequently disease burden and morbidity, with the support of global initiatives and significant donor and national financing. Act to End NTDs | West is a USAID-funded project supporting 11 countries in West Africa to achieve elimination and control goals for all five PC-NTDs. As national NTD programs (NTDPs) near elimination targets, sustaining gains may be challenging as Ministries of Health prioritize other high-prevalence diseases (e.g., HIV, malaria) with higher visibility and mortality. If programs are unable to secure sufficient national support, NTD gains may be threatened, leading to the reemergence of "eliminated" NTDs and erasing the success achieved thus far through coordinated action and sustained investments. NTDPs use the Tool for Integrated Planning and Costing (TIPAC), a Microsoft Excel-based program that helps NTDPs accurately estimate their program's costs and funding gaps. TIPAC is important for programmatic decision making as the tool can be used in conjunction with existing national NTD strategic plans and budgets to effectively plan and coordinate future program resources. Additionally, TIPAC data is a key driver for advocacy and resource mobilization activities. This poster presentation will illustrate how TIPAC data is used by Act | West programs to inform budgeting, planning, and costing and is an essential factor for resource mobilization and NTD elimination. Such activities contribute to country ownership, a key pillar of the WHO 2030 Roadmap.

0528

INTRODUCING A SPATIAL SYNDROMIC SURVEILLANCE APPROACH TO IDENTIFY EMERGENT CLUSTERS OF COVID 19

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Identifying emergent patterns of coronavirus disease 2019 (COVID-19) at the local level presents a geographic challenge. The need is to identify meaningful spatial patterns, especially in vulnerable settings where even small numbers and low rates are vital for the targeting of early intervention. This presentation identifies a gap in current spatial analytical approaches that tend to rely on maps describing broader patterns of disease rather than a near-real time assessment of emerging situations. This new technique, Geographic Monitoring for Early Disease Detection (GeoMEDD), is either used as a stand-alone analysis or part of an "enterprise" solution integrating a spatial database, by providing multiple spatial and temporal perspectives on an ever-changing disease landscape by connecting cases using different spatial and temporal thresholds. GeoMEDD, has proven effective in revealing different cluster sizes, as well as the influencers and accelerators that give insight as to why a cluster exists where it does, and why it evolves. In this paper we will show how the technique was developed at the beginning of the pandemic to support a single hospital system, but soon evolved into the syndromic surveillance tool for the entire US state of Ohio. We will go on to show how it can be used to sort through spatial noise in periods of surge, or for identifying flare-ups when cases recede. In this regards it has also proven useful for the spatial targeting of vaccine deployment. The technique had been developed for maximum flexibility for different data environments, making

it ideal for any global setting including locations with limited resources. We will end this presentation with an example of how GeoMEDD can be used to address other health situations beyond the current pandemic.

0529

BUILDING CAPACITY ON INFECTION, PREVENTION AND CONTROL (IPC) IN HEALTHCARE SETTINGS DURING THE COVID-19 PANDEMIC IN BANGLADESH

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The COVID-19 pandemic underscored the lack of capacity in infection, prevention and control (IPC) among frontline health care workers (HCWs) in Bangladesh. Upon request of the Communicable Disease Control of the Directorate General of Health Services, USAID Medicines, Technologies, and Pharmaceutical Services (MTaPS) program provided IPC training to 4,250 HCWs from 259 health facilities across the country between March and December 2020. Capacity building activities included training on IPC knowledge and practices, how to practice IPC along with triage, and hands-on demonstrations on safety measures while treating COVID-19 patients in health care settings. In February 2021, MTaPS evaluated the effectiveness of its COVID-19 IPC capacity building efforts through a cross-sectional study using a mixed-method design. Ninety-seven doctors and 30 nurses from a random sample of trainees from 12 COVID-19 health facilities in Dhaka were surveyed on IPC knowledge and practices. Key informant interviews were also conducted with 6 doctors and nurses to help contextualize the survey findings. The findings showed that the IPC knowledge retention rate among the doctors and nurses was high; over 80% of the respondents correctly answered the IPC knowledge assessment survey questions. Key informants explained that the training improved their IPC knowledge and encouraged them to practice IPC. Nearly all study participants agreed on the relevance and necessity of the training program and its contribution to proper management of COVID-19 patients at health facilities. Doctors (50%) and nurses (60%) reported that they were able to use all their learnings from the IPC training in their practice during COVID-19 pandemic. The findings demonstrate that the training improved reported IPC knowledge and practices of the trainees. However, the findings also underscore the need to invest in more sustained IPC capacity building of HCWs, invest in behavioral change and functional IPC committees to ensure implementation of IPC activities at the facilities as IPC is a key component in effectively managing the COVID-19 pandemic and other public health emergencies.

0530

A CONTINUOUS, SCALABLE SEROSURVEILLANCE PLATFORM TO MONITOR EXPOSURE TO PATHOGENS AND IDENTIFY HEALTH DISPARITIES USING ELECTRONIC HEALTH RECORDS

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Serosurveillance provides a unique opportunity to quantify the proportion of the population that has been exposed to pathogens. However, "gold standard" (probabilistic) population-based serological surveys are often too expensive and time consuming to quickly and frequently implement in response to an emerging epidemic. We developed and piloted *Serosurveillance for Continuous, ActionabLe Epidemiologic Intelligence of Transmission* (SCALE-IT), a platform through which we systematically selected and tested remnant samples from routine blood draws in two major health networks in San Francisco for SARS-CoV-2 antibodies from March to June 2020 and during February 2021. Importantly, these remnant samples were linked to detailed electronic health records, enabling careful algorithmic selection based on demographic, geographic and clinical variables, improving their representativeness to the general population. Between March 28 and June 26 2020, we collected a total of 5,244 samples and calculated raw and adjusted seroprevalence estimates stratified by space, time, and socio-demographic indicators including age, health insurance status, gender, homelessness status and race/ethnicity. These data provide estimates of the overall population-weighted seroprevalence in San Francisco during the initial phase of the SARS-CoV-2 outbreak (4.2%, 95% Credible Interval: 2.1% - 6.3%) and highlight important heterogeneities in transmission by neighborhood, homelessness status, gender and race/ethnicity. We will also present updated results that incorporate additional sampling efforts, providing a picture of how seroprevalence and the health disparities we identified varied as the pandemic progressed. Serosurveillance efforts globally will be fundamental to monitor transmission rates of emerging infectious diseases, including SARS-CoV-2, and evaluate the impact of interventions. Due to the ease and low cost of implementation, this hybrid serosurveillance approach has strong potential for scaling its application beyond this local context and for diseases other than SARS-CoV-2.

0531

EPIDEMIOLOGY OF INFLUENZA IN GHANA: 2011-2019

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The earliest reports of influenza in Ghana date back to the 1918-1919 global pandemic. Influenza data from Africa was limited for multiple decades after this pandemic until an outbreak of highly pathogenic influenza A H5N1 among poultry in 2007, which led to the initiation of a national surveillance program. Here we present influenza surveillance data in Ghana from 2011-2019. Systematic data on influenza was collected at the population level to describe the epidemiology of influenza as an etiology for influenza like illness (ILI) and severe acute respiratory infection (SARI) in Ghana from 2011-2019 as part of the Integrated Disease Surveillance and Response (IDSR) system. The IDSR utilized modified WHO case definitions for ILI and SARI, including individuals with measured fever or history of fever with cough or sore throat, with symptom onset within the prior 10 days for outpatients (ILI) and inpatients (SARI). A maximum of five ILI and all SARI samples were analyzed weekly for seasonal influenza A and B lineages (A[H1], A[H3], A[H1] pdm, B Yamagata and B Victoria). A total of 25,176 samples were collected from patients who met the case definitions of ILI (21,747) and SARI (3,429). Of those meeting ILI criteria, 14% were positive for influenza with the peak incidence of 21% occurring in the 5-14-year-old group. The observations for both ILI and SARI surveillance show that in Ghana, influenza is most prevalent in children 5-14 years old. Based on global influenza data, extremes of age tend to be most affected resulting in a U-shaped incidence curve, but this pattern is not evident in the surveillance data. There are years where Ghana mirrors the global and/or West African case peaks and influenza lineages, but many years with significant variability. This unpredictability limits the generalizability of global and West African data in predicting influenza trends within Ghana, highlights the need for multiple surveillance sites, potentially has significant implications for vaccine strain selection and effectiveness, and impacts public health preparedness and military force health protection.

0532

A SYSTEMATIC LITERATURE REVIEW AND META-ANALYSIS OF THE SEROPREVALENCE AND SPATIAL DISTRIBUTION OF MEDICALLY IMPORTANT TICK-BORNE DISEASES IN EAST AFRICA (1901-2020)

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Tick-borne diseases (TBDs), including anaplasmosis, babesiosis, and East Coast fever, pose a significant threat to livestock production and both human and animal health in East Africa. Despite their importance, remarkably little is known on the seroprevalence and spatial distribution of TBDs in this region. This study represents the most comprehensive regional meta-analyses to date on the prevalence of TBDs impacting populations in Chad, Djibouti, Ethiopia, Kenya, and Uganda. A systematic literature review was conducted to identify peer-reviewed articles reporting tick collection findings, including pathogen screening results and TBD seroprevalence data. Over 6,000 manuscripts were screened, of which more than 200 contained high-quality occurrence data regarding TBD seroprevalence. Surveillance data were georeferenced and assigned a spatial resolution. These findings will be linked with additional datasets detailing the spatial distribution of tick vector species and the pathogens they carry. Using this approach, we can characterize the prevalence, geographic distribution, and temporal trends of TBD exposure among wildlife, livestock, and human populations across East Africa. The resultant dataset reflects a significant improvement to our regional knowledge of TBD prevalence and provides a valuable platform for future TBD risk assessment, prevention, and mitigation in this region. Such data further allows us to identify and address associated gaps in disease surveillance.

0533

DOCUMENTING TICK AND TICK-BORNE DISEASE DISTRIBUTIONS IN EAST AFRICA THROUGH ADVANCED SYSTEMATIC LITERATURE SEARCHES

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Ticks pose a significant risk to human health across the globe. To diminish these risks, high quality tick and tick-borne disease surveillance data is needed to develop accurate risk assessments and effective mitigation plans. However, despite the large quantity of tick research performed in East Africa, no standardized, comprehensive, review has been conducted for this region. Our goal was to produce a comprehensive review of historic tick and tick-borne disease studies conducted in five countries in East Africa—Chad, Djibouti, Ethiopia, Kenya, and Uganda. Eligible reports were those that contained mappable tick collection data such as detailed locality descriptions, GPS coordinates, and map-displayed data. To generate our review, we searched for original research articles, published in English, between 1901 and August 2020. We searched four databases (PubMed, Web of Science, Scopus, and CABI VetMed Resource) using 19 search terms related to ticks and tick-borne diseases in each library, with the corresponding MeSH terms used in PubMed. Over 6,000 articles were initially captured in our search, yet after scrutiny, nearly 300 met our eligibility criteria. Tick collection records gleaned from these articles were subsequently georeferenced using point-radius method to standardize the locality data, and then combined with historic collection records from the Walter Reed Biosystematics Unit (WRBU) VectorMap platform (vectormap.si.edu), which includes our current surveillance records. The result was a comprehensive, novel dataset comprised of high-quality collection events from over 1300 unique point locations representing over 100 tick species in the five countries. These data were used to map tick and tick-borne disease presence in these countries, providing regional oversight and detailed risk maps for tick-borne diseases in East Africa.

0534

TICKS AND TICK-BORNE DISEASE THREATS OF KENYA

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There are several documented tick-borne disease (TBD) risks in Kenya, impacting both humans and livestock. Despite the persistence of these threats, there are few data resources available for assessing TBD risk. A comprehensive understanding of the tick vector threats in this country is essential. Kenya-specific data from our ongoing surveillance and a systematic literature review of over 6000 tick and tick-borne disease research papers in East Africa were sourced and over 120 met our inclusion criteria for Kenya. This effort revealed over 70 tick species, spanning 8 genera, were reported from Kenya between 1901 and August 2020. Additionally, over 50 disease-causing parasitic, bacterial, and viral agents were detected across these species. *Coxiella burnetii* and *Rickettsia* spp. were some of the most frequently detected agents. Preliminary results indicate vast distribution of several tick species such as *Amblyomma variegatum* (Fabricius, 1794), *Rhipicephalus decoloratus* Koch, 1844, *R.*

evertsi Neumann, 1897, and *R. appendiculatus* Neumann, 1901. Further investigation of this rich dataset will contribute to a more complete understanding of the tick vector threats of Kenya and will drive future tick surveillance and tick-borne disease mitigation efforts.

0535

FIRST RECORD OF THE GENUS CULICOIDES (DIPTERA: CERATOPOGONIDAE) IN CHIHUAHUA, NORTHWEST MEXICO: A STUDY OF DNA BARCODE, HUMAN-LANDING RATE, ARBOVIRUS DETECTION, AND PUBLIC HEALTH IMPORTANCE OF CULICOIDES FURENS

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The genus Culicoides (Fam. Ceratopogonidae), commonly named biting midges, are the smallest hematophagous dipterans with public health importance worldwide. Culicoides transmit multiple pathogens to humans and animals, but as vectors of arboviruses, they achieve their principal importance; more than 50 viruses have been isolated from Culicoides species. From the almost 1400 morphospecies identified, Mexico has 84 morphospecies. However, some regions of Mexico are still scantily studied. This is the case of Chihuahua, located in Northwest Mexico, where does not have any record for Culicoides species. In the present study, DNA barcode, bionomic parameters, and arboviral diagnosis of Culicoides from San Buenaventura, Chihuahua, Mexico were estimated. Here, collections of Culicoides were carried out from April through September 2020 in San Buenaventura, Chihuahua, by using the human-landing-collection (HLC) method, CDC, and mosquito magnet traps. Morphological keys and corroborated by DNA barcoding were performed to Culicoides identification. The daily pattern of total landing activity of Culicoides was estimated. Also, pools of Culicoides were achieved for the screening of viruses by metagenomic analyses. In this research, *Culicoides furens* was identified as unique species. DNA barcode reveals a possible complex compared with those in DNA databases. Host-seeking females of *C. furens* showed a unimodal human-landing activity pattern between 4-8 pm. The human daily landing rate was directly proportional to temperature and solar radiation, but there was a significant negative association with wind speed. Overall, a daily landing rate (DLR) of 88 lands/person/day was estimated. Several sequences of possible arbovirus associated with *C. furens* were detected. We conclude that *culicoides furens* is the first species reported in Chihuahua, northwest Mexico. Also, this is the first study of metagenomics analyses in Culicoides in Mexico. The data offer insights into the ecology of *C. furens*, a potential vector of arbovirus in Mexico.

0536

SPOTLIGHT REPORT: TICK VECTOR THREATS OF DJIBOUTI

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VectorMap is the largest online repository for global arthropod disease vector distribution data. In an effort to improve tick surveillance coverage in East Africa, a systematic literature review was conducted, targeting publications from 1901-2020. This study supports an ongoing regional effort to better characterize the distribution of ticks and the pathogens they carry in Djibouti. Scientific literature was compiled from PubMed, Scopus, WOS, and CABI search engines. Over 6,000 manuscripts were screened for relevance according to title and abstract, of which 7 met inclusion criteria. Information captured included tick species, collection locality, collection method and pathogen detections. Thirty-two species/subspecies of ticks from 5 different genera were reported in Djibouti. Pathogens detected include *Rickettsia africae* and Crimean-Congo hemorrhagic fever virus. Most ticks reported were removed from animals and in many instances, these animals had migrated into Djibouti from neighboring countries. Considering this trend, and the role Hajj plays in human and animal movement through this region, enhanced surveillance is recommended for early detection and monitoring of select agents. Findings from this study highlight major gaps in our understanding of tick-borne diseases within Djibouti that warrants further investigation.

0537

GENETIC DIVERSITY AND POPULATION STRUCTURE OF PHLEBOTOMUS ARGENTIPES, THE VECTOR OF LEISHMANIASIS IN SRI LANKA

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Vector of leishmaniasis in Sri Lanka is *Phlebotomus argentipes* which transmit *Leishmania donovani* and the disease is a growing health problem. A proper understanding of the population genetic structure of sand fly vectors is considered important prior to planning and implementation of a successful vector control program. Thus, the present study was conducted to determine the population genetic structure of sand fly vectors in Sri Lanka. Two mitochondrial genes viz. *Cytochrome c oxidase subunit 1 (CO1)* and *Cytochrome b oxidase (Cytb)*, and the internal transcribed spacer 2 (ITS2) region from the nuclear ribosomal DNA were used for molecular characterization. Analysis was done using maximum likelihood method, Network analysis and DNA polymorphisms. The analysis revealed unique sequences of all genomic regions studied except the *CO1* region in 21 flies that aligned with those from Kerala, India and *Cytb* region of 4 flies that aligned with those isolated earlier from Sri Lanka and 3 from Madagascar. *CO1* and ITS 2 region analyses revealed gene flow between the study sites whereas *Cytb* gene region analysis indicated genetically distinct populations of *P. argentipes* in each of the study sites. Populations of *P. argentipes* in Sri Lanka are in most part distinct when compared to sand flies from elsewhere. Gene flow with lack of population differentiation of *P. argentipes,* even between geographically distant sites is likely to pose future challenges for leishmaniasis control due to the likelihood of gene transfer between locations. Such occurrences affecting functionally important genes such as those which confer insecticide resistance can facilitate the spread, of insecticide resistance across the country, which in turn will make vector control difficult.

0538

QUANTUM CASCADE LASERS FOR MOSQUITO SURVEILLANCE

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Fourier Transform Infrared (FTIR) spectroscopy has shown a great potential as a tool for mosquito surveillance. Mid-Infrared spectroscopy coupled with machine learning analysis allow us to identify mosquito age and species. However, current FTIR technology has various limitations: very expensive and bulky equipment, low signal-to-noise ratio and low speed. These limitations make them unsuitable for on-site measurements in remotes areas where surveillance is needed. New technologies in the field of semiconductor lasers have emerged in the last decade with the potential of overcome these limitations. Quantum Cascade lasers (QCLs) can emit infrared radiation with high power over a broad spectral range with a size of no more than a few millimetres. QCLs have been applied mainly in the development of portable system for biomedical diagnosis and monitoring, gas sensing and analysis of pharmaceutical formulations.

Here we present a portable system using QCLs for mid-IR spectroscopy measurements in mosquitoes. Our set up consists of a single QCL 3 mm in size chip mounted in an external cavity. The laser chip can be tuned using a scanning galvanometer from 950 to 1150 cm^{-1}. This set up can scan samples with speeds up to 125 Hz (~100 scans per second). Our set up successfully measure mid-infrared spectra from mosquitoes in KBr pellets. Currently, we are working on non-destructive methods to measure mid-IR spectra on other mosquito tissues. Our results highlight the potential of QCLs for a cheaper, compact option for current tools for spectroscopy. Moreover, this technology can be expanded to other areas such as malaria diagnosis.

0539

CULTURE-DEPENDENT AND -INDEPENDENT METAGENOMIC ASSEMBLIES FROM MALAYSIAN CTENOCEPHALIDES SPP. FLEAS UNCOVER NEW COMPLETE GENOMES FOR BARTONELLA CLARRIDGEIAE, RICKETTSIA ASEMBONENSIS AND MULTIPLE CLADES OF WOLBACHIA

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Ctenocephalides spp. fleas are recognised as globally distributed vectors of human diseases including cat-scratch disease (bartonellosis), dipylidiasis, and several rickettsioses, such as murine typhus (*Rickettsia typhi*) and flea-borne spotted fever (*Rickettsia felis*). They may also harbour other microorganisms, including arthropod-specific bacterial symbionts, which remain poorly characterised. Scant information exists on the geographical variation of pathogen and symbiont carriage in *Ctenocephalides* spp. fleas, especially outside Western countries, and the risk these vectors pose for disease transmission in the tropics. In this study, fleas were collected from cats and dogs in an Orang Asli (aboriginal) community in Peninsular Malaysia. Cat flea tissues were cultured with IDE8 tick cells to isolate bacterial symbionts and positive cultures were sequenced on the Oxford Nanopore MinION platform. Fleas from dogs (*Ctenocephalides orientis*) were subjected to culture-independent metagenomics on the same sequencing platform. Two *Wolbachia* strains from cat fleas were successfully isolated in IDE8 cells and genomic analysis demonstrated these constituted a novel supergroup F symbiont closely related to *w*Cle from bedbugs, and a strain highly similar to *w*CfeJ, recently sequenced from *C. felis* in the USA. From *C. orientis*, complete genomes were obtained for *Bartonella clarridgeiae*, *Rickettsia asembonensis*, and a third *Wolbachia* strain that resembled *w*CfeT from North American cat fleas. The *B. clarridgeiae* genome was highly similar to Western cat-scratch disease isolates except for polymorphisms in flagellin genes. For *R. asembonensis*, which has been previously associated with a human clinical case in Malaysia, our assembly represents the first circularised genome obtained worldwide and was accompanied by a distinct plasmid with low similarity to the published sequence for this species. Taken together, our data highlight the complexity and knowledge gaps pertaining to *Wolbachia* symbionts in *Ctenocephalides* spp. and the potential for these vectors to transmit bacterial pathogens in aboriginal communities.

0540

ALERTACHIRIMACHA: SHIFTING COMMUNIY-BASED VECTOR SURVEILLANCE TO SOCIAL MEDIA DURING COVID-19

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COVID-19 pandemics have brought new challenges for the surveillance and control of many other infectious diseases. The social distancing measures and the social immobilization imposed by the national government to avoid the transmission of SARS-CoV-2 virus in Arequipa-Peru have greatly affected the entomological surveillance of kissing bugs, the vectors of the etiological agent of Chagas disease: *Trypanosoma cruzi*. As a way to maintain surveillance activities, we created "AlertaChirimacha" a new system of communication and reporting that educes the contact between the community and vector control staff. We have used facebook advertising to promote this new reporting system via phone calls and WhatsApp messages. If an infested house is detected, we delimit a risk area around it and distribute fliers requesting householders to check their homes for the presence of triatomines and report it through the phone line. After the two paid posts and promotion around an infested dwelling, we have received 82 reports of insects that people considered to be kissing bugs, two of which resulted in the uncovering of 2 new infestation foci--a substantial finding in a city where the insects are very near elimination Compared to the 7 months prior to the launch of "AlertaChirimacha" the number of reports had increased by 575%. Reports received came from all across the city. These preliminary results demonstrate the possibility of using social media to keep disease surveillance active in situations where physical contact must be reduced.

0541

RESULTS: SYSTEMATIC LITERATURE SEARCH TARGETING TICK AND TICK-BORNE DISEASES IN FIVE COUNTRIES IN EAST AFRICA

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As part of an ongoing Armed Forces Health Surveillance Division-Global Emerging Infections Surveillance (AFHSB-GEIS) regional East Africa project, a large systematic review of ticks and their associated pathogens in five countries (Chad, Djibouti, Ethiopia, Kenya, and Uganda) was undertaken to augment current field data and recover maximal verified data points for mapping and risk modelling purposes. Over 6,000 peer-reviewed articles were screened for reports of tick and tick-borne pathogen surveillance published between 1901 and 2020. Articles containing surveillance data were mined and collection data geo-referenced and combined into a single database. The findings from the database are presented in a large matrix of identified tick species and pathogens for the five countries of interest. While over one hundred articles each were sourced for Ethiopian and Kenyan, few articles were available for tick studies in Chad, Djibouti and Uganda, highlighting the need for increased surveillance efforts in these under sampled countries. With data now readily available on vector - pathogen associations and their geographic occurrences, highly specific tick-borne disease maps and models can be created for a variety of study types using a single database. The longitudinal data accumulated in this study provides a digital, mappable, and relatable database of tick collections across 5 neighboring countries in East Africa that can immediately inform tick-borne disease risks and surveillance priorities at both country and regional levels.

0542

THE FIRST PROSPECTION FOR VECTOR BREEDING SITES OF ONCHOCERCIASIS IN LIBERIA: IDENTIFYING PRODUCTIVE SITES OF SIMULIUM DAMNOSUM S.L. SPECIES IN 2018

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Onchocerciasis has been earmarked as one of the five neglected tropical diseases (Onchocerciasis, Lymphatic Filariasis, Trachoma, Schistosomiasis and STH) amenable to preventive chemotherapy targeted for elimination. Unlike the other diseases where the target is elimination as a public health problem, onchocerciasis has a goal to eliminate transmission. Towards this goal, the World Health Organization (WHO) has come out with a verification of onchocerciasis elimination guideline. The guideline places emphasis for verifying the elimination of onchocerciasis on transmission assessment (Entomology), infection prevalence (Epidemiology) and transmission zone. Liberia begun treatment for onchocerciasis in 2000, however, due to the civil unrest (2001-2003) and the Ebola outbreak (2014-2015), the program experience interruption in the treatment round. After 12 rounds of Mass Drug Administration (MDA), the country along with experts decided to identified vector productive breeding sites, vector *Simulium* species, first line communities and the development of a breeding sites map for Liberia. The survey was completed in 10 counties constituting two regions (northwest & southwest) showing possible transmission zones in Liberia. The research was undertaken in the dry season when most rivers especially the smaller ones were dry while larvae collected were processed involving an initial sorting of the samples into members of the *S. damnosum* complex. During the research, 17 breeding sites were identified, 4 different species seen, 204 samples (larvae) collected and the percent of forest cytospecies predominate with only a few sites having savannah species. Simulium yahense was observed to be the most widespread species in Liberia. The research results have generated valuable data to support the onchocerciasis control and elimination programme in Liberia. It shows the classification of species found in Liberia and have guided the program to the next step of conducting impact assessment for communities' dwellers living within 5km of a productive breeding site.

0543

EVALUATING THE EFFECTS OF BREEDING WATER BACTERIA ON THE LIFE HISTORY OF MALE AEDES AEGYPTI MOSQUITOES

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The yellow fever mosquito *Aedes aegypti* presents a threat to human health around the globe, making the development of sustainable and effective methods of mosquito population control necessary. One such strategy is the sterile insect technique (SIT), in which sterile males are released into the environment to compete for mates, ultimately reducing the population. Boosting the fitness of these sterile males would improve the efficacy of SIT strategies. Our work seeks to evaluate the role that different larval microbes play in the development and life history of male mosquitoes. During development, *Ae. aegypti* larvae regularly ingest bacteria and bacteria from breeding water are also found in adult mosquitoes. These bacteria support development, provide nutritional benefits, and impact female mosquito physiology in many ways. We hypothesize that bacteria species will differ in the effects they have on larval and adult physiology, leading to differences in overall male fitness. We are rearing larvae in the presence of a single bacterial species. From here, we are measuring the development time, adult longevity, and wing length (as a proxy for body size) of single individuals. The effects of larval bacteria on male longevity and body size will be discussed, as these data may be used to identify specific bacteria species that could enhance the current mass rearing practices employed for SIT. These data will also be compared to data collected from female individuals resulting from the same treatments to investigate the possibility of sex-specific effects of larval bacterial presence.

0544

VECTORS TAKING FLIGHT; THE INFLUENCE OF INVASIVE TICK SPECIES, BIRD MIGRATION AND CLIMATE CHANGE ON SOUTH CAROLINA'S COASTAL ZONES

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Climate change is having a profound impact globally, including changes in migratory bird patterns. Along the eastern North American coastline, a substantial number of migratory birds have expanded their ranges to adapt to more southern latitudes, increasing their exposure to novel tick vectors in tropical regions. Further, certain species have created new geographic routes altogether, allowing for a second pathway of potential exotic tick introduction into new environments. Recent state-wide tick surveillance in collaboration between the University of South Carolina and the South Carolina Department of Health and Environmental Control has identified invasive and exotic avian-feeding tick species within the state. This presentation will review the public health importance of *Haemaphysalis longicornis*, *Haemaphysalis leporispalustris*, *Ixodes scapularis, Ixodes brunneus*, and *Ixodes affinis H. longicornis* is a recently invasive species, and the others are native but have been documented as moving into new ranges. These four tick species were recently identified in South Carolina, and all four are known causes of clinical or veterinary disease and can be geographically displaced by migratory birds. Understanding the relationship between these tick vectors and changing migratory bird patterns due to climate change can serve to inform vector surveillance and public health officials on possible new trends in vector-borne disease spread.

0545

GRADIENT OF ONCHOCERCIASIS ENTOMOLOGIC INDICES FROM RIVERSIDES TO THE CENTRE OF TWO FIRST LINE COMMUNITIES IN THE MBAM VALLEY: IMPLICATION FOR OPTIMAL VECTOR CONTROL USING TRAPS

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The reduction of human-blackfly contact can interrupt onchocerciasis transmission. Esperanza window trap (EWT) has been shown to be effective in reducing blackfly densities. Several shape-based improvements to this trap have been made to optimize its performance. However, its placement within a community generally only meets the criteria of high-density areas and the presence of humans without taking into account blackfly transmission potentials (MTP and ATP, monthly and annual transmission potentials) and parity rates (PR). We investigated entomological indices at different points within the communities for optimal use of the EWT. Monthly blackfly collection was carried out for one year using human landing method at three catch points along a transect from riverside toward the center of two first-line communities (Biatsota and Bayomen) in Cameroon. Collected blackflies were counted and dissected, and entomologic indicators (MBR and ABR, monthly and annual biting rates, PR, MTP and ATP) were computed and compared using Kruskal Wallis test. A total of 80,732 blackflies (37,242 at Biatsota and 43,490 at Bayomen) were caught, of which 57,517 (71.2%) were

dissected (27,508 at Biatsota and 30,008 at Bayomen). A total of 2,743 (4.8%) were parous and 44 (1.6%) were infective, with a total of 51 infective larvae (Li) in the head. Regarding the distance to the river, we observed a decreasing vector density gradient, with the highest ABRs recorded at the two riversides (489,009 bites/human/year). The highest ATPs were also recorded at the two riversides (420 Li/man/year). The comparison of MBRs between the three catching points in each village revealed a significant difference (p: 0.0001 and 0.001 for Bayomen and Biatsota respectively). Globally, the highest PR was recorded at the riverside in Biatsota (5.1%) where various human activities take place all day long, while in Bayomen it was recorded in the centre of community (6.36%). This study showed that entomologic parameters where highest at riversides and indicates that EWT or other traps should be set up in priority on the riverside for optimal performance in onchocerciasis control.

0546

FIRST REPORT OF N1575Y MUTATION IN PYRETHROID RESISTANT ANOPHELES GAMBIAE S.L. IN NIGERIA

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In view of recent findings that codon 1575 mutation increases the L1014F-mediated resistance in *Anopheles* mosquitoes, we investigated the presence of this mutation in four States with intense pyrethroid resistance in Nigeria. Permethrin and deltamethrin susceptibility tests were carried out using 2-3 day old adult female *Anopheles* from larval collections. Based on the susceptibility test results, intensity tests with 5x and 10x concentrations were carried out at sites with high resistance (% mortality>85). Resistant specimens were identified by PCR and screened for the presence of L1014F and N1575Y mutations using allele specific PCR and Taqman assay respectively. The frequency of *kdr* mutation for permethrin varied from 0.71-0.75 for 5x and 0.73-0.75 for 10x. The kdr frequency for deltamethrin was not significantly different (P=0.157): 0.54-0.60 for 5x and 0.66-0.71 for 10x. Percentage increase in *kdr* frequency between 1x and 5x permethrin doses ranges from 1.4% to 11.9% in all sites except Edo and Anambra; while only Lagos had percentage increase of 1.4% between 5x and 10x. For deltamethrin, Lagos and Niger had the same (3.8%) percentage increase in *kdr* frequency between 1x and 5x, while 5x and 10x gave 23.6% and 31.5% respectively. Resistant *Anopheles gambiae* at 5x and 10x concentrations of permethrin showed the presence of homozygous (Y) and heterozygous (NY) mutant allele in two sites. From this study, the mutation has no significant association with resistance intensity ($\chi 2$=0.8, P=0.3711). There is need for frequent monitoring to forestall the effect this may have on national malaria control programs.

0547

PRE-INTERVENTION CHARACTERISTICS OF THE MOSQUITO SPECIES IN BENIN IN PREPARATION FOR A CLUSTER RANDOMIZED CONTROLLED TRIAL ASSESSING THE EFFICACY OF DUAL ACTIVE-INGREDIENT LONG-LASTING INSECTICIDAL NETS FOR CONTROLLING INSECTICIDE-RESISTANT MALARIA VECTORS

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This study provides detailed characteristics of vector populations in preparation for a cluster randomized controlled trial aiming to compare the impact of dual active-ingredient long-lasting insecticidal nets (LLINs) that combine two novel insecticide classes - chlorfenapyr or pyriproxyfen - with alpha-cypermethrin to improve the prevention of malaria transmitted by insecticide-resistant vectors compared to standard pyrethroid LLINs. The study was carried out in 60 villages across three districts, south Benin. Mosquito collections were performed using human landing catches. After morphological identification, a sub-sample of *Anopheles gambiae* s.l. were, analyzed by PCR for species and presence of L1014F *kdr* mutation and by ELISA to identify *Plasmodium falciparum* sporozoite infection. WHO susceptibility tube tests were performed by exposing adult *An. gambiae* s.l., collected as larvae from each district, to 0.05% alpha-cypermethrin, 0.75% permethrin, 0.1% bendiocarb and 0.25% pirimiphos-methyl. Synergist assays were conducted with exposure first to 4% piperonyl butoxide followed by alpha-cypermethrin. Overall, *An. gambiae* s.l. was the main malaria vector complex. It was comprised of *An. coluzzii* (53.9%) and *An. gambiae* s.s. (46.1%), both displaying a frequency of the L1014F *kdr* mutation >80%. Human biting rate in *An. gambiae* s.l. was higher indoors [26.5 bite/person/night (95% CI: 25.2-27.9)] than outdoors [18.5 b/p/n (95% CI: 17.4-19.6)], as were the trends for sporozoite rate [2.9% (95% CI: 1.7-4.8) vs 1.8% (95% CI: 0.6-3.8)] and entomological inoculation rate [21.6 infected bites/person/month (95% CI: 20.4 - 22.8) vs 5.4 (95% CI: 4.8-6.0)]. *An. gambiae* s.l. was resistant to alpha-cypermethrin and permethrin but, fully susceptible to bendiocarb and pirimiphos-methyl. Synergist assays induced a higher 24 hour mortality compared to alpha-cypermethrin alone. The intense malaria transmission and high pyrethroid resistance with involvement of multiple mechanisms (L1014F *kdr* and mixed function oxidases) suggest that novel insecticide classes are required to tackle issues of pyrethroid resistance in the study area.

0548

PERSONAL PROTECTION WITH PBO-PYRETHROID SYNERGIST TREATED NETS AFTER TWO YEARS OF HOUSEHOLD USE AGAINST PYRETHROID-RESISTANT ANOPHELES IN TANZANIA

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Synergist nets combine pyrethroid and piperonyl-butoxide (PBO) to enhance potency against resistance mediated by mono-oxygenase mechanisms. We assessed personal protection of Olyset Plus versus standard Olyset net against pyrethroid resistant malaria vectors in North West Tanzania after 20 months of household use. From household survey, 39 standard Olyset net and 39 Olyset Plus houses were selected, physical integrity and hole index (HI) of nets assessed, resting mosquitoes collected from inside nets and room walls, indoor abundance estimated using CDC light traps and species identified using PCR. Bioefficacy of PBO and standard LLINs against wild *Anopheles* was assessed using 30 minutes cylinder bioassays. Of 2397 *Anopheles* collected, 8.9% (n=213) were resting inside standard Olyset nets while none were found inside Olyset Plus of any HI category. Resting density of blood-fed mosquitoes was higher on walls of sleeping rooms with Olyset net compared to Olyset Plus (0.62 vs 0.10, density ratio: 0.03, 95% CI: 0.01-0.13, p<0.001). Mosquitoes were found inside Olyset nets of all WHO HI categories, but more were collected inside the more damaged nets (HI≥ 643) than in less damaged (HI 0-64) nets (DR: 6.4, 95% CI: 1.1-36.0, p=0.037). In bioassay, mortality of *An. gambiae* s.l. was higher with Olyset Plus than with Olyset net for new nets (76.8% vs 27.5%) and 20 months' nets (56.8% vs 12.8%); similar trends were observed with *An. funestus*. The Olyset Plus provided improved protection after 20 months of household use irrespective of hole index category, as compared to Olyset nets.

0549

RESISTANCE TO PYRETHROID INSECTICIDES IN ANOPHELES GAMBIAE S.S AND AN. ARABIENSIS IN UGANDA

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The effectiveness of malaria control interventions is threatened by widespread insecticide resistance. The World Health Organization (WHO) advocates resistance management through use of combinations of insecticides. In Uganda, long lasting insecticidal nets (LLINs) with pyrethroids have been distributed nationwide every 3-4 years, and indoor residual spraying (IRS) with bendiocarb or pirimiphos-methyl implemented in selected districts. We assessed if different vector control combinations were associated with resistance patterns in *An. gambiae s.s* and *Anopheles arabiensis* raised from larvae collected from 11 districts in Uganda, including those with no IRS history (3 districts), where IRS was conducted in 2014-17 then discontinued (4 districts), and where IRS has been sustained since 2014 (4 districts). All sites received two rounds of LLINs (2013-14 and 2017). Susceptibility to permethrin and deltamethrin was assessed using the standard WHO tube test; analyses were stratified by IRS status. Overall, 1971 Anopheles mosquitoes were assessed; 773 *An. gambiae* s.s. (240 No IRS, 525 IRS stopped, 8 IRS active) and 1198 *An. arabiensis* (249 No IRS, 254 IRS stopped, 695 IRS active). Mortality to pyrethroids was significantly lower for *An. gambiae* s.s. than *An. arabiensis* in sites with no history of IRS (permethrin 16.3% vs 69.0%, p<0.0001; deltamethrin 23.1% vs 83.8%, p<0.0001), and in sites where IRS was discontinued (permethrin 11.1% vs 69.4%, p<0.0001; deltamethrin 24.9% vs 88.6%, p<0.0001). In IRS active sites, nearly all Anopheles were *An. arabiensis* (98.9%); mortality to permethrin and deltamethrin was 63.4% and 88.6%, respectively. Comparing mortality for *An. gambiae* s.s. across different IRS site categories, no statistically significant differences were observed for permethrin or deltamethrin. The same was true for *An. arabiensis*. Overall, resistance to pyrethroids was widespread, particularly in *An. gambiae* s.s. In sites with active IRS, *An. arabiensis* predominated. There was no evidence that resistance patterns varied with different vector control combinations once data were stratified by species, but larger studies are needed.

0550

INTERACTION BETWEEN INSECTICIDE RESISTANCE ASSOCIATED MARKERS AND MALARIA TRANSMISSION PATTERNS IN ANOPHELES GAMBIAE S. L. POPULATION AROUND BOUAKÉ, CÔTE D'IVOIRE.

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There is evidence that the Kdr L1014F and G119S Ace-1^R mutations involved in pyrethroids and carbamates resistance in *Anopheles gambiae* in sub Saharan Africa influence malaria transmission. Such influence is likely due to changes in behavior, life history, vectorial competence and vectorial capacity. In the present study, we investigated the distribution of insecticide target site mutations and their association with the infection status in wild *Anopheles gambiae* s.l populations.Mosquitoes were captured indoor and outdoor in 40 villages around Bouaké by human landing catches (HLC), from May 2017 to April 2019. Sample of 1,392 mosquitoes collected (686 infected with *Plasmodium* sp. And 706 uninfected randomly selected) were identified to species and then genotyped for the L1014F Kdr and G119S Ace-1^R mutations using quantitative polymerase chain reaction (qPCR) assays. The frequencies of the two alleles were compared between *An. coluzzii* and *An. gambiae* and then between infected and uninfected groups for each species. The presence of *An. gambiae* (49 %) and *An. coluzzii* (51 %) was confirmed in Bouaké. There was significant difference in the allelic and genotypic frequencies of Kdr L1014F and Ace-1^R G119S mutations between *An.gambiae* and *An. coluzzii* (p<0.05). The probability of the Kdr L1014F resistance allele (R) to occur in *An. gambiae* was 56 times higher than in *An. coluzzii* population. The two mutations showed no significant association with *Plasmodium* infection status in wild population. Further studies should consider more complex interactions between resistance and other aspects of the mosquito biology to assess the impact of insecticide resistance markers on malaria transmission in wild population of *An. gambiae* s.l targeted by vector control interventions.

0551

"TRICK OR TREAT": DEVELOPMENT OF A FIELD-FRIENDLY IVERMECTIN BIO-EFFICACY ASSAY BY EXPLOITING SWITCHING MECHANISMS IN MOSQUITO FEEDING

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Ivermectin is currently being considered as a novel malaria vector control tool due to its endectocidal properties. Several trials are ongoing to evaluate the efficacy of ivermectin mass drug administration (MDA) at reducing malaria transmission. Malaria transmission is expected to be disrupted through reduction in mosquito survival and fitness post feeding on ivermectin-treated hosts. Monitoring of ivermectin bio-efficacy is necessary to evaluate the effect of the intervention on local vector populations. Endectocides are taken up in blood meals, thus requiring wild-caught mosquitoes to either feed directly on a treated host's skin, raising ethical issues; or feed on blood through a membrane which is unfeasible due to low feeding rates. In this experiment, ivermectin bio-efficacy was compared when administered in glucose solution using soaked filter paper; spiked blood fed through a membrane; and in glucose solution laced with blood soaked in filter paper. Insectary-reared *Anopheles gambiae* (Kilifi strain) were exposed to five ivermectin concentrations in the different solution types: 85 ng/ml, 64 ng/ml, 43 ng/ml, 21 ng/ml, 11 ng/ml and control (0 ng/ml). Mosquito survival was monitored for 28 days and three replicates were carried out per treatment and solution type. Survival rates were presented using Kaplan-Meier curves and compared across groups using Log-rank tests. It was observed that when ivermectin was ingested in a sugar meal, survival rates were significantly different to when ingested in blood, suggesting that the digestion process plays a determinant role in ivermectin's bio-efficacy. By adding blood to the sugar solution mortality rates resembled those observed in spiked blood, suggesting that the presence of blood in the sugar solution may have "tricked" the mosquito to divert the meal to the midgut rather than the diverticula through a "switching mechanism". This study presents the development of a field-friendly assay to monitor ivermectin bio-efficacy and will inform programmes on how to best monitor the effect of ivermectin MDA on malaria vectors in the field.

0552

SEASONAL ABUNDANCE AND INSECTICIDE RESISTANCE STATUS OF AEDES MOSQUITOES IN GHANA

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Vector control is the main intervention for arboviral diseases transmitted by *Aedes* mosquitoes, because for most there are no effective vaccines or treatment. Vector control relies heavily on the use of insecticides, effectiveness of which may be impacted by resistance in the vector. This study investigated the spatio-temporal distribution and insecticide resistance status of *Ae. aegypti* in seven study sites (Ada Foah, Tema, Accra, Konongo, Larabanga, Navrongo and Paga) from across ecological extremes of Ghana. Indoor and outdoor adult sampling were done using BG traps, human landing catch, and prokopack aspirator during the dry and rainy seasons of 2017/2018. Distributions of immatures and adult *Aedes* mosquitoes were determined indoors and outdoors during dry and rainy seasons at all sites. Phenotypic resistance status of *Aedes* mosquitoes to insecticides was determined using WHO bioassays. A total of 16,711 *Aedes* immatures were sampled from car tires (73.9%), discarded containers (18.8%), air-condition saucers (4.4%), buckets (1.4%), tanks (1.3%) and drums (0.3%). There were more positive habitats during the rainy season 50 (61.73%) compared to the dry season 31 (38.27 %) (df = 5; χ^2 = 19.4435; p = 0.001). A total of 1,895 adult *Aedes* mosquitos were collected consisting of *Ae. aegypti* (97.8%), *Ae. africanus* (2.1%) and *Ae. luteocephalus* (0.1%). Adult *Aedes* mosquitoes were more abundant during the rainy season 1,257 (66.3%) compared to the dry season 638 (33.7%) (z = -1.433; p = 0.1519). *Aedes aegypti* populations were resistant to DDT at all study sites (0-88%). Vectors showed suspected resistance to bendiocarb (96-97%), permethrin (90-96%) and deltamethrin (91-96%) and were susceptible to the organophosphate malathion at all study sites. About 90% of vectors had taken a blood meal from humans. *Aedes* mosquitoes were found at high densities in all ecological zones of Ghana. Resistance to pyrethroids and carbamates may limit the efficacy of current vector control tools and requires constant monitoring.

0553

MAKING THE CASE FOR METHOD VALIDATION IN VECTOR CONTROL

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Next generation insecticide treated nets (ITN) are vital to malaria control. Multiple ITNs with differing product specifications are now PQ listed, and many are being evaluated in RCTs and pilot deployment schemes. However, the introduction of new AIs and product classes is exacerbating the methodological shortcomings that already exist in evaluation of vector control tools. Several products that are currently undergoing evaluation rely on entomological effects that differ from the pyrethroids' fast acting knockdown. There is little consensus on the methods used to monitor performance of these entomological effects, and although a lot of data is being generated, a lack of validated methodologies to provide comparable data could delay evaluation of these tools. Using durability monitoring methods for next generation nets as a worked example, we demonstrate a pipeline used to collate and interrogate current methods to produce a 'consensus SOP', and how data will be used to validate the method before it is made available for use. Overall, a renewed focus on developing, updating or validating testing methodologies is needed to efficiently evaluate novel tools, streamline access and monitor performance.

0554

ANALYSIS OF METABOLIC RESISTANCE AGAINST INSECTICIDES IN CULEX QUINQUEFASCIATUS IN HOUSTON, TEXAS

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Resistance against insecticides is widely reported in mosquitoes around the world, including in the West Nile vector Culex quinquefasciatus. In Houston, Texas, this mosquito is subjected to extensive insecticide-based control by the Harris County Public Health Department. In order to assess to what extent Culex quinquefasciatus has developed metabolic resistance against insecticides we conducted a gene expression analyses of detoxification gene families between susceptible and resistant mosquitoes. Culex quinquefasciatus were sampled from Harris County in 2019. Mosquitoes were classified into 'malathion resistant' and 'malathion susceptible' groups based on a standard WHO bottle assay. RNAseq data was obtained for five pools of 10 mosquitoes for each group. In addition, RNAseq data was obtained for five pools of 10 field collected C. quinquefaciatus and susceptible Sebring colony samples. Differential gene expression in EdgeR found1,339 differential expressed genes in comparison between insecticide resistant and susceptible groups, and 1,915 differential expressed genes in comparison between wild and susceptible groups in Culex mosquito. Within these, we identified 16 candidates from the three gene families (P450, GST and esterase) known to be associated with metabolic resistance. Several of these candidate genes were implicated in insecticide resistance previously. For others we will validate their role in metabolic resistance using RNAi. This work will assist in planning future vector control work.

0555

YEAST RNAI-BASED ATTRACTIVE TARGETED SUGAR BAITS FOR CONTROL OF ANOPHELES MOSQUITOES

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The development of transformative, environmentally friendly, cost-effective new mosquito control tools is critical for preventing the spread of malaria parasites. Attractive targeted sugar baits (ATSBs), a new paradigm for vector mosquito control, exploit the sugar feeding behavior of female and male mosquitoes that are lured to feed on a sugar source containing an insecticide. The mosquito-specific interfering RNA pesticides (IRPs) identified by our investigative team are a novel class of insecticides that can overcome rising incidences in pesticide resistance, significantly enhance existing ATSB technology, and reduce outdoor residual transmission. Recent high-throughput screens in the Scheel laboratory identified hundreds of IRPs, a subset of which have 25 bp target sites that are conserved in many species of disease vector mosquitoes, but that are not found in humans or other non-target organisms. We are developing an ATSB target product with IRP(s) as the mosquitocidal agent. To this end, we have generated yeast strains which can be used for economical IRP production and are developing yeast formulations that can be delivered to mosquitoes in sugar baits. Laboratory trials confirmed the activity of five different yeast IRPs in sucrose-based ATSB trials conducted on *Anopheles gambiae* females. Target gene silencing and a mode of action was confirmed for each of the five IRPs in the adult *A. gambiae* brain. Although the yeast-based ATSBs were toxic to *A. gambiae*, the insecticides, which lack known target sites outside of mosquitoes, were not found to be toxic in assays conducted on select non-target arthropods. The results of these studies suggest that yeast IRP-ATSBs could one day be a beneficial addition to integrated mosquito control programs. Future studies will include evaluation of these broad-based mosquito adulticides in additional *Anopheles* species, outdoor semi-field trials, efforts to scale yeast production, and development of commercial formulations.

0556

FIGHT THE BITE: APPLYING REMOTE SENSING TECHNOLOGIES TO DETECT MOSQUITO BREEDING HABITATS OF IMPORTANCE

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Mosquitoes are the world's most deadly animal. The arboviruses they transmit result in an estimated 300 million infections and 500,000 deaths annually. With few effective therapeutics or preventative vaccine available, public health officials rely on targeted mosquito abatement efforts to reduce the burden of disease. Effective insecticides have been developed to reduce the mosquito population and therefore reduce disease transmission. However, widespread insecticide application is inhibited by limited budgets for mosquito control and the fear of inducing insecticide resistance in the mosquito population. Targeted mosquito abatement efforts are critical to effectively reducing the burden of disease in affected areas. Our current collaborative project built a predictive geospatial model to detect mosquito breeding habitats in Harris County, Texas. Our models focused on *Culex sp*. mosquitoes, the known vectors for West Nile virus in the southern US. We have built a series of models using low (Landsat-8), medium (Sentinel-2), and high (WorldView-2/3) resolution imagery to conduct a comparative analysis. For each image type, we derived variables using remote sensing techniques in ArcGIS Pro 2.6. Our models were constructed using 8 different machine learning techniques and the optimal technique was determined based on overall accuracy and major error analysis. Finally, we conducted a comparative analysis to determine the optimal imagery type and model building technique for implementation for vector control. We hope this innovative approach to mosquito control will be employed to develop targeted abatement efforts that will reduce the burden of mosquito-borne disease not only in the US but globally.

0557

ANOPHELES PRODUCTIVITY OF AQUATIC HABITATS CREATED THROUGH ARTISANAL CAPTURE FISHING ON MAGETA ISLAND IN WESTERN KENYA

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The effect of physicochemical characteristics of mosquito larval habitats on *Anopheles* productivity is not well known. To fill this knowledge gap, a cross-sectional survey was carried out on Mageta Island, western Kenya. Habitats were classified either as those created by or associated with artisanal capture fishing (ACF) activities on the Island or non-ACF. Physicochemical parameters and *Anopheles* mosquito larval density were recorded for each habitat type. The degree of association between *Anopheles* larvae/pupae and the physicochemical factors was tested using generalized linear models. High numbers of *Anopheles* pupae and larvae were found in habitats associated with ACF activities. Habitats with wooden bottom surfaces had more larvae than mud and rock bottom surfaces. Perimeter, TSS, temperature, and conductivity influenced larval abundance significantly. Other factors interacted resulting in significant associations with *Anopheles* larval abundance. These were TDS with conductivity, TSS with DO, and TSS with temperature. Even though individual physicochemical characteristics could be linked to the density of *Anopheles* larvae and productivity of mosquito habitats, the results indicate that certain variables interact to regulate mosquito abundance. Malaria control measures tailored towards manipulating physicochemical characteristics in mosquito breeding sites should be adopted in integrated mosquito control programmes.

0558

UPWARDLY MOBILE: MICROCLIMATE AND THE VERTICAL STRATIFICATION OF POTENTIAL VECTORS OF MOSQUITO-BORNE VIRUSES IN A CENTRAL AMAZONIAN FOREST BORDERING MANAUS, BRAZIL

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The emergence and resurgence of Zika, chikungunya, and dengue viruses in Latin America brings with it the threat of arbovirus spillback into neotropical forests. To discern which mosquito species may bridge urban and sylvatic transmission cycles, we investigated the vertical stratification of mosquitoes and their relationships with microclimate in a rainforest reserve bordering Manaus, Brazil. We collected mosquitoes at heights of 0, 5, and 9 m using handheld nets in a tower constructed at the edge of a treefall gap and compared our findings with BG-Sentinel trap collections previously made in the same area. Mosquito taxa sampled with each method were broadly similar and included *Psorophora*, *Haemagogus*, and *Sabethes* species. Net collections were dominated by *Haemagogus janthinomys* and *Psorophora amazonica*, which were detected more frequently when 7-day cumulative rainfall was higher at one week prior to collection, and at one and four weeks prior to collection for respective species, supporting our earlier findings. *Hg. janthinomys* was significantly more abundant above ground (Kruskal-Wallis test, $P = 0.007$), whereas *Ps. amazonica* showed no significant vertical stratification. The mean temperature when *Hg. janthinomys* was collected in nets was 29.9°C at each height, which was greater than the mean daily temperature at ground level (29.0°C). Nominal logistic regressions revealed that the occurrence of this species increased with increasing temperature at 0 m ($DF = 1$, $\chi^2 = 13.9$, $P = 0.0002$) and 5 m ($DF = 1$, $\chi^2 = 17.0$, $P < 0.0001$), but not at 9 m ($DF = 1$, $\chi^2 = 2.0$, $P = 0.157$). Our results show that *Hg. janthinomys* prefers warm temperatures, such as those associated with forest clearings, while the high relative abundance of both *Hg. janthinomys* and *Ps. amazonica* at each height sampled suggests that both are potential bridge vectors in this setting.

0559

A DIVERSE GROUP OF ANOPHELINES FORAGE INDOORS AND OUTDOORS BEFORE 22:00 IN A HOLOENDEMIC SETTING IN NCHELENGE DISTRICT, ZAMBIA

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In Nchelenge District, Zambia, malaria is transmitted year-round, with 50-80% of individuals parasitemic at any given time despite a decade of intensive annual indoor residual spraying (IRS) and insecticide-treated bed net (ITN) distributions. The limited effectiveness of these primarily indoor vector-targeted interventions has cast doubt on our understanding of the foraging behaviors of the major vector, *Anopheles funestus*, and led to a concerning hypothesis that this vector may be feeding outdoors and before people go to sleep. We performed mosquito collections over eight weeks in 2019 in Nchelenge District during the *An. funestus* s.s.

population peak in the dry season, by setting CDC light traps indoors, outdoors where people gather, and near animal pens from 16:00-22:00. We also assessed human risk by performing surveys to collect information about evening behaviors, and household and environmental data to identify other covariates associated with high numbers of early-evening foraging mosquitoes. Human surveys revealed that before 22:00, 78% of people were not asleep, leaving them unprotected by their bed nets. At least nine species of *Anopheles* were identified foraging outdoors and indoors during this time, including a large proportion of *An. gibbinsi* - a species not previously described in this region of Zambia. 1% of samples were positive for *P. falciparum* sporozoites by CSP ELISA, and were composed of *An. gambiae* s.s. and *An. funestus* s.l. Positive specimens were captured indoors, in outdoor gathering spaces, and animal pens, indicating that infection risk may not be limited to indoor exposure. These data underscore the necessity for further evaluation of outdoor vector surveillance and control tools that are effective outside net use and independent of IRS.

0560

COMPARISON OF AEDES AEGYPTI AND ANOPHELES DIRUS FEEDING SUCCESS AND PREFERENCE USING AN IN VITRO BLOOD-FEEDING SYSTEM

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Blood-feeding of female mosquitoes is a basic part of laboratory colonization since blood it is required for female egg production. Artificial membrane feeding is the standard for mass rearing of colony mosquitoes. In this study, *Aedes aegypti* and *Anopheles dirus* were allowed access to human blood via three natural membranes (pig intestine, cow intestine, and fresh frog skin) and two synthetic membranes (Parafilm M and latex condom). This study was designed under two different test conditions, namely no-choice and choice tests to determine 1) feeding success and 2) membrane preference. In the no-choice test, pig intestine proved to be the most effective membrane (92.4%) for blood-feeding *Ae.aegypti*, while, *An.dirus* displayed no significant differences in the feeding rate between pig intestine (80.0%) and cow intestine (72.4%). In the preference experiment by choice test, feeding rates of both *Ae.aegypti* and *An.dirus* females that fed on pig intestine and cow intestine were greater than the other pair membranes. In conclusion, the rates of feeding were higher when using natural membranes of pig intestine, cow intestine and fresh frog skin perhaps due to mosquito preference. These data thus demonstrate the efficiency of the artificial feeder, especially if combined with natural membranes and human blood.

0561

SEASONALITY AND ABUNDANCE OF ANOPHELES STEPHENSI IN DJIBOUTI

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The presence of the invasive malaria vector, *Anopheles stephensi*, in Djibouti has been a significant concern to health authorities over the past 5 years. Urban malaria cases are expected to increase in the overall human population due to the introduction of this vector. The increased malaria risk also threatens the health of military personnel at the Camp Lemonnier, Djibouti. Vector control measures have been initiated on the installation, with control efforts mandating regular monitoring of vector populations to identify areas on the installation with the highest vector densities. Mosquito were collected weekly using CDC Light and Mosquito Magnet® traps from October 2019 to July 2020 across 32 sites on the installation. Mosquito collection data were analyzed and correlated with metrological records monitored at the geographic position of latitude 11.54733 and longitude 43.15948. In 2019 and 2020 mean temperature ranged between 24-38 degree centigrade and precipitation rates were between 0 to 155 mm^2. Temperature and rain fall records have been separated into categories based on the weekly temperatures and precipitation rates. Analysis of trap collection rates found Mosquito Magnet® Traps were significantly more effective (P=0.02) in collecting *An. stephensi* than CDC traps (5.0 ±5.8; 3.4 ±3.5). The vector numbers were negatively correlated (R=-0.5; P=0.004) with temperature ranges (24-38°C), whereas the rainfall did not affect the numbers of *An. stephensi*. Conversely, the populations of other mosquito species increased significantly in the weeks after heavy rains. The insignificant role of rain fall on *An. stephensi* populations is likely due to its presence in human dwellings and modified environments, which may provide the vector an independent niche to breed in and adapt to the conditions in Djibouti. The presented updates on *An. stephensi* data at Camp Lemonnier is of profound interest to the Djiboutian health authorities due its proximity to Djibouti airport and to Camp Lemonnier in informing their vector control operations. Continuing efforts are ongoing to expand the surveillance to cover Djibouti City and other districts.

0562

MATING COMPETITIVENESS IS REDUCED IN ANOPHELES GAMBIAE MALES MARKED WITH RHODAMINE B IN LABORATORY TRIALS

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Mosquito marking can be a valuable tool to study physiological and behavioral traits in field and laboratory settings. One of the most common techniques for marking mosquitoes is fluorescent dust; however, it can increase mortality, decrease mobility, and affect sensory organs. Rhodamine B, a thiol-reactive fluorescent dye, has been shown to successfully mark mosquito sperm, among other tissues, with a red-violet and fluorescent stain after providing males with a sugar meal containing rhodamine B. Rhodamine B has been used to study mating by dissecting and observing the presence/absence of fluorescence in spermathecae of females exposed to rhodamine-marked males. However, the effect of the dye on the capacity of males to compete for mates remains uncertain. We tested whether rhodamine B impacted the mating competitiveness of *Anopheles gambiae* (G3) males when exposed to virgin females for three nights. Rhodamine B's effect was assessed according to cage size (24cm3 versus 50cm3) and ratios of unmarked males: rhodamine-marked males: females of 50:50:50 versus 50:50:100, respectively. Approximately 35% of mated females mated with rhodamine-marked males whereas approximately 65% mated with unmarked males. These percentages were consistent regardless of cage size or male: female ratio, suggesting that rhodamine-marked males are less competitive than unmarked males. Interestingly, we did not find higher mortality of rhodamine-marked males compared to unmarked males during the mating period. Further studies addressing other variables, such as the effect of rhodamine B concentration on male mating competitiveness, are underway and will be reported.

0563

AN ECOLOGICAL NICHE MODEL TO PREDICT THE GEOGRAPHIC DISTRIBUTION OF THE YELLOW FEVER AND MAYARO VIRUS VECTOR, HAEMAGOGUS JANTHINOMYS, IN SOUTH AMERICA

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Yellow fever virus (YFV) has a long history of impacting human health in Latin America. Mayaro virus (MAYV) is an emerging arbovirus and its full impact to the region is yet unknown. Both YFV and MAYV are maintained via a sylvatic transmission cycle and both can be transmitted to humans

opportunistically, by forest dwelling *Haemagogus* janthinomys mosquitoes. To understand the potential risk of YFV more fully and MAYV transmissions to human populations, a more detailed estimation of this vector species' distribution is needed. This study compiled a comprehensive database of *Hg. janthinomys* collection records from published literature, museum specimens and publicly accessible mosquito surveillance data. A covariate analysis was performed to optimize a selection of climatic variables associated with predicting habitat suitability. Using a maximum entropy approach, collection data was used to model this species distribution across South America. Our results indicate that suitable habitat for this species can be found across all countries in the region and that more surveillance is needed to better define where this species is most likely to encounter humans.

0564

EFFECTS OF LARVAL EXPOSURE TO SUBLETHAL DOSES OF IVERMECTIN ON ADULT FITNESS AND SUSCEPTIBILITY TO IVERMECTIN IN ANOPHELES GAMBIAE

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Since its discovery in 1987, ivermectin has been widely used as a parasiticide both in veterinary and human medicine. The last 30 years have seen a scale up in ivermectin coverage through mass drug administration (MDA) for the control of onchocerciasis and lymphatic filariasis. In addition, the effects of ivermectin on adult mosquito survival have prompted it to be considered as a potential malaria control tool. The current considerations for malaria control are based on ivermectin's ability to reduce adult mosquito survival and in turn alter malaria transmission. However, the effects of ivermectin to the malaria vector larval stages are not properly understood. The increased use of ivermectin could cause increased exposure of larval habitats to ivermectin either directly through livestock excreta or indirectly through leaching or run-off from contaminated soil. Therefore, it is important to understand how exposure to ivermectin during the aquatic larval stages affects both the larvae and the emerging adults. Herein we evaluated the effects of exposure to sublethal doses of ivermectin during the larval stages of *Anopheles gambiae* on larval survival and development. We subsequently assessed the fitness of the emerging adults by evaluating their survival and fecundity. To identify the sublethal doses, we first subjected larvae to five concentrations of ivermectin; 100ng/ml, 10ng/ml, 1ng/ml, 0.1ng/ml and 0.01ng/ml and monitored larvae survival. The concentrations producing about 20% mortality (LC20) were 0.1ng/ml and 0.01ng/ml were selected as the sublethal doses. Larvae were exposed to these sublethal doses at either the first instar stage or third instar stage. Larval development was assessed as well as oviposition rate, number of eggs laid and egg hatch rate for the emerging female adults. Additionally, the susceptibility of the emerging adults to ivermectin was evaluated by blood feeding females on ivermectin spiked blood.

0565

BITING BEHAVIOR OF ANOPHELES MOSQUITOES IN ZANZIBAR: IMPLICATIONS FOR THE MALARIA ELIMINATION PROGRAMME

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Understanding mosquito behavior is key in implementing an effective malaria vector control intervention. The present work highlights the important malaria vectors species present in Zanzibar, their host-seeking behavior, and their role in malaria transmission. Monthly adult *Anopheles* sampling was conducted from October 2018 to September 2019 using human landing catch (HLC), CDC light-trap, pyrethrum spray catch (PSC), and pit trap collection methods. Collected female mosquitoes were identified based on morphology and categorized according to their physiological status. Members of *Anopheles gambiae* s.l. and *An. funestus* were separated using PCR-based methods. *Plasmodium falciparum* sporozoites were detected by enzyme-linked immunosorbent assay (ELISA). Overall, 2754 female *Anopheles* mosquitoes were collected. Of these, 65.5% were collected using HLC. Morphological identification showed catches comprised *Anopheles gambiae* s.l. (86.7%), *Anopheles funestus* (12.2%), and the remaining 1.1% were *An. maculipalpis*, *An. coustani, An. nili,* and *An. ziemanni. Anopheles gambiae* s.l. were trapped more abundantly outdoors (69.2%) than indoors using HLC. *Anopheles gambiae* s.l. demonstrated high outdoor biting activity which peaked during the night between 2200-2300 hours whilst the indoor biting activity remained at low levels throughout the night. The majority of the *An. gambiae* s.l. were *An. arabiensis* (96.4%) followed by *An. merus* (2.9%), *An. gambiae* s.s. (0.7%) and *An. quadriannulatus* (0.1%). The *An. funestus* group comprised *An. leesoni* (68.5%), *An. funestus s.s.* (2.8%), *An. parensis* (1.2%) and *An. rivolorum* (27.5%). The *P. falciparum* sporozoite rate was 0% (n=2712). We observed increased exophilic and exophagic behavior of the local malaria vectors in Zanzibar. Before the introduction of insecticide treated nets (ITNs) and indoor residual spraying (IRS) in 2006, malaria vectors were highly endophilic and endophagic. This change might be attributed to the high coverage of ITNs and IRS. Using supplementary outdoor vector control interventions with ITNs and IRS might reduce the exophilic and exophagic mosquitoes in Zanzibar.

0566

BLOOD-MEAL SOURCES OF MALARIA VECTORS FOUND IN ENDEMIC AREAS OF ZIMBABWE

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Understanding vector behaviour is critical to informing effective vector control decisions. Zimbabwe has a comprehensive vector control programme spanning seven decades, based on mainstay indoor residual spraying (IRS) and, more recently long lasting insecticidal nets (LLINs). Long term exposure to IRS insecticides and LLINs can lead to shifts in the biting or resting behaviour of mosquitoes. The present study examined the blood meal preferences of mosquitoes in endemic areas of Zimbabwe where vector control methods are implemented. A total of 428 mosquitoes were collected in 2019 and 2020 in Vumba (no vector control), Dendera, Arcturus, Burma Valley where IRS is done, and Makarara where LLINs are used. Mosquito collection methods used were CDC light traps, pit shelter and PSC. The samples were identified morphologically followed by PCR confirmation prior to blood meal PCR assay. 32% of the anophelines were collected indoors and 67% collected outdoors. *An. funestus s.s.* constituted 57% of the samples collected indoors with a human blood index (HBI) of 36% observed on Vumba mosquitoes. The most common species found outdoors were *An. pretoriensis* (27%) and *An. parensis* (20%) and the primary vectors were found in low proportions. 37% of the anophelines captured outdoors mostly fed on cow blood while those indoors had fed on humans. It was also observed that other anophelines found outdoors, especially *An. parensis,* fed on multiple hosts, exhibiting combinations of human and animal/s blood meals (25%). Multiple feeding was not observed in *Anopheles* species found indoors. *An. funestus s.s.* predominantly biting humans may reflect the effects of having no vector

control in Vumba, indicating that there is need for vector control in that area. The abundance in the cow blood meal for exophilic anophelines might be influenced by availability of cattle in Zimbabwe. Vector control in potential malaria vector species like *An. parensis* feeding on multiple hosts may prove to be a challenge in the control of malaria vectors. More studies are recommended to ascertain timing and feeding behaviour patterns that can be exploited to guide malaria control interventions.

0567

MALARIA VECTOR ECOLOGY ON THE LIHIR GROUP OF ISLANDS, PAPUA NEW GUINEA, WITH A FOCUS ON THE IMPACT OF A LARGE-SCALE MINING OPERATION

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There is ongoing efforts control and eliminate malaria in the Lihirian communities of Papua New Guinea, a country that accounts for 79% of the malaria cases in the Western Pacific region. Aniolam, the main island of the Lihir group of islands, New Ireland Province, hosts one of the largest global gold mining operations with clear demarcation of development and different vector control interventions between mine impacted and non-impacted communities. This study provides insights into the vector ecology and transmission dynamics in Lihir to guide future elimination strategies. A comprehensive larval and adult mosquito survey including standard insecticide resistance tests was conducted in 2019. All mosquito samples were identified to morphospecies level using standard identification keys. Species typing and *Plasmodium* infection will be done by PCR-RFLP and quantitative PCR. In Aniolam, *An. farauti* was the predominant vector in the mine impacted area, while *An. punctulatus* was the main species outside it, with the exception of one outer island that was also colonized by *An. farauti*. Both species showed exophagic behavior with peak biting periods from 9-10PM at a biting rate of 0.24bites per person/hour (p/h) by *An. farauti* within impact areas and 6-7PM outside of it (2.5bites p/h). *An. punctulatus* was biting mostly at 0.45bites p/h around 9-10PM. Both species were fully susceptible to deltamethrin and bendiocarb (100% mortality) but showed low grade DDT resistance in vector populations outside the impact area (95% mortality). *An. farauti* and *An. punctulatus* are the main vector species found in Lihir Islands with different biting profiles and distribution. Vector control could be improved by using alternative outdoor interventions and by expanding larval source management strategies across communities outside the mine impacted area. Future experiments to quantify risks of malaria transmission between mine impacted and on-impacted areas will provide further insights to individualize malaria control strategies to accelerate malaria elimination.

0568

SUSTAINING EFFICIENT MALARIA VECTOR CONTROL WHILE ACCOUNTING FOR BREEDING SITE STAGES: A PREDICTIVE MODELLING APPROACH

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Malaria is one of the three biggest infectious diseases amidst tuberculosis and HIV/AIDS terrorising the world and has been a global concern for decades. In an effort to reducing malaria incidence, the malaria vector is one of the cornerstones its contribution needs to be controlled. Targeting entomological features distinguishing malaria vector species is crucial in vector control interventions and a key component in malaria control that need to be considered appropriately. In fact, breeding habits and choice of breeding sites vary among mosquito species, hence understanding the breeding habits of targeted species can facilitate cost-efficient and optimal vector control measures. Additionally, unlike *Aedes* mosquitoes, *Anopheles* (An.) are less tolerant to desiccation and drought. Mosquitoes breed in water puddles formed when rain falls. Over time, predating organisms such as fish or tadpoles cohabit the breeding site with the mosquito juvenile stage and contribute to the depletion of nutrients and the mortality of mosquitoes in the larval stage. Typically, these critical breeding habits are overlooked or not adequately addressed in predictive models. Here, we introduce an extended SEIR model that incorporates the dynamics in the breeding site of the An. mosquito accounting for (i) larvae competition resulting in a reduction in the number of emerging late-stage larvae, (ii) effect of larvivorous fish as a predator on the late-stage larvae, (iii) effect of insecticide-treated bednets as an adult-stage control intervention, and (iv) the implication of introducing genetically modified mosquitoes to the wild. In our model, it is supposed that gravid mosquitoes find a suitable breeding site to oviposit. We derive the basic reproduction number in the mosquito population and study the effect of different mosquito control interventions. The model was exemplified by parameters from the literature.

0569

HABITAT MICROBIOTA SHAPES HETEROGENEITY IN MOSQUITO POP DYNAMICS IN THE FIELD

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Mosquito population dynamics play a fundamental role in the chain of vector-borne disease transmission, and these dynamics are rooted in conditions during the aquatic larval stage. Larval development is shaped by gut microbiota recruited from the environment, and many surveys of environmental microbiota associated with mosquitoes are available. However, to understand the implications of patterns of microbial diversity for vector-borne disease, it is necessary to connect patterns of microbial diversity to mosquito population dynamics. Here, we combine 16S-rRNA gene amplicon sequencing-based characterization of bacterial communities from mosquito larval habitats with monitoring of larval productivity through time, analyzing local variation in the Madison, WI, USA area. Our results highlight a dominant role for the community composition of habitat microbiota in shaping larval productivity in the field. These findings underscore the importance of future work to identify the mechanisms of microbiota action on mosquito populations and physiology under natural conditions.

0570

SPATIOTEMPORAL DYNAMICS OF CULEX SPECIES ON AN URBAN TO RURAL GRADIENT IN GREATER WASHINGTON DC

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In the mid-Atlantic region of North America, a community assemblage of *Culex* species, including *Cx. pipiens*, *Cx. restuans,* and *Cx. quinquefasciatus* transmits West Nile Virus among vertebrate hosts. In this study, we described the influence of both seasonality and environment on the *Culex* community in the greater Washington DC region along an urban to rural gradient. Because mosquitoes with diverse life-history traits and feeding patterns can sustain, amplify, and transmit the virus between hosts and vectors across the landscape, species distributions in a heterogeneous environment can have a strong impact on disease transmission. In 2019, we collected over 6,000 mosquitoes using gravid traps at 5 urban, 5 suburban, and 5 rural site classes in Washington DC

and Maryland across eight occasions per site. A subset of cryptic *Culex* mosquitoes were identified to species using molecular methods (N=788). Species distributions differed by site class (Pearson's X^2=126.44, df=4, p<0.001) and season (Pearson's X^2=51.901, df=4, p<0.001), according to a contingency table analysis. *Culex quinquefasciatus* associated with urban environments, whereas *Cx. restuans* associated with rural environments, most strongly in the early season. Furthermore, both season and site class predicted differences in the relative frequency of *Cx. pipiens* according to analysis by generalized linear model. There was considerable by-site variation within each of our urban, suburban, and rural site classes, which motivated us to use redundancy analysis (RDA) to explore the environmental factors that contribute to this variation. Normalized difference vegetation index (NDVI), distance to city center, and site class explained 58% of the variance in species distribution (p=0.021). As observed in our contingency analysis, our RDA indicated that *Cx. quinquefasciatus* was associated with urban environments. *Cx. restuans* was associated with increasing NDVI and increasing distance to city center. *Cx. pipiens* did not have strong relationships to any of these variables. This may be due to the consistently high relative frequency of *Cx. pipiens* spatially and phenologically.

0571

INNOVATIVE METHODS AND SOFTWARE FOR THE ANALYSIS OF MOLECULAR XENOMONITORING SURVEYS AND OTHER POOL-TESTED DATA

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Pooled specimen testing has a long history of use in public health surveillance and screening where prevalence of the condition is low and the cost of the diagnostic tests are prohibitive, e.g., pooled stool samples for soil-transmitted helminths, and recently in COVID-19 testing. Pooled-testing is often part of molecular xenomonitoring (MX) surveys, where surveillance of vector-borne diseases is conducted by capturing, pooling, and testing large numbers of vectors. MX can be used to map disease prevalence, evaluate the efficacy of interventions, and identify locations with residual transmission in the endgame of elimination campaigns. MX surveys often include hierarchical sampling designs, with vector traps placed at locations around the study area of interest. We have developed an R package, PoolTestR, to meet the needs of large and complex MX surveys and other data involving pooled testing. PoolTestR includes user-friendly and flexible tools to estimate prevalence and fit a range of regression models for pooled data in frequentist and Bayesian frameworks, including fixed- and mixed-effect models and Gaussian process models. Mixed-effect models can be used to account for the hierarchical sampling designs in surveys. The regression modelling capabilities allow for the identification of trends and variables associated with high or low infection prevalence (using odds ratios), while extensions for Gaussian process modelling enable Bayesian geostatistical modelling. This presentation will illustrate the key features of the PoolTestR package and its benefits compared to existing software and methods, including application to real and simulated data from MX surveys. We show that failing to adjust for the hierarchical nature of MX surveys leads to overly confident estimates of infection prevalence with confidence intervals that often may not include the true value. We show that using a regression framework for estimating infection prevalence for different vector species and primary sampling units can improve the precision of prevalence estimates even when sample sizes are small.

0572

COMPLEMENT-FIXING ANTIBODIES ARE PRODUCED IN RESPONSE TO A TETRAVALENT DENGUE VACCINE

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The complement system is an arm of innate immunity that functions as an effector mechanism of the humoral response. Mechanisms of viral neutralization mediated by antibodies are highly dependent upon complement activation, which involves aggregation, opsonization, lysis and phagocytosis. The classical pathway of the complement system has been demonstrated to contribute to protection against flaviviruses through virus opsonization, B-cell activation, antibody production and inhibition of antibody-dependent enhancement of infection. Takeda's attenuated tetravalent dengue vaccine candidate TAK-003 demonstrated 80% efficacy against virologically-confirmed dengue due to any serotype in an ongoing phase 3 study (ClinicalTrials.gov NCT02747927). TAK-003 elicits neutralizing antibodies against all four dengue viruses (DENV). We investigated the ability of antibodies produced in response to TAK-003 to bind complement as an indicator for activation of the complement system. Samples were collected from baseline seronegative and seropositive study participants from two phase 2 clinical trials (DEN-203, NCT01511250 and DEN-204, NCT02302066), before and after vaccination with two doses of TAK-003 at months 0 and 3. The sera were evaluated for the presence of complement-fixing antibodies against all four DENV serotypes using a Luminex multiplex platform-based assay. In both trials, TAK-003 elicited complement-fixing antibodies against all four DENV serotypes which persisted for up to one year post-vaccination, irrespective of baseline serostatus. In general complement-fixing antibody levels were correlated with neutralizing antibody titers, as well as virus-specific IgG concentrations and avidity. Taken together, these results indicate that antibodies produced after vaccination can fix complement system components of the classical pathway and may contribute to protection mediated by TAK-003.

0573

VALIDATION OF A MICRONEUTRALIZATION TEST TO QUANTIFY DENGUE VIRUS NEUTRALIZING ANTIBODIES AFTER VACCINATION WITH THE TAK-003 TETRAVALENT DENGUE VACCINE CANDIDATE

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Reliable assays to measure functional antibody responses to the four dengue virus serotypes (DENV-1–4) are essential for dengue research and vaccine development. The gold standard plaque-reduction neutralization test (PRNT) is the method most commonly used to measure neutralizing antibody responses to each of the four DENV serotypes. The dengue microneutralization test (MNT) was developed based on WHO PRNT guidelines and is essentially a miniaturized PRNT conducted in 96-well cell culture plates to increase sample throughput when measuring neutralizing antibodies to DENV-1–4 in human sera. The MNT was validated for intra- and inter-assay precision, accuracy/linearity, limits of quantitation, and specificity using serum samples containing a wide range of anti-dengue antibody levels. The assay met the pre-specified acceptance criteria for intra-assay (percent geometric coefficient of variation [%GCV] < 30) and inter-assay precision (≥ 91% of the samples with median neutralizing titers within the limits of quantitation had %GCV < 60). The MNT also met the prespecified acceptance criteria for accuracy (the predicted bias between the geometric mean and expected titers was 0.62–1.88-fold for ≥ 80% of samples) and dilutional linearity (the overall fold-decrease in titer per 2-fold dilution increase was 2.03–2.16-fold for DENV-1–4). Specificity for the presence or absence of dengue antibodies was demonstrated.

Possible cross-reactivity was observed in samples positive for antibodies to Japanese encephalitis, West Nile, and yellow fever viruses. The assay has been validated for quantitating DENV-1–4 neutralizing antibodies and has been used to measure immune responses to the tetravalent DENV vaccine candidate TAK-003.

0574

ADRENAL DYSFUNCTION IN PATIENTS WITH DENGUE: A HOSPITAL BASED STUDY FROM JODHPUR, INDIA

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Stress response during dengue fever usually results in secretion of cortisol mediated by cytokines like interleukin-6, interleukin-1, tumour necrosis factor- alpha. It is hypothesized that the adrenal gland may be affected either due to hemorrhage or functional insufficiency due to cytokine effects on hypothalamic-pituitary-adrenal axis. We did a cross-sectional study in which all patients >18 years of age attending the in-patient services at our centre with diagnosis of dengue fever were included. Basal and stimulated cortisol were measured in all patients. We used the following criteria for diagnosis of adrenal insufficiency: Post-ACTH stimulated serum cortisol < 18 mcg/dl, basal cortisol <3 mcg/dl or increment during ACTH stimulation < 7 mcg/dl. Total 50 patients were enrolled in the study out of which 44 were male and 6 were female. Median age of patient was 29.5 (±12.75) years. Thirty-eight patients had dengue without warning signs and twelve had dengue with warning signs. All patients survived. Eight out of 50 (16%) patients had adrenal insufficiency according to definition; out of which 7 had stimulated cortisol < 18 mcg/dl. The median basal cortisol value in dengue without warning signs cohort was 9 mcg/dl and the median stimulated cortisol was 22 mcg/dl. The median basal cortisol value in dengue with warning sign cohort was 6.5 mcg/dl and the median stimulated cortisol was 25 mcg/dl. The median value was significantly reduced in dengue fever with warning signs (6.5 + 2.75 mcg/dl) compared with those without warning signs (9.0 ± 4.0 mcg/dl) [p = 0.04, Mann Whitney U test]. However, there was no significant difference in post-ACTH stimulated cortisol between the two groups. Our results suggest that adrenal insufficiency is more common than previously reported in dengue fever. Lower serum cortisol values are associated with dengue with warning signs suggesting an imbalance in the stress response and may be an important predictor of severity. However, lower levels of stimulated cortisol in adrenal insufficiency suggest a direct viral mediated suppression on adrenal gland than a hypothalamic-pituitary-adrenal axis mediated effect.

0575

AEDES-BORNE VIRUS IMMUNE PROFILES IN A PEDIATRIC POPULATION IN MERIDA, YUCATAN, MEXICO: A BASELINE SEROLOGIC ANALYSIS OF THE TIRS TRIAL

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Aedes-borne viruses (ABVs) like dengue (DENV), Zika (ZIKV) and Chikungunya (CHIKV) viruses are mosquito vector-borne diseases responsible for substantial health burden in many tropical areas. While DENV infection can be controlled by robust antibody (Ab) responses, the presence of four serotypes of DENV, the potential for co-transmission of the related flavivirus Zika virus, and cross-reactivity of neutralizing and non-neutralizing Abs complicates infection control among individuals and populations. Indeed, cross-reactive but weakly neutralizing Abs may lead to worse disease outcomes due to Ab dependent enhancement of DENV infection. We characterized the immune state of a cohort of children in Merida, Yucatan, Mexico, participating in the TIRS trial and living in 50 clusters located in an area identified as high risk for ABV transmission. Children were categorized as naïve or previously infected by DENV, ZIKV, and/or CHIKV according to flavivirus IgG ELISA and neutralization or CHIKV IgG ELISA tests. We analyzed binding affinity and virus neutralization across four DENV serotypes and ZIKV in a subset of samples. Following reported infections among the participants, we analyzed newly collected serum samples. Finally, by collecting socio-economic data and spatial statistics, we generated a heatmap of risk for DENV and ZIKV prevalence by serotype. The map indicates areas of high susceptibility to both DENV and ZIKV, which may inform future infection control efforts.

0576

VIRAL PARKINSONISM: AN UNDERDIAGNOSED NEUROLOGICAL COMPLICATION OF DENGUE VIRUS INFECTION

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Dengue virus (DENV) is a flavivirus that is a significant cause of human disease. It is spread by the mosquito *Aedes aegypti*, and humans are crucial to the viral life cycle. Up to 20% of patients with DENV infections exhibit neurological symptoms, including encephalitis and encephalopathy. DENV infection has also caused the rare complication of viral-parkinsonism. Viral-parkinsonism occurs when infection of the brain causes degeneration of neurons. Approximately 11% of DENV encephalitis cases have a movement disorder associated with their condition. One of these is the lesser-known condition of post-DENV parkinsonism or DENV-parkinsonism. For viral-parkinsonism to be considered as a diagnosis, the patient must exhibit at least two of the following symptoms: tremor, bradykinesia, rigidity, and postural instability. Little is known about the condition, and current knowledge is confined to case reports. We examined the indexed knowledge from Pubmed, Scopus, CINAHL, Embase, LILACs, Google Scholar, and gray literature from medical and governmental agencies. We also included DENV cases with an encephalitis diagnosis containing at least 2 symptoms of parkinsonism occurring during or immediately following acute infection but without a diagnosis of parkinsonism. We performed logistic regression analysis to obtain odds ratios of specific clinical features to determine which specific symptoms prompted an official diagnosis of DENV-parkinsonism. The data show that a specific serotype could not be linked to any manifestation of parkinsonism. Cases of DENV-parkinsonism were almost exclusively reported in southern Asia, specifically in India, Sri Lanka, and Malaysia. Men were significantly more likely to develop viral-parkinsonism, and age was not associated with disease. Expressionless face, speech problems, and slow movements were significantly associated with patients acquiring an official DENV-parkinsonism diagnosis. The diagnosis of dengue encephalitis is vague, considering the broad range of symptoms exhibited by patients. DENV-parkinsonism is likely more prevalent than reported due to misdiagnosis as encephalitis.

0577

THE RESURGENCE OF DENGUE VIRUS AFTER THE ZIKA EPIDEMIC IN BRAZIL

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After Zika virus (ZIKV) was introduced in the Americas, it caused major outbreaks in 2016, and the number of reported dengue cases in Brazil dropped in 2017-2018. However, in 2019, dengue resurged, reaching

high incidence across the country. In this study we investigate the causes of dengue decline, and uncover the dynamics and origins of dengue virus 1 (DENV-1, genotype V) and DENV-2 (genotype AA) causing recent outbreaks in the country. To investigate dengue dynamics in recent years we used distinct data types. Based on epidemiological data, we modelled the annual force of infection of dengue from 2002 to 2019. Using ecological data, we estimated the level of environmental suitability for mosquito populations, in distinct regions. Finally, by sequencing 69 dengue virus genomes, we performed phylogeographic analyses to uncover the evolutionary history of DENV-1 and -2 in Brazil. Estimates of force of infection revealed that dengue transmission was very low in 2017 and 2018. This likely indicates the population immunity acquired in prior ZIKV and/or DENV infections likely played a role determining the dengue decline. Furthermore, environmental conditions in 2017/2018 did not change in comparison to other years, and were favourable for mosquito reproduction. Phylogenetic analyses revealed that the 2018-2019 dengue outbreaks were mainly caused by local DENV lineages, circulating and causing recurrent outbreaks in Brazil since 2012 (DENV-1, BR5) and 2013 (DENV-2, BR4). We revealed the dynamics of DENV before and after the major 2016 Zika outbreaks in Brazil and uncovered the causes of dengue decline and resurgence. The 2018-2019 DENV outbreaks in the country were caused by viruses circulating locally prior to (and despite) the Zika outbreaks. Dengue transmission in 2017/2018 was low, likely due to a decline in the number of susceptible individuals because of population immunity from years of high dengue incidence. Moreover, public health interventions in response to ZIKV may have also impacted the transmission of other arboviruses. Thus, the dengue decline in 2017-2018 is more likely explained by immunological and anthropological factors.

0578

GLYCOSYLATION ON PREMATURE AND ENVELOPE PROTEIN DETERMINE DENGUE VIRUS MATURATION

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Dengue virus (DENV) is a vector-borne, single-stranded positive-sense RNA virus with four distinct serotypes (DENV1-4). While DENV maturation affects both the intrinsic viral infectivity and antibody neutralization extrinsically, the generation and evolution consequences of DENV maturation are largely unknown. The lack of reagents hinders our understanding of DENV maturation and the only known maturation determinant is the furin cleavage site of the premature (prM) protein. Given the major structural conformational change between the immature and mature DENV, we hypothesize post-translational structural modification, such as N-linked glycosylation could affect DENV maturation. The prM and Envelope (E) region possess three highly conserved surface exposed N-glycosylation sites, prM-N69, E-N67, and E-N153. To investigate the impact of prM and E glycosylation on viral maturation, we systematically ablated the three glycosylation sites in DENV4. We identified prM-N69 as a lethal mutation and prevented viral growth while E-N67 and N153 did not. Subsequently, we were able to rescue the prM-N69 ablated mutant by adding a prM-N57 glycosylation site proximally near the prM-N69 region. All our variants exhibited attenuated phenotype in tissue culture compared to the wildtype DENV4 strain (WT), but the degree of attenuation is species dependent. Importantly, compared to WT, the E-N153K variant is more mature while E-N67Q and prM-N69D-V57N variants generate immature viruses. Our result suggests surface glycosylation determines DENV maturation and viral fitness. Our attenuated DENV viruses have the potential to serve as live attenuated vaccine candidates and our genetically encoded mature and immature DENVs are great tools to delineate DENV maturation.

0579

DENGUE SEROTYPE 4 -2 ENVELOPE DOMAIN CHIMERIC VIRUSES: DESIGN, RECOVERY, AND ANTIBODY MAPPING

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Dengue is the most prevalent vector-borne virus plaguing our world. In 2010, there were an estimated 390 million infections worldwide, 25% of which were symptomatic. Properties of antibodies that correlate to protection after natural infection or vaccination have not been fully described, which hinders our ability to measure vaccine efficacy. Only two human type-specific neutralizing antibodies against dengue serotype 2 (DENV2) have been mapped. In an attempt to identify additional type-specific neutralizing DENV2 epitopes, we have developed three chimeric viruses with envelope domain I, II, and III (EDI, EDII, and EDIII) transplants from DENV2 in a DENV4 backbone. Full-length viral RNA of each construct was electroporated into C636 cells and serially passaged in C636, Vero 81, and furin-overexpressing Vero 81 cell lines to compare maturation status and viral titers. Sanger-sequencing confirmed preservation of the DENV2 EDI, EDII, and EDIII footprint, respectively, on the DENV4 backbone. Neutralization assays by DENV2-specific, DENV4-specific, and cross-reactive antibodies showed phenotypic preservation of each DENV2 envelope domain as well. Our DENV4-2 chimeric viruses were then used to identify domain-specific epitopes of unmapped monoclonal antibodies. In the future, this panel of reagents can be used to identify viral targets by type-specific and polyclonal human sera.

0580

MAJORITY OF PEDIATRIC DENGUE VIRUS INFECTIONS IN KENYA DO NOT MEET THE 2009 WHO CRITERIA FOR DENGUE DIAGNOSIS

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From 1975-2009, the WHO guidelines classified symptomatic dengue virus infections as dengue fever, dengue hemorrhagic fever, and dengue shock syndrome. In 2009 the case definition was changed to a clinical classification after concern the original criteria was challenging to apply in resource-limited settings and not inclusive of a substantial proportion of severe dengue cases. More recently, the 2009 clinical classification has been criticized for being overly inclusive and non-specific, different from our experience in Kenya. Between 2014 and 2019 as part of a child cohort study of febrile illness in our four clinical study sites (Ukunda, Kisumu, Msambweni, Chulaimbo) we identified 369 dengue-PCR positive symptomatic cases and characterized whether they met the 2009 revised WHO diagnostic criteria for dengue with and without warning signs and severe dengue. Of the 369 identified cases, we found 7% met criteria for dengue without warning signs, 29% met criteria for dengue with warning signs, 2% met criteria for severe dengue, and 62% did not meet criteria for diagnosis. The mean ages were 4.99, 4.47, 4.7, and 5.5 years, respectively and the most common warning signs were lethargy paired with aches/pains and nausea/vomiting. For severe dengue all cases had impaired consciousness but none were notable for hemorrhagic symptoms. This correlates with our experience that dengue disease in children in Kenya is less severe as reported in other parts of the world. These findings also suggest the 2009 WHO dengue case definition may miss more than 50% of symptomatic infections in Kenya and may require further modification to include the African experience.

0581

TISSUE SPECIFICITY OF FLAVIVIRUS NS1 BINDING AND NS1-INDUCED ENDOTHELIAL PERMEABILITY IS MEDIATED BY DISTINCT PROTEIN DOMAINS

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Vascular leak is a hallmark of severe dengue caused by the positive-sense RNA flavivirus, dengue virus (DENV). DENV non-structural protein 1 (NS1) is a secreted glycoprotein that causes endothelial hyperpermeability directly by disrupting the endothelial glycocalyx-like layer (EGL) *in vitro*, as well as inducing vascular leak *in vivo*. We have shown that NS1 proteins from different flaviviruses bind to human endothelial cells (EC) and induce vascular leak in a tissue-specific manner, consistent with their respective disease tropism. For example, DENV NS1, but not West Nile virus (WNV) NS1, binds to human pulmonary microvascular endothelial cells (HPMEC), as DENV but not WNV induces leakage in the lungs. However, the molecular determinants of NS1 driving differential EC tissue specificity remain unknown. Using chimeric NS1 proteins, we exchanged the wing and β-ladder domains between DENV and WNV NS1. The DENV NS1 with WNV wing domain (D-W^{Wing}) chimera lost the capacity to bind to HPMEC, whereas its counterpart, WNV NS1 with DENV wing domain (W-D^{Wing}) chimera exhibited gain-of-function HPMEC binding. Interestingly, the DENV NS1 with WNV β-ladder domain (D-$W^{\beta\text{-ladder}}$) chimera was able to bind to HPMEC like DENV NS1 but was unable to induce endothelial permeability. Conversely, the W-$D^{\beta\text{-ladder}}$ chimera ablated binding but could induce endothelial hyperpermeability in HPMEC. Taken together, these results suggest that the wing domain of DENV NS1 drives its capacity to bind to HPMEC, while the β-ladder domain mediates its ability to induce endothelial permeability. Further mutational analysis to identify specific residues responsible for these characteristics is underway, as well as work to elucidate the implications in relation to viral infection.

0582

SAFETY, TOLERABILITY AND PHARMACOKINETICS OF A NOVEL PAN-SEROTYPE DENGUE ANTIVIRAL SMALL MOLECULE IN A PHASE 1, DOUBLE-BLIND, RANDOMIZED, DOSE-ESCALATION STUDY

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Dengue is a growing global health threat. JNJ-1802 is a novel pan-serotype dengue antiviral small molecule that blocks the formation of the viral replication complex. Preclinical results demonstrated promising safety data and excellent efficacy against dengue virus infection in murine and non-human primate models. This first-in-human, double-blind, randomized, placebo-controlled study aimed to examine the safety, tolerability and pharmacokinetics of increasing single and multiple oral doses of JNJ-1802. Eligible healthy participants 18-55 years of age were randomized to receive oral JNJ-1802 in fasted conditions as: 1) single doses (50 to 1200 mg; n=29) or placebo (polyethylene glycol 400; n=10); or 2) once daily doses (50 to 560 mg for 10 consecutive days or 400 mg for 31 days; n=38), or placebo (n=9). Safety and tolerability were evaluated throughout the study. Plasma (all cohorts) and urine (selected cohorts) samples were collected at pre-determined timepoints to characterize the pharmacokinetics of JNJ-1802. No deaths, serious adverse events (SAEs), or AEs leading to JNJ-1802 discontinuation were reported. No clinically relevant changes were observed in electrocardiograms, laboratory tests or vital signs. Two grade 2 events of rash, which were considered very likely related to JNJ-1802 by the investigator, occurred and resolved. JNJ-1802 exposure after single or multiple dose increased dose-proportionally from 50 to 150 mg and less than dose-proportionally for higher doses. The terminal half-life was 6.3 to 9.2 days and the accumulation factor varied from 4.3 to 7.3 after 10 days and 14.6 after 31 days. Renal elimination of JNJ-1802 was very limited as low amounts of unchanged compound were excreted via urine. Oral JNJ-1802 administered as single or multiple doses was generally well tolerated and no safety concerns were identified, supporting its further clinical development for the treatment and/or prevention of dengue. To our knowledge, JNJ-1802 is the first dengue-specific antiviral small molecule to complete a first-in-human study.

0583

ASSOCIATION BETWEEN MAGNITUDE OF ANTI-DENGUE VIRUS ANTIBODY AVIDITY AND PROTECTION AGAINST SYMPTOMATIC DENGUE VIRUS INFECTION

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Dengue is a mosquito-borne disease caused by four dengue virus serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) with an estimated 390 million infections annually, of which approximately 96 million are symptomatic. The degree of antibody affinity maturation driven by vaccination or natural infection is one measure of the overall evolution of the polyclonal antibody repertoire. Previously, we reported the development of a novel avidity assay employing bio-layer interferometry and dengue virus-like particles to assess the affinity maturation of antibodies in sera from naturally infected individuals and recipients of the Takeda tetravalent dengue vaccine candidate (TAK-003). The avidity index measured by the assay increased after infection and after vaccination and remained high through one-year post-vaccination. The Nicaraguan Pediatric Dengue Cohort Study (2004 to present) is a community-based study with an average active cohort of ~3,700 children aged 2–14 years old in Managua, Nicaragua. Blood samples are collected from all participants each year, and symptomatic DENV infections are confirmed by molecular, virological, and serological assays. Sera were selected from 40 study participants prior to a secondary DENV-3 infection (20 symptomatic and 20 asymptomatic infections) and analyzed for polyclonal antibody avidity and neutralizing antibody titers. The DENV-3 avidity index and neutralization titers were higher in asymptomatic compared with symptomatic study participants. Study participants with a high avidity index were more likely to have experienced a subsequent inapparent infection, regardless of neutralizing antibody titer. Study participants with both a low avidity index and low neutralizing antibody titer were more likely to have experienced a subsequent symptomatic infection. These results suggest that the magnitude of the avidity index, a measure of the degree of polyclonal antibody affinity maturation, is associated with prevention of symptomatic DENV-3 infection.

0584

DETECTION OF SPATIO-TEMPORAL HETEROGENEITY OF DENGUE IN COLOMBO CITY, SRI LANKA: AN ASSESSMENT OF URBAN RISK FACTORS AND DRIVERS USING POINT PROCESS SPATIAL MODELLING

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Colombo, the capital of Sri Lanka, is a highly dense area with a heterogenous residential land use pattern. Endemic for dengue transmission, it has the highest yearly burden of dengue in the country and dengue clustering persists in certain areas of the city. As part of their surveillance system, the Colombo Municipal Council records the location of dengue cases in Colombo city to pinpoint areas of high incidence and to administer vector control measures. The aim of this research was to confirm the spatio-temporal heterogeneity of dengue within the city at a high resolution of 100x100m grids and relate these to ecological risk covariates of population density, type of residential area and residential density, land use, and proximity to known vector breeding risk sites such as construction, markets, and schools. Particular focus was how the built environment and land use changes relate to dengue incidence. Spatial analysis of individual confirmed geolocated dengue cases was done for the period 2000-2016 using point pattern analysis methods to develop a spatially continuous map of disease risk. Bayesian inference was used to estimate the model parameters using Integrated nested Laplace approximation (INLA) and a Poisson process. The model estimated dengue incidence at 100m x month adjusted for spatial effects. The analysis highlighted the clustering and risk present in certain areas of the city. These findings help the dengue control program target its activities and inform control.

0585

ARBOVIRUS SURVEILLANCE AND JAPANESE ENCEPHALITIS VIRUS GENOTYPE IV ISOLATION FROM CULEX VISHNUI MOSQUITOES COLLECTED ON BALI ISLAND, INDONESIA

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Japanese encephalitis virus (JEV) remains to be the leading cause of children's encephalitis in its endemic countries in Asia, including Indonesia. Indonesia is the only country with the most diversity of Japanese encephalitis virus (JEV) genotypes, with the detection of four out of five genotypes available in the world. That being the case, knowledge on the arthropod-borne virus (arbovirus) disease surveillance in Indonesia may be crucial in elucidating the mechanism behind the JEV genotypes' evolution and spread, and ancient arboviruses' spread in general. Nonetheless, a limited number of studies in the country indicated that we are still far from understanding the arbovirus evolution. In this study, we conducted cross-sectional arbovirus surveillance by collecting mosquitoes in four sites in Tabanan Regency, Bali Island, Indonesia in 2019. An extensive approach of high-throughput RNA-sequencing combined with virus isolation in mosquito cells was utilized to determine viruses harbored in the mosquitoes. Subsequently, the results were verified by PCR and followed by phylogenetic analysis. We successfully identified 35 putative and known viral genomes, including one JEV isolate. Some novel viruses belonged to divergent families, notably Reoviridae and Rhabdoviridae. The JEV isolated belonged to Genotype IV based on its envelope (E) gene sequences and was closely related to JEV identified from swine in Bali in 2018 and human patient in Queensland in 2020. We are currently further characterizing viruses in the mosquitoes and also determining their correlation with the most restricted JEV genotype in the world, i.e. JEV GIV, if any. This study not only adds up the knowledge pool of arbovirus studies on Bali Island, Indonesia, but also supports the evidence of JEV GIV active transmission currently occurring on the island. Established national surveillance and vaccination in Indonesia, Bali Island in particular, is greatly encouraged.

0586

URINE AS A POSSIBLE RESOURCE FOR YELLOW FEVER DIAGNOSIS

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From 2016 to 2018, a huge yellow fever (YF) outbreak was detected in Southern Brazil, causing 2166 confirmed human cases and 752 deaths. Laboratory molecular diagnosis of YF is usually based on RT-qPCR to detected YFV RNA in sera within ten days post symptoms (DPS) onset. The presence of YFV RNA has already been demonstrated in urine of patients. To investigate the presence of RNA in urine from YF naturally infected patients, we tested urine samples collected during the last YF outbreak in Brazil from patients in Minas Gerais state. A total of 74 urine samples, from 74 patients, were collected (55 from acute phase and 19 from convalescent phase of YF infection). A total of 140 µL from each urine sample was used for total RNA extraction followed by RT-qPCR targeting part of the YFV NS5 gene. Fifteen urine samples from different patients from the acute phase (from 2 to 21 DPS) and two urine samples from the convalescent phase (66- and 68- DPS) were YFV positive. Although international guidelines advocate using molecular tests in sera until ten DPS, the limited window of detection with serum may be extended with evaluation of urine for molecular tests, as demonstrated in our case, where YFV RNA was detected in urine up to 68 DPS onset. Whereas the two positive samples from the convalescent phase of YF were from patients who vaccinated against YF 10 days before symptoms, we genotyped the samples. They grouped with YF-SAI genotype, indicating the presence and persistence of WT YFV for a longer time in urine. To our knowledge, this is the first report of WT-YFV-RNA detected in the urine this far out from symptom onset. Other studies previously detected the RNA of YFV WT in urine, but in a smallest window (up to 45 DPS). With a prolonged period of detection beyond the viremic phase, we recommend using urine as an alternative for YF diagnosis. The use of urine samples after YF acute phase, altogether serology tests, epidemiology inquiry, and clinical assessment could provide a longer assertive YF diagnosis, especially in lower-resourced communities or remote areas, where blood collection can be problematic and in situations where trained personnel or proper facilities are unavailable.

0587

NON-CONSERVATIVE MUTATIONS AT THE HIGHLY CONSERVED ALA54 RESIDUE OF YELLOW FEVER VIRUS ENVELOPE PROTEIN MODULATES THE YIELD OF INFECTIOUS VIRUSES IN CELL CULTURE

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Yellow fever virus (YFV) is the prototypic flavivirus, that can cause a fatal viscerotropic disease in humans. The disease is prevented by live attenuated vaccines. Infection of cells with YFV is initiated after viral and cellular membrane fusion followed by the release of positive-sense RNA genome into the cytoplasm. Membrane fusion involves conformational changes of the envelope (E) protein from a dimer to a trimer in the acidic environment of endosomes. This is achieved through the coordination of the EDI-EDII hinge region. Three amino acids with small hydrophobic side chains are highly conserved in the EDI-EDII hinge region of YFV and other pathogenic flaviviruses: Ala^{54}, Gly^{260}, and Gly^{279} of YFV. The Ala^{54} residue has been shown to tolerate genetic mutations; whereas, substitutions of the Gly^{260} and Gly^{279} residues cause lethal phenotypes or compensatory mutations in the E protein of related flaviviruses. The live-attenuated French neurotropic vaccine (FNV) was derived from wild-type (WT) strain French Viscerotropic virus (FVV) and was used in the vaccination campaign in French-speaking Africa until 1982. Intriguingly, FNV contains a stable E-A54V mutation. The objective of this study is to determine the phenotypic changes caused by the substitutions of the Ala^{54} residue in YFV E protein. Seven mutations including: A54S, A54V, A54D, A54G, A54K, A54P and A54Y were tolerated by WT YFV. The non-conservative A54D and A54K mutations with electrostatically charged side chains reduced the yield of infectious virus and prevented the formation of plaques in Vero cells, resembling the phenotypes associated with the A54V mutation found in FNV. The substitution of the Ala^{54} residue with amino acids with hydrophobic or neutral side chains had no demonstrable differences in the yield of infectious viruses in electroporated BHK-21 cells. Whilst the A54V mutation in FNV is a conservative mutation, its impacts on the phenotype of WT YFV are similar to those caused by other non-conservative mutations. Reduced viral yield suggests that the A54V mutation in the E protein is a candidate attenuation determinant responsible for the loss of virulence in FNV.

0588

SUBSTITUTIONS OF CONSERVED HYDROPHOBIC AMINO ACIDS IN THE ENVELOPE PROTEIN DOMAIN I AND DOMAIN II HINGE REGION IMPAIR THE REPLICATION OF YELLOW FEVER VIRUS IN CELL CULTURE

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Flaviviruses are a group of small, enveloped RNA viruses with diverse tissue tropisms and pathogenic mechanisms. All flaviviruses have evolved to utilize the same pH-driven viral membrane fusion mechanism that releases the positive-sense RNA genome into susceptible cells. In the acidic environment of endosomes, the fusion loop (FL) structure located in domain II of the envelope protein (EDII) is exposed and inserted into the host cell membrane to initiate viral membrane fusion. This process requires the relative movement between envelope domain I (EDI) and EDII and is coordinated by the EDI-EDII hinge region that consists of four linear chains of amino acids. As a functionally important element, the EDI-EDII hinge region contains several conserved residues, the majority of which are hydrophobic amino acids. We hypothesized that the substitutions of conserved hydrophobic residues in the EDI-EDII hinge region will interfere with the infection process of flaviviruses and produce mutants with attenuated phenotypes. To test this hypothesis, eight conserved hydrophobic residues, Ala^{54}, Val^{130}, Ile^{135}, Val^{188}, Ala^{261}, Val^{264}, Leu^{273}, and Leu^{276}, in the EDI-EDII hinge region of wild-type yellow fever virus Asibi strain were substituted with amino acid residues with polar side chains. Four single-site mutations, A54S, V188T, V264T and L273N, produced genetically stable mutants that did not contain compensatory mutations in the E protein. Multiplication curve experiments conducted in Vero and BHK-21 cells demonstrated that the E-V264T mutant produced significantly lower infectivity titers than the parental Asibi virus. Our findings suggest that substitution of conserved hydrophobic amino acids in the EDI-EDII hinge region is an effective strategy in generating genetically stable attenuated YFV mutants. Current studies are evaluating virulence of the mutants in mouse models.

0589

REEMERGENCE OF POWASSAN VIRUS LINEAGE 1 IN NEW YORK STATE

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Ixodes scapularis, the black-legged tick, is an important vector in the northeastern United States able to transmit up to seven human pathogens including Powassan virus (POWV). POWV was first isolated in 1958 in Ontario, Canada from a young boy with encephalitis. In 1997, a POWV-like agent was isolated from *I. scapularis* ticks in New England and determined to be genetically distinct from the original POWV isolate. This discovery revealed the existence of two lineages: lineage 1, prototype POWV (POWV-1) and lineage II, deer tick virus (DTV). It is suggested that POWV-1 is maintained in a cycle between *I. cookei* and groundhogs and *I. marxi* and arboreal squirrels, while DTV is found in an *I. scapularis* and small mammal host cycle similar to that of *Borrelia burgdorferi*. This distinction in nature suggests an evolutionary progression of POWV-1 into the *I. scapularis* transmission cycle and subsequent evolution into DTV. A majority of tick and mammalian host isolates from New York State are identified as DTV; however, for the first time in 30 years three new POWV-1 isolates were detected in both *I. scapularis* and *I. cookei*. This study aimed to understand the genetic differences between historic POWV isolates (POWV-1 and DTV) and our recent New York POWV-1 isolates and further investigate genetic determinants of host-specificity in both lineages. POWV-1 isolates LB (Ontario, Canada 1958) and 64-7062 (New York, 1964) were compared to our recent POWV-1 isolates through sequence analysis and replication kinetics in mammalian and *Ixodes spp*. cell lines. Our sequencing data revealed genetic distinctions between the New York POWV-1 isolates from both *I. scapularis* and *I. cookei* compared to the historic POWV-1 isolates. Additionally, the recent POWV-1 isolates showed a decrease in viral growth in mammalian cells compared to historic POWV-1 and DTV isolates. Of note, a new POWV-1 isolate from *I. cookei* displayed significantly decreased viral growth in mammalian cells but reached the highest titers in tick cell lines. These data suggest a reemergence of unique POWV-1 isolates in New York State that may display fitness costs due to host-specific adaptations.

0590

PREVALENCE OF SELECTED INFECTIONS IN PREGNANT WOMEN AND LIVEBORN INFANTS IN THE ZIKA EN EMBARAZADAS Y NIÑOS (ZEN) PROSPECTIVE COHORT IN COLOMBIA

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Some maternal and fetal infections are associated with adverse pregnancy and infant outcomes. However, little data exist on the frequency of these infections and their impact on birth outcomes in Colombia. The Colombian *Instituto Nacional de Salud* (INS) in collaboration with the U.S. Centers for Disease Control and Prevention (CDC) conducted a prospective cohort study in three regions in Colombia to study the prevalence of congenital infections and characterize adverse pregnancy and infant outcomes related to Zika virus (ZIKV) and other infectious agents. Between January 2017 and February 2018, pregnant women in their first trimester were recruited from 13 participating prenatal care clinics. All samples were processed to detect arbovirus RNA (dengue [DENV], ZIKV, and chikungunya) using CDC´s Trioplex assay. Maternal serum samples collected at study enrollment and delivery were also tested to determine the presence of ZIKV IgM and serologic response indicating recent cytomegalovirus (CMV) infection, rubella, toxoplasmosis, and syphilis. Available samples from fetuses and liveborn infants were tested by Trioplex and for ZIKV IgM. Presumptive ZIKV positive samples were determined by the presence of seroreactivity against ZIKV and the absence of seroreactivity against DENV. A total of 1,519 pregnant women and 1,108 liveborn infants were enrolled. Evidence of confirmed or presumptive ZIKV infection was present for 95/1,519 (6.3%) pregnant women and 42/1,108 (3.8%) liveborn infants. Evidence of active syphilis and possible acute toxoplasmosis infection was present in 16/1,519 (1.1%) and 3/1,519 (0.2%) pregnant women, respectively. Recent rubella and CMV infection was present in 2/1,519 (0.1%) and 12/1,519 (0.8%) pregnant women, respectively. Clinical review of adverse birth and infant outcomes is ongoing. This study provides epidemiologic data on the prevalence of congenital infections during the waning ZIKV outbreak in Colombia, which can be used to implement health interventions to mitigate their impact at individual and population levels.

0591

IMPACT OF MOSQUITO SALIVA IN THE PATHOGENESIS OF JAPANESE ENCEPHALITIS VIRUS

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Japanese encephalitis virus (JEV), a neurotropic flavivirus, is primarily transmitted through the bite of infected *Culex* species mosquitoes. During blood feeding, JEV-infected mosquitoes inoculate virus with salivary components into the vertebrate hosts. Mosquito saliva has been widely recognized to modulate the host's hemostatic, inflammatory, and immune responses. In comparison with needle inoculation, when arboviruses are delivered with mosquito saliva, this modulatory activity can result in differences in the severity and pathogenesis of the arbovirus infection. Whilst the modulation of flavivirus infections by mosquito saliva has been studied in various laboratory models, the majority of challenge studies involved in the pathogenesis of JEV have been conducted through virus-only needle inoculations. Using weanling pigs as a model for human Japanese encephalitis and immune response, the impact of mosquito saliva in JEV infection was investigated through the simultaneous co-injection of the virus and mosquito salivary gland extract (SGE), an established method to mimic mosquito feeding. Fever kinetics and duration of viral nasal shedding were altered by the presence of SGE. Interestingly, while general trends suggest the co-injection of JEV and SGE led to a lower viral load among tissues in the central nervous system, viremia, clinical signs, and tissue dissemination patterns were not affected. Our results provide us a better understanding of how mosquito saliva modulate the pathogenesis of JEV in its enzootic hosts.

0592

HUMAN NEURON INFECTION WITH ZIKA VIRUS AND HYBRID IGG4 ANTIBODY RESPONSE TO AEDES AEGYPTI SALIVARY PROTEINS

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Zika virus (ZIKV) and Dengue virus (DENV) are arboviruses transmitted by aedes mosquitoes including *Aedes aegypti.* ZIKV has been linked to the development of microcephaly in fetuses of infected pregnant women, along with other serious neurological complications. DENV is endemic in many of the regions affected by ZIKV, and it has been suggested that prior immunity against DENV may have an impact on ZIKV infection. The aim of this study is to determine whether pre-existing DENV antibodies bispecific for viral particles and *Ae. aegypti* salivary proteins act to decrease ZIKV infection of human neuron cells by activating anti-inflammatory pathways or increase infection through antibody dependent enhancement. Our results have shown that IgG antibodies against total *Ae. aegypti* salivary gland extract are much higher in exposed individuals living in DENV endemic areas than in non-exposed samples. In addition, there were fewer antibodies expressing λ light chain in exposed patients, indicating a bias in the immune response that is different than that seen in non-exposed individuals. Neuroblastoma cells infected with ZIKV strain MR-766 (original African strain) in the presence or absence of IgG4 antibodies were used to determine antibody effect on viral entry and replication. We found that

ATP levels were significantly greater in ZIKV infected neurons incubated with IgG antibodies, which correlates with previous studies suggesting that ATP production is increased during ZIKV replication. Thus, our preliminary data suggest there is a role of pre-existing DENV immunity in ZIKV neuronal infection through hybrid antibodies and establish a better understanding of the neurological pathology of this disease.

0593

MEASUREMENT OF ZIKA VIRUS-SPECIFIC ANTIBODIES USING A MICROSPHERE-BASED COMPETITIVE IMMUNOASSAY

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Zika virus (ZIKV) re-emergence and spread into flavivirus (FV) endemic regions have caused sero-diagnostic challenges due to antibody cross-reactivity among the FVs. Currently there are no viral envelope protein-based assays available to quantitatively measure ZIKV-specific antibody responses in individuals with prior FV exposure. Here, we describe the development of a ZIKV-specific competitive microsphere-based immunoassay to support ongoing clinical trials of Takeda's purified inactivated ZIKV vaccine (PIZV) candidate. The assay design allows ZIKV-specific antibodies present in plasma or serum samples to compete with the binding of an anti-ZIKV EDIII monoclonal antibody to a specific epitope on ZIKV virus-like particles that are coupled to Luminex magnetic microspheres. ZIKV-specific antibody levels in the presence or absence of antibodies to other FVs can then be determined in the samples. Human and non-human primate (NHP) sample panels with exposure to different FVs including ZIKV, dengue virus (all 4 serotypes), Yellow Fever virus, Saint Louis Encephalitis virus, West Nile virus, Japanese Encephalitis virus, and Tick-Borne Encephalitis virus were assessed using this assay. The assay quantified ZIKV-specific antibodies in all presumptive or confirmed ZIKV or PIZV-immune samples, while all other FV-immune samples were negative in the assay. Of note, the assay was also able to quantify PIZV-elicited ZIKV-specific antibodies in the presence of cross-reactive antibodies from prior FV vaccination. Collectively, our data suggest that the assay can differentiate ZIKV-specific antibodies from antibodies to other FVs elicited by natural infection or vaccination. Availability of a ZIKV-specific antibody-based immunoassay will improve differential diagnosis, serosurveillance and support development and implementation of ZIKV vaccines in FV endemic regions.

0594

IMPORTATION OF STAMARIL VACCINE PREVENTED A YELLOW FEVER VACCINE SHORTAGE IN THE UNITED STATES, 2017-2021

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In 2017, Sanofi Pasteur experienced an interruption in YF-VAX (Yellow Fever Vaccine) manufacturing, causing a stock-out in the United States (US). Sanofi Pasteur, FDA and CDC agreed to importation of STAMARIL (Yellow Fever Vaccine [Live]), through an Expanded Access IND Program (EAP), to ensure continued availability of yellow fever (YF) vaccine. STAMARIL is prepared by culturing the 17D-204 strain of YF virus in chicken eggs. It is approved in > 100 countries and has a long history of safety and effectiveness with over 600 million doses distributed since 1986. Travel clinics with YF vaccine-certified providers were invited to join the EAP based on location and prior YF-VAX usage. Participation required completion of training, IRB approvals and adherence to protocol requirements, including obtaining informed consent and reporting the following: doses administered, demographic information, suspected adverse reactions, serious adverse events, vaccination during pregnancy and vaccination of women who breastfed infants during the 14 days after vaccination. The EAP began in June 2017, enrolling > 250 US civilian travel clinics as EAP sites. As of June 2020, a total of 609,010 STAMARIL doses were administered. There have been seven cases of YF vaccine-associated acute neutrotropic disease (YEL-AND) and two cases of YF vaccine-associated acute viscerotropic disease reported (reporting rate: 1.1 and 0.3/100,000 vaccinees, respectively), which, with the exception of one case of YEL-AND, all occurred in individuals at increased risk. One vaccinee developed an anaphylactic reaction. No safety concerns were identified from inadvertent vaccine exposure during pregnancy or potential neonatal exposure via breast milk. No case of YF disease has been reported in a returning US traveler during the EAP. The EAP supported the public health need for YF vaccination by making STAMARIL available to travelers during a complete stock-out of the US-licensed YF-VAX. With over 600,000 doses administered, no new safety concerns were identified. Serious adverse reactions remain very rare and consistent with the known safety profile of STAMARIL.

0595

EVALUATION AND APPLICATION OF NOVEL SEROLOGIC TOOLS FOR ASSESSING ZIKA VIRUS SPECIFIC IMMUNITY FOR SURVEILLANCE AND TRANSLATIONAL RESEARCH

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Since the Zika epidemic first recognized in Brazil in 2015, transmission of Zika virus (ZIKV) continues to be a global public health threat. Although most ZIKV infections are mild, there is the risk of developing Guillain-Barré syndrome and adverse fetal outcomes from congenital infection. Although RT-PCR testing is available, ZIKV RNA is detectable during a narrow window. Serology can detect recent or remote infections, but traditional assays are complicated by cross-reactive antibodies elicited by other co-circulating flaviviruses like dengue virus (DENV). Thus, there is a critical need for serologic tools for reliable diagnosis and surveillance of ZIKV infection and to better understand protective immunity for vaccine development. We developed a blockade-of-binding (BOB) ELISA using A9E and G9E, two ZIKV envelope protein-binding monoclonal antibodies (mAbs), which are strongly and specifically neutralizing against ZIKV. We assessed BOB diagnostic performance by ROC curve analysis after running a panel of positive and negative control sera, classified by RT-PCR and/or neutralization assays. The A9E BOB ELISA has a sensitivity of 93.5% (95% CI: 79.3,98.9) and specificity 97.8 (95% CI: 92.2,99.6). The G9E BOB ELISA has a sensitivity and specificity of 100% (95% CI: 89.0,100.0) and 100% (95% CI: 95.9,100). We applied these assays to test samples from a cohort in Risaralda, Colombia and found that the assay sensitivity was similar, but specificity was notably reduced (A9E spec: 86%, G9E spec: 66%) due to a high proportion of multitypic DENV immunity. Finally, the assay was applied to samples from participants in a phase 1 RCT for a ZIKV DNA vaccine. Serum samples collected 30-day post-vaccination exhibited significantly less A9E and G9E BOB than those with natural ZIKV immunity (mean BOB % reduction: A9E: -90%, G9E: -94%). We are further testing the hypothesis that A9E and G9E BOB assays may identify a quality of neutralizing antibody response that will correlate with protective immunity against ZIKV and mapping BOB activity for additional ZIKV-neutralizing mAb in these sample sets and sera from an additional ZIKV vaccine trial.

0596

TRANSFER AND DECAY OF MATERNAL ZIKA EDIII ANTIBODIES IN A BIRTH COHORT FROM NICARAGUA

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Zika virus (ZIKV) exposure in utero is a cause of birth defects and neurodevelopmental disorders and incident ZIKV infection may also be detrimental to children. The role of antibodies induced upon in-uterus exposure or transferred from mothers to children in early life protection have been barely explored. A longitudinal cohort of 512 pregnant women was established in León, Nicaragua following Zika virus emergence in this country. A proportion of 253 of these women were pregnant mainly during the high Zika circulation (cohort 1) and 259 during low or no Zika circulation (cohort 2). Blood and umbilical cord samples were taken from mothers and their children during delivery. Subsequently, the children were followed up every 3 months up to 12 months. Serological tests based on ELISA to detect anti-ZIKV IgG and anti-ZIKV envelope domain III (Z-EDIII) were performed to investigate antibody transfer and decay over time. A 2-fold OD increased between 3 and 6 and between 6 and 9 months were defined as incident ZIKV infection. There was strong correlation between the maternal blood and cord blood anti-ZIKV and Z-EDIII IgG antibodies (p <0.0001). Also, Z-EDIII IgG is specific for ZIKV, and dengue-elicited antibodies do not bind Z-EDIII. A higher frequency of children (78.8%) from cohort 1 have Z-EDIII antibodies in their cord-blood as compared with 64.7% of children from cohort 2 (OD geometric mean 0.820 vs 0.480, p <0.0001). In cord blood, Z-EDIII IgG OD mean declined from 1.070 in cohort 1 to 0.700 in cohort 2 and from 0.470 and 0.390 in the first three months of life. In the group of children whit no Z-EDIII antibodies 22 from cohort 1 and 18 from cohort 2. Incidental ZIKV infections were observed in 27.3% (6/22) of the children in cohort 2 between 6 to 9 month of life, as compared 5.5 % (1/18) in the children from cohort 1 between 3 – 6 month of life. This study shows the transplacental transfer of specific antibodies against Zika during the epidemic in Leon, in addition to the decay of these antibodies at 3 months and the increase at 6 to 9 months in almost 1/3 in children with no Z-EDIII antibodies.

0597

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

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We have characterized the immune response to ZIKV infection and vaccines by epitope mapping over 90 anti-ZIKV MAbs at amino acid-resolution, using a comprehensive ZIKV prM/E library of 672 single alanine mutants expressed in human cells. Published studies include epitopes of MAbs isolated from a Brazilian patient, with a highly neutralizing MAb protective in animal models of ZIKV fetal disease. Epitope locations suggest that some MAbs act by binding across adjacent E proteins, preventing rearrangements necessary for ZIKV infectivity. Mapping also reveals epitopes specific for ZIKV or common to DENV, information that can help create better vaccines and therapeutics. To provide critical reagents, we developed ZIKV reporter virus particles (RVPs) capable of one round of infectivity, with luminescent or fluorescent readout, and demonstrated reproducible neutralization titer data (NT_{50} values) across different RVP production lots, volumes, time frames, and laboratories. We have also identified mutations that increased ZIKV RVP budding, which may aid the design and production of anti-ZIKV vaccines, by screening each individual ZIKV library prM/E variant for ZIKV particle budding and infectivity. We also isolated anti-ZIKV MAbs for ZIKV-specific immunodetection, diagnostic applications, and ZIKV neutralization studies. After immunization with DNA and sub-viral particles, and phage library panning with RVPs, we isolated 48 ZIKV-specific conformational MAbs against prM/E, including one that potently neutralized ZIKV RVPs (IC_{50} 45 ng/ml) with a quaternary epitope spanning adjacent E proteins. We have also used ZIKV RVPs to identify cellular receptors and attachment factors that enable ZIKV entry. ZIKV RVPs were tested on our Membrane Proteome Array (MPA), comprising 6,000 unique human membrane proteins individually expressed in live human cells. Known receptors and attachment factors were identified (validating the approach), as well as a number of membrane proteins not previously known to enable ZIKV entry. These newly identified proteins help explain viral tropism and pathogenesis, and may be useful as therapeutic targets.

0598

ZIKA VIRUS EFFECTS ON CREB3L1 NEUROPROTECTION

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Zika virus (ZIKV) flavivirus can be vertically transmitted and cause congenital Zika syndrome. It reduces the ability of human fetal nerve cells to survive or trigger self-repair mechanisms. Human neural progenitor cells' (hNPCs) ability to induce neuroprotective effects may be affected due to its permissiveness to ZIKV. Up-regulated cAMP responsive element binding protein 3-like 1 (CREB3L1) have been reported in hNPCs, suggesting this neuroprotective pathway is altered in nerve cell survival processes during an infection. CREB3L1 is synthesized endogenously as a membrane-bound precursor outside the nucleus. Later on, gets translocated into the nucleus where it activates transcription of genes encoding cell cycle inhibitors to block proliferation of the cells infected by ZIKV. The objective of our study is to determine the effects a ZIKV infection has on CREB3L1 by evaluating its protein and gene expression levels depending on the cellular compartment location and viability state. SHSY-5Y neuroblastoma cells were infected with ZIKV at a multiplicity of infection (MOI) of 0.1 for 48 hours (h). The gene expression of CREB3L1 pathway was assessed using qRT-PCR and western blot to assess the protein levels. The main mediators of CREB3L1 showed differences in protein expression in the nucleus/cytoplasm compared to controls and no alterations in their RNA levels. However, no alterations were observed in viable cells compared to apoptotic cells in any sample. Preliminary results indicate that a ZIKV infection alters CREB3L1 pathway at a protein level depending on the cellular compartment in ZIKV infected SH-SY5Y cells 48 h post infection. Analyses on protein and RNA levels will be optimized to assess the alteration of the CREB3L1 pathway in a time dependent manner. We will further evaluate CREB3L1 role in SH-SY5Y cells during a ZIKV infection.

0599

UNIQUE INTERACTIONS BETWEEN VIRAL GENOTYPE AND MOSQUITO POPULATION DETERMINE REGIONAL VARIABILITY IN THE EFFECT OF INCREASED TEMPERATURE ON VECTORIAL CAPACITY OF CX. PIPIENS FOR WEST NILE VIRUS

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Arthropod-borne viruses are associated with over 140 human diseases. The most common arboviruses vectored by mosquitoes are those which belong to the *Flaviviridae* family, including West Nile virus (WNV), Zika virus (ZIKV) and dengue virus. Global temperatures are increasing, which directly impacts arboviruses vectored by ectothermic organisms. There is a correlation between increases in WNV prevalence and increased temperature in New York State, but the extent to which this effect varies among populations and regions is not fully understood. Temperature has been shown to influence blood feeding, fecundity, development, survival and vector competence, yet this influence is variable among *Culex* spp. populations. This variation suggests that there is a genetic basis that drives a heterogeneous relationship among temperature and flavivirus prevalence and that temperature sensitivity will likely be subject to evolutionary pressures with changing environments. The purpose of this study is to assess complex interplay among population–by–genotype–by-environment interactions in NYS. To test that variability in temperature sensitivity exists in naturally occurring populations and among viral strains, two unique *Culex pipiens* populations from upstate and downstate NYS were reared at mean temperatures of 22, 25 or 30°C, mimicking current and future regional means. Life history traits were monitored at each temperature for both populations and vector competence was assessed using representative historic and contemporary WNV strains. This data was used to calculate vectorial capacity of these unique *Cx. pipiens* populations at varying temperatures. Overall, Culex populations were found to be unique in their capacity to vector WNV at increased temperature. Accelerated development time and decreased survival were identified at higher temperatures, yet the extent of this effect was population-dependent. Temperature effects on vector competence were also found to be both strain and population-dependent. These differences will likely contribute to regional differences in transmissibility under climate change.

0600

FLAVIVIRUS SEROPREVALENCE AND CONTINUOUS TRANSMISSION OF WEST NILE VIRUS AMONG FEMALE SEX WORKERS IN SENEGAL BETWEEN 1991 AND 1999

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West Nile Virus (WNV) is a flavivirus known to circulate in the African continent, including the West African country of Senegal. Seroprevalence studies of WNV have been reported in various animal species, however, the seroprevalence and transmission of WNV in humans remains elusive. We recently used Western blot (WB) analysis containing antigens of 6 flaviviruses (dengue virus serotypes 1 to 4 [DENV1-4], WNV and Zika virus [ZIKV]) to study antibody responses following confirmed flavivirus infections. We found that anti-premembrane (prM) antibodies can discriminate WNV, DENV and ZIKV infections with a sensitivity/specificity of 88.9/96.5%, 89.5/100% and 100/98.6%, respectively (Hsieh et al. submitted). In this current research we employed WB analysis to investigate the seroprevalence of WNV in a previously described female sex worker cohort in Dakar, Senegal between 1992 and 2004. After initial DENV WB screening of the latest 236 samples, we identified 109 samples that were positive for the cross-reactive flavivirus envelope protein. Further WB analysis with 6 flavivirus antigens revealed 24 WNV infections (21 WNV and 3 WNV/DENV infections), 32 DENV infections, 5 ZIKV infections, and 3 DENV/ZIKV infections. From those 24 WNV infections, previous blood draws 6 to 36 months prior to the latest samples were tested by both WB and WNV virion-IgG ELISA. WB analysis identified 2 seroconversions between 1991 and 1999, which was further confirmed by ELISA. The seroprevalence of WNV in this cohort was 11.0%, and the seroconversion rate was 0.8%. We also noted one possible reinfection with WNV, further implicated by a four-fold increase in WNV antibodies from an endpoint titer experiment. The seroprevalence of DENV and ZIKV were 16.1% and 3.4%, respectively. These findings demonstrate continuous transmission of WNV in humans in Senegal. We intend to test the rest of the samples from each individual for seroconversions and reinfections with other flaviviruses as well.

0601

EXPERIMENTAL FITNESS EVALUATION OF THE MAIN WEST NILE VIRUS GENOTYPES IN THE UNITED STATES USING REVERSE GENETICS

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Following the initial introduction of West Nile virus in New York in 1999, the virus rapidly emerged and spread across the United States. Evolution may be at least partially responsible for the rapid expansion and subsequent outbreaks, but this process is not well defined. Even the strongest current evidence for West Nile virus adaptation, the displacement of the original genotype (NY99) by locally derived genotypes (WN02 and SW03), requires further investigation. Here, we aimed to investigate if the West Nile virus genotypes WN02 and SW03 may have displaced the original NY99 genotype due to vector- or host-specific transmission advantages. The commonly cited hypothesis is that the emergence and spread of West Nile virus in the United States was facilitated by adaptation to local mosquito vectors. A key reason why there is a discrepancy in comparing the mosquito transmission potential between NY99 and WN02 is likely because previous studies have used different West Nile virus isolates. While all of these studies provided valuable information about West Nile virus fitness, it is difficult to discern the impact of any single amino acid substitution. Therefore, our approach was to use reverse genetics to engineer the genotype-defining amino acid substitutions (WN02: E-V159A; SW03: NS4A-A85T and NS5-K314R) into a NY99 West Nile virus infectious clone to examine their phenotypic impacts in *in vitro* and *in vivo* transmission models. Examining transmission phenotypes within mosquito and avian model systems allows us to understand the role of evolution during spread and establishment of West Nile virus.

0602

FORECASTING MUTATIONS OF CONCERN: A SURVEY OF SARS-COV-2 INTRA-HOST VIRAL DIVERSITY ACROSS THE NCBI SEQUENCE READ ARCHIVE DATA

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Despite increased sequencing and surveillance of SARS-CoV-2 across the world, timely prediction of the emergence and spread of novel variants of concern remains a great challenge. Thus, development of tools that can give an early warning of sudden increases in variant prevalence remains a high priority. The amount of publicly available SARS-CoV-2 raw whole genome sequencing datasets has become extremely large. As of April 2021, over 500,000 raw genome sequencing runs are publicly available in the NCBI SRA repository. We sought to survey these data for intra-host mutations of concern and explore if any patterns may predict emerging mutations of concern. We developed a bioinformatics pipeline using kmer methods to relatively quickly and efficiently screen the large number of samples in the NCBI SRA to retrieve samples that contained intra-host mutations belonging to the lineages of concern. Comparing across months revealed that certain mutations of concern surge in frequency as intra-host variants just prior to or while lineages of concern arose. For instance, we detected that the N501Y mutation that is nearly fixed in the B.1.1.7, P.1, and B.1.351 lineages of concern was present as a minor variant in a high number of samples in October 2020 (834 samples). This coincides with timing of the first detected samples within the B.1.1.7 and B.1.351 lineages. Our analysis detected another mutation, L452R, which is nearly fixed in the B.1.429 and B.1.427 lineages of concern was present as a minor variant in 68 samples in September 2020 prior to the rise of the B.1.429 and B.1.427 lineages in late 2020. Other clinically important mutations such as E484K, were not detected as minor variants prior to the emergence of this mutation in several lineages of concern. Detecting increases in intra-host minor variant prevalence prior to their increase on the consensus global levels might provide an early forecast, thus giving additional time to adjust public health policies and control measures prior to variant spread, as well as providing additional time for testing of phenotypic/functional changes these mutations might induce.

0603

VALIDATION OF MACHINE LEARNING MODELS FOR OUTCOME PREDICTION IN LASSA FEVER PATIENTS USING THE LARGEST RETROSPECTIVE CLINICAL DATASET AVAILABLE TO DATE

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Recent Lassa fever (LF) outbreaks of increasing severity in Nigeria highlight the need for reproducible data-driven methods to inform better control and treatment. We present a retrospective, observational cohort study that validates prognostic models using the largest LF patient dataset to date, collected at the Irrua Specialist Teaching Hospital (ISTH), in Edo State, Nigeria, during standard clinical care. Data includes demographic information, clinical symptoms, vital signs, laboratory results, and treatment interventions for patients who tested positive for LASV with RT-PCR and were admitted to ISTH from 2016 to 2020. Of 727 patients enrolled in the study, 620 patients with known outcome (died or survived) were included in the analysis. The median age was 30 years and 39.19% (243/620) were female. The overall case-fatality rate was 18.54% (115/620). Age was strongly predictive of mortality, with a 1.42 increase in risk for each 10 years of age ($p<10^{-5}$). The complications most associated with adverse outcome were bleeding (odds ratio [OR] 10.16, $p<10^{-5}$) and renal failure (OR 3.36, $p<10^{-5}$). We trained several models on data collected at ISTH between 2011 and 2013, comprising 284 cases with known outcome. Predictor variables in these models were selected under the premise that physicians order laboratory tests to investigate two distinct clinical scenarios: (1) inflammation and end organ damage and (2) electrolyte imbalances. The two best performing models use age, severe central nervous system symptoms, and bleeding as clinical predictors together with creatinine and aspartate aminotransferase in model 1, and creatinine and potassium in model 2. The accuracy on the new 2016-20 validation data is comparable to the accuracy on the 2011-13 training data: 87% vs 83% for model 1 and 83% vs 82% for model 2. Other performance measures (AUC, sensitivity, specificity) show a similar trend. The prediction threshold can be adjusted according to the clinical requirements for sensitivity and specificity. Our results support the use of these models as validated tools for risk stratification and could be further improved by recalibration on the new data.

0604

GETTING READY FOR THE NEXT EBOLA OUTBREAK: A PREPAREDNESS TOOL TO PREDICT THE EFFECTS OF CONTACT TRACING, CASE ISOLATION, AND SAFE BURIAL CEREMONIES

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Ebola outbreaks in West Africa in 2014 followed by those Uganda and DRC in 2016 challenged the global health community. Ebola virus disease (EVD) is a severe zoonotic filovirus infection caused by viruses of the genus Ebolavirus, with the Zaire Ebola Virus (ZEBOV) being the deadliest species. EVD is one of the deadliest known viral infections, with a fatality rate that varied between 25%-90% in the past outbreaks. Ebola spreads by smear infections and is most contagious in the late stage of the infections, the corps of deceased individuals remain highly infectious. During the initial phase of an epidemic, public health-emergency management needs to respond swiftly, in the absence of reliable epidemiological data and adequate treatment and vaccination infrastructure. Preparedness tools such as predictive models help to support decision-making by assessing the impact of potential control interventions. In the case of EVD, contact tracing and systemic isolation of infected individuals from the general population have been the cornerstone of the surveillance strategies with the aim to curtail its spread at the early stage of the epidemic. Since the major outbreak of 2014, different models have been developed to predict the impact of different control interventions, e.g., case isolation, contact tracing, assuming different transmission scenarios, e.g., safe and unsafe burial ceremonies. However, the combination of these interventions and scenarios has not been comprehensively studied. Here, we use an extended SEIR model to study the effect on the spread of EVD accounting for combinations of different control interventions and transmission scenarios. Novel to the model is its realistic response dynamics, that eliminates the naïve exponentially-distributed waiting times that typically lead to inaccurate quantitative predictions. We illustrate the model using parameters obtained from the literature (from the West African and DRC outbreaks), for a range of intervention scenarios. The results suggest that the effect of case isolation is substantially improved by combining isolation with contact tracing and safe funeral ceremonies.

0605

IMMUNOLOGICAL EVALUATION AND COMPARISON OF DIFFERENT CACHE VALLEY VIRUS VACCINE CANDIDATES

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Cache Valley virus (CVV; genus *Orthobunyavirus*, family *Peribunyaviridae*), first isolated in Cache Valley, Utah in 1956, is now enzootic throughout the New World. Although transmission of CVV occurs through an enzootic cycle including more than 30 species of competent arthropod vectors, serological evidence has indicated white-tailed deer as the amplification host for the virus in nature. Whilst CVV is primarily known to cause severe disease in pregnant ruminants resulting in abortions, fetal malformations, and embryonic lethality, it has also been recently recognized for its expansion as a zoonotic pathogen. Other viruses in the bunyamwera serogroup are thought to be of minimal concern. However, during an outbreak of Rift Valley fever virus, Ngari virus was found to have infected some of the goats being tested, and Oropouche virus, has been responsible for febrile disease outbreaks in Central and South America. With this increased emergence of bunyaviruses with human and veterinary health importance, there have been significant efforts dedicated to the development of bunyavirus vaccines. In this study, immunogenicity of a CVV vaccine candidate based on the deletion of NSs and NSm genes was evaluated and compared to a vaccine candidate created through the inactivation of CVV using binary ethylenimine with the addition of an aluminum hydroxide adjuvant in sheep. Immunization of 20 sheep with one of the two vaccine candidates was performed followed by two booster immunizations. Plaque reduction neutralization test was then used to monitor the development of neutralizing antibodies elicited by each vaccine candidate. The development of a vaccine for ruminants could lead to less human exposure and a platform for CVV and other emerging bunyaviruses that have already or could potentially cause future outbreaks.

0606

ENSEMBLE MACHINE LEARNING METHOD AS A HIGH ACCURACY APPROACH TO COVID-19 SIMULATION

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The COVID-19 pandemic shows that only timely implemented scientifically based measures can stop it. Mathematical modeling is an effective tool for studying the epidemic process. However, models suffer from low accuracy and are short-term in nature. Ensemble machine learning methods help optimize predictive models by combining several base models of epidemic processes. Different modeling approaches have different disadvantages, but their combined use makes it possible to eliminate these drawbacks. We used ensemble machine learning models to see if we could improve the accuracy of COVID-19 morbidity forecasting using data provided by the Coronavirus Resource Center of John Hopkins University & Medicine. The result was a cluster analysis method grouping countries based on similar COVID-19 morbidity characteristics, forecasting COVID-19 morbidity for each group using neural network, and combining the results into one country database. For clusterization we developed a neural network with 60 input neurons, 100 hidden neurons with Fermi activation function, and 4 output neurons. For forecasting, a neural network was built with 6 pairs of layers Dense with an activation function ReLu and 64 neurons, a layer Dropout and the last layer Dense with one output, RMSProp method as optimizer and Mean Squared Error as error function. The final ensemble machine learning model was realized using Python programming. Employing ensemble machine learning model, we improved the accuracy of the forecast of COVID-19 morbidity to 98.11% compared to other models with accuracy of 85.12% (compartment models) or 87.30% (machine learning based models). High accuracy forecasting will improve the investigation of short-term dynamics and long-term trends of COVID-19 epidemic processes. In turn, this will allow for timely implementation of scientifically grounded measures to decrease COVID-19 dynamics. These results indicate that ensemble machine learning is useful tool for understanding the main drivers of COVID-19 dynamics and may better inform decision-making processes to prevent new waves of morbidity and deterioration of the epidemic situation.

0607

COVID-19 SEROSURVEYS IN MASSACHUSETTS: CALL FOR ACTION

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Seroprevalence studies (i.e. serosurveys) are essential public health tools that help determine the extent of an infectious disease outbreak and map its distribution among specific populations. COVID-19 serosurveys are particularly important, because (i) they capture asymptomatic infections, which are thought to account for 40-45% of all SARS-CoV-2 infections, and (ii) they indicate the level of existing immunity among affected populations. We implemented a laboratory developed test (LDT) that measures IgM, IgG and IgA antibodies by ELISA against the SARS-CoV-2 Spike Receptor Binding Domain (RBD) to determine the seroprevalence among health care workers (HCW) and return-to-work employees (RTW) at the University of Massachusetts Medical School. We enrolled and collected blood from 553 HCWs between April 27 and June 4, 2020 and 335 RTWs between Aug 3 and Aug 6, 2020. Our initial findings based on RBD IgG antibody levels, which is the most specific compared to PCR-confirmed cases, found a 14.1 % and 5.3 % seropositivity among HCWs and RTWs, respectively. The correlation between IgG and IgA levels was modest (r=0.4, Spearman Correlation, p<0.0001), supporting other studies that have shown divergent development of IgA in lieu of IgG for this respiratory infection. In contrast, IgM was the least sensitive and specific as a serosurveillance metric. As expected, we found that the overall positivity was higher among HCWs who were at higher risk of SARS-CoV-2 exposure as compared to employees who had been working remotely. This LDT ELISA has also been adapted for nucleocapsid and spike trimer protein in order to compare the sensitivity and specificity of each antigen and isotype, alone and in combination. In summary, we found that most of the HCWs and RTWs who enrolled in our study did not have antibodies to RBD, emphasizing the need for vaccines in order to reach population-wide immunity. This study shows that it is possible for academic medical centers to rapidly deploy LDTs and contribute to public health surveillance strategies for COVID-19.

0608

UTILIZATION OF A PRIMER-TEMPLATE MISMATCH APPROACH FOR PCR-BASED IDENTIFICATION OF TWO CRITICAL SPIKE MUTATIONS IN THE SARS-COV-2 VARIANT B.1.1.7

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the pathogenic agent for coronavirus disease 2019 (COVID-19), an acute respiratory disease that threatens human health and public safety. The spike protein on the virus surface plays a key role in the pathogenesis of COVID-19. During the COVID-19 pandemic, SARS-CoV-2 viruses have acquired mutations in the spike protein gene and other parts of its genome, giving rise to many new lineages/variants. The B.1.1.7 variant was first identified in the United Kingdom and is currently reported in 94 countries, as well as being dominate in the US due to its 59~74% increase of transmissibility. Among nine mutations (two deletions and seven non-synonymous mutations) in the spike gene of B.1.1.7, del HV 69-70, N501Y, and P681H impact the 3D structure of the protein and its interaction with the host receptor, ACE2, and neutralizing antibodies. Like del YY 144-145, A570D, T716I, S982A and D1118H, del HV 69-70 has not been detected in any other SARS-CoV-2 variants. Using a primer-template mismatch approach, we developed PCR assays (conventional PCR and SYBR Green-based quantitative PCR) that effectively identify del HV 69-70 and N501Y, two critical spike mutations in B.1.1.7. Further optimization of reverse transcriptase quantitative PCR (RT-qPCR) conditions will enable us to detect B.1.1.7 variants without the need for Next Generation Sequencing (NGS). Although NGS is recognized as the gold standard for identifying new SARS-CoV-2 variants, this platform may not be readily available and practical for enhanced temporal monitoring to detect new variants, especially in resource-challenged settings. Therefore, application of the RT-qPCR assays for clinical samples can greatly facilitate the detection of B.1.1.7 variants.

0609

IDENTIFICATION OF SARS-COV-2 RNA IN STILLBIRTHS AND NEONATAL DEATH CASES IN BANGLADESH

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Since the COVID-19 pandemic began, the toll of SARS-CoV-2 in human lives has been extraordinary. Studies reveal that age is by far the strongest predictor of the risk of dying- with older adults at the highest risk and children usually spared from severe disease. Knowledge regarding the effects of SARS-CoV-2 infection on fetuses and neonates is very limited. Child Health and Mortality Prevention Surveillance (CHAMPS) aims to understand the causes of stillbirths and under-five deaths in sub-Saharan Africa and South Asia. Laboratory diagnosis (post-mortem specimen - blood, CSF, nasopharyngeal swabs, lung tissue), verbal autopsy, and clinical data from each death are reviewed by an expert panel (DeCoDe) to determine the cause of death. From September 2020 to March 2021, a total of 62 cases from stillbirths and deceased neonates were retrospectively tested using real-time RT-PCR for SARS-CoV-2 N- and RdRp-genes. Of those, four nasopharyngeal swabs were positive, including three early neonates (2-4 days) and one late neonate (8 days). Among other specimens, three blood, two CSF, and one lung tissue were positive. Overall, 6 (9.7% of 62 with all specimens tested) cases had at least one specimen positive. Five of the six positive cases were detected during January-February 2021. Positive cases included two stillbirths, three early neonatal and one late neonatal death. Most of the positive specimens showed Ct values >30, indicating a very low viral load. However, the late neonate was positive for all specimens tested, and the NPOP sample had the lowest Ct value (Ct=14.1). The spike gene sequence analysis revealed its similarity with the Wuhan D614G variant. One of the cases had gone through the DeCoDe, and SARS-CoV-2 was decided to be the co-morbid factor. CHAMPS project has a unique opportunity to relate microbiological findings with histopathological changes and thus explore the association of SARS-CoV-2 with child death and manage COVID-19 infected children during the early days of life.

0610

THE PRESENTATION OF INFLUENZA AND CLINICAL SEVERITY IN KAMPHAENG PHET HOSPITAL, THAILAND

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Influenza is clinically indistinguishable from other respiratory viruses. The department of Virology, USAMD-AFRIMS has conducted influenza surveillance from April 2012 to present. The demographic and clinical data then nasal and throat swabs were collected to identify Flu A (pdmH1 and H3) and Flu B influenza subtypes and measured by cycle-to-threshold (Ct) value. This presentation analyzed the clinical and laboratory findings of different severities of influenza. We enrolled 2,136 subjects, of which 906 (42.4%) tested positive for influenza by RT-PCR from April 2012 to March 2021. Among these, the median age was 8.6 years (range from 6 months-72 years), 53.5% were male. Fever duration from onset until presentation at hospital was 1.9 days (range from 0 to 5 days). Influenza severity is classified into upper (URI) and lower (LRI) respiratory infection by either chest x-ray findings or clinical diagnosis; 89.1% of subjects were diagnosed with an URI. RT-PCR those samples: 36.7% were Flu B, 32.2% were Flu A/H3, 30.7% were Flu A/pdmH1, and 0.4% were Flu A and Flu B co-infections. The data were analyzed and compared between groups by non-parametric statistics. The baseline age and gender did not differ between groups ($p = 0.116$ and 0.089). The fever duration was significantly different ($p<0.05$) at 1.9 days in URI and 2.4 days in LRI. The overall Ct values of upper and lower respiratory diagnosis were significantly different ($p< 0.05$) at 20.5 [8.4, 36.7] and 23.7 [14.3, 35.6] respectively. Specifically, the URI and LRI Ct values of Flu B were 18.4 [8.4, 34.9] and 21.6 [14.3, 32.7], Flu A/H3 were 19.2 [10.1, 34.0] and 22.3 [14.3, 33.6], and Flu A/pdm H1 were 24.5 [15.9, 36.7] and 26.5 [19.6, 35.6] respectively. There were significant Ct value differences between the URI and LRI for each subtype at $p < 0.05$. URIs had significantly shorter fever duration and lower Ct values in all subtypes than LRIs, which suggests that nasal and throat swabs may be more appropriate to measure upper respiratory tract viral infection than the lower ones. These findings could potentially be utilized in formulating control strategies for emerging disease such as SARS-CoV-2 in the near future.

0611

CHARACTERIZING THE HUMORAL RESPONSE OF A RECOMBINANT SUBUNIT EBOLA VIRUS VACCINE IN NON-HUMAN PRIMATES

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Ebola virus (EBOV) causes lethal hemorrhagic fevers with case fatality rates of up to 90%. Outbreaks are sporadic and unpredictable and vaccination has been a much awaited tool to controlling spread of disease. However, due to limited stocks of approved vaccines and requirement of stringent and ultra-cold cold-chain storage, there is a need to continue developing new, more practical vaccine candidates. Although all vaccine candidates use the surface glycoprotein as the main antigen, the modality by which protection is induced by the EBOV glycoprotein remains unclear. We have

developed a recombinant subunit vaccine that combines an increased safety profile with excellent thermostability, allowing easier deployment in endemic regions. Our vaccine has shown full protection in non-human primates, however, we have yet to fully characterize the immune responses elicited by our vaccine with a focus on identifying correlates of protection. Subunits of the EBOV glycoprotein (GP) have been produced in *Drosophila* S2 cells including full length GP, GPΔmucin-like domain, GP1, GPcl and sGP. These subdomains were used to characterize the binding fractions of antibodies elicited by our GP subunit vaccine. The various Ebola GP subunits were used in a multiplex immunoassay format and detect antibody populations from non-human primate sera. Approximate concentrations of antibody fractions in sera binding to individual subunits were calculated. We found more consistent humoral responses in NHPs receiving 3 doses over 2 as well as a shift towards stronger binding of the GPΔmucin-like domain one year after immunization. Ongoing analysis focuses on samples from NHPs immunized with EBOV GP + adjuvant that will be analyzed to differentiate the pre-challenge antibody binding patterns to the different EBOV subdomains. These binding patterns will be compared to neutralization titers and survival data to reveal subdomains that are more protective. This can be used to guide further vaccine development and provide insights into correlates of protection that are still unknown for filoviruses.

0612

RESOURCE CONSTRAINTS IN AN EPIDEMIC: A GOAL PROGRAMMING AND MATHEMATICAL MODELLING FRAMEWORK FOR OPTIMAL RESOURCE SHIFTING IN SOUTH AFRICA

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The COVID-19 pandemic has had devastating consequences across the globe, and has led many governments into completely new decision making territory. As such, developing models which are capable of producing realistic projections of disease spread under extreme uncertainty has been paramount for supporting decision making on many levels of government. In South Africa, this role has been fulfilled by the South African COVID-19 Modelling Consortium's generalised Susceptible-Exposed-Infectious-Removed compartmental model, known as the National COVID-19 Epi Model. This project adapts and contributes to the Model; its primary contribution has been to incorporate the notion of limited capacity, which allows for a more holistic and accurate picture of the resource-scarce context of a pandemic. This project further designs and implements a goal programming framework to shift ICU beds amongst districts in a way that aims to minimise deaths caused by the non-availability of ICU beds. The results showed a drastic decrease in lives lost when ICU beds were shifted under various scenarios. Although there are limitations to the scope and assumptions of this study, it demonstrates that it is possible to combine mathematical modelling with optimisation in a way that may save lives through optimal resource allocation.

0613

PREDOMINANCE OF SARS-COV-2 B.1.1.7 AND B.1.351 VARIANTS IN METRO MANILA, PHILIPPINES

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The first case of the SARS-CoV-2 B.1.1.7 variant was detected from a returning Filipino who arrived at the Philippines on 7 January 2021 and y March 2021, B.1.1.7, B.1.351, and P.1 variants were detected with local transmission of the B.1.1.7 and B.1.351 variants. Here, we report the molecular evolution of SARS-CoV-2 in the Philippines and document viral lineages and introductions events into the country. NP/OP swabs were collected at the V Luna Medical Center from April 2020-April 2021. PCR testing was done at the VLMC Molecular Laboratory or the AFP-AFRIMS Collaborative Molecular Laboratory. SARS-CoV-2 positive samples were sequenced at the AFP-AFRIMS Collaborative Molecular Laboratory and at the Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand using Illumina Iseq100 or MiSeq. Reference mapping was performed by BWA-MEM aligner using Wuhan-Hu-1 genome sequence(NC_045512.2) as the reference sequence. Primer regions trimming and call variants (Q ≥25) were performed using iVAR v.1.2.2 and samtools, respectively. Consensus sequences were generated by iVAR v.1.2.2 (Q ≥25 and depth of coverage≥10). Gaps, deletion, and ambiguous bases were identified and confirmed by genome-guided assembly with the reference sequence using Trinity v2.8.5 and Sanger sequencing. Lineage and clade were determined using Pangolin v.2.3.8, GISAID clade nomenclature, and phylogenetic analysis. We used MEGA 7.0 to examine nucleotide and amino acid substitutions. All tools were run with default parameters. A total of 77 SARS-CoV-2 genome sequences were generated. Our data shows multiple introduction events into the country. All of the sequenced samples from March-April 2021, were either B.1.1.7 aor B.1.351 variants which provides evidence of sustained community transmission of these variants. The surge of COVID-19 cases starting early March in the National Capital Region and surrounding provinces may be explained by the increasing prevalence of these variants in addition to the relaxation of quarantine measures, increased mobility of the population and lower compliance with health standards concurrent with the opening up of the economy.

0614

PROFILING OF EPITOPE-SPECIFIC ANTIBODY RESPONSES AND BIOMARKER DISCOVERY IN VIRAL AND PARASITE INFECTIONS UTILIZING HIGH-DENSITY PEPTIDE ARRAYS

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Sensitive diagnostic tests and the identification of targets for vaccine development are fundamental to fight against infections. The significance of humoral responses is multi-facetted. Antibodies do not only play an important role in combating a wide range of infectious diseases but can also be utilized for serology. Hence, a comprehensive analysis of humoral responses can ultimately uncover novel target antigens of protective immune responses and disease-specific biomarkers for the development of innovative serological tests. High-density peptide arrays can display large numbers of antigen proteins translated into overlapping peptides. Antibody responses to linear and conformational epitopes can rapidly be analyzed yielding high and low immunogenic specific epitopes. As examples, we chose SARS-CoV-2, HEV and *Plasmodium* infections to conduct extensive epitope mappings for antigen characterization and biomarker discovery. Profiling epitope-specific antibody responses of COVID-19 patients with mild versus life-threatening disease progression revealed significant epitope reactivities associated with severe COVID-19.

The herein identified epitopes may serve as serological markers able to discriminate severe from mild disease courses. In case of *Plasmodium* infections, we analyzed the antibody responses of individuals from malaria-endemic areas to better characterize epitopes associated with naturally acquired immunity. The results show distinct antibody patterns according to immune status of infected individuals. For HEV, we discovered common immunogenic regions in patients that clearly distinguish infected from non-infected individuals. The corresponding peptide epitopes can potentially serve as starting points for peptide based diagnostic tests. In conclusion, high content peptide microarrays are an ideal tool for the identification of reliable epitope-based biomarkers for infectious diseases. The discovery of novel linear or conformational epitopes can lead to the development of future multiplex serological assays and novel vaccine candidates.

0615

GENOMIC SURVEILLANCE OF HUMAN CORONAVIRUSES SARS-COV-2 AND 229E AT THE UNIVERSITY OF MARYLAND CAMPUS

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SARS-CoV-2 pandemic has led to a renewed interest in the spread of seasonal human coronaviruses, as the latter may give insights into the patterns of SARS-CoV-2 transmission. We investigated genomic epidemiology of human coronavirus 229e collected from cohorts of students on the University of Maryland College Park (UMD) campus two consecutive years prior to the SARS-CoV-2 pandemic, in 2018 and 2019, as well as genomic epidemiology of SARS-CoV-2, collected June-October 2020 from students, faculty, staff and members of surrounding communities. We performed phylogenetic analyses of the UMD SARS-CoV-2 and 229e full genomes, and the S gene for 229e, in combination with reference genomes. Coronavirus 229e analyses indicated at least two separate introductions of the virus, all resulting in successful spread to three or more individuals within the student cohorts. Epidemiological information revealed eight contacts, confirming all 2018 genomic connections, and confirming five connections in 2019. Genomic clustering indicated that the six remaining individuals also belonged to the same 2019 cluster, thus pointing to missing epidemiological links. SARS-CoV-2 analyses indicated five separate introductions, with two of the introductions resulting in clusters of four or more individuals. A major cluster comprising of nine individuals sampled in Sept-Oct 2020 also contained a Maryland reference genome sampled in early November. The genetic and spatiotemporal proximity of these genomes would suggest that the single additional genome may be a previously unreported case belonging to the same epidemiological cluster. Our analyses show that both 229e and SARS-CoV-2 coronaviruses are continuously introduced onto the UMD campus. Furthermore, we show how genomic epidemiology may aid in identification of missing epidemiological links.

0616

IMPACT OF THE COVID-19 PANDEMIC ON THE INCIDENCE OF DENGUE IN PERU

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Dengue is a mosquito-borne viral infection endemic in our country. The world is currently mobilizing to respond to the 2019 coronavirus pandemic (COVID19) caused by Severe Acute Respiratory Syndrome type 2 coronavirus (SARSCoV2). Both viruses coexist in dengue-endemic countries, so it is important to take the necessary preventive measures to control the spread of both diseases. This study aims to compare the temporal pattern of dengue incidences before and during the COVID-19 pandemic in Peru. An ecological study was undertaken. Time series analysis was performed. Incidence was estimated by t-student with variance correction. Poisson regression was applied to obtain dengue incidence rates before and during the COVID-19 pandemic. Dengue cases increases during the COVID-19 pandemic in all of Peru. Higher incidence rates in Huánuco (IRR= 58.3), Ica (IRR= 53.93) and Ucayali (IRR= 30.66), except in Piura (IRR=0.7). The highest increase in dengue cases per million population was in Ucayali (393.38), Tumbes (233.19), Ica (166.08), and Loreto (129.93). The slope of dengue cases was positive in all endemic regions during the COVID19 pandemic in Peru. In conclusion, the number of dengue cases per million increased during the COVID19 pandemic throughout Peru and in several of the endemic regions, except in Piura, where it was found to have decreased. The dengue incidence rate has increased during the COVID19 pandemic in all dengue-endemic regions in Peru.

0617

EVALUATION OF FTA CARDS FOR THE PRESERVATION OF YELLOW FEVER VIRAL RNA FOR ENHANCED MOLECULAR DIAGNOSTICS

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Yellow fever virus (YFV) is a flavivirus that frequently causes outbreaks of hemorrhagic fever in Africa and South America and is considered a reemerging public health threat. Accurate diagnosis of YFV is critical as one confirmed case constitutes an outbreak and results in the initiation of mass vaccination campaigns. Highly sensitive and specific molecular diagnostics have been developed; however, these assays require cold chain transport to prevent the degradation of viral RNA. Maintaining a cold chain can be problematic in YFV endemic regions. In this study, we investigated FTA cards as an alternative stabilization method of YFV RNA for use with molecular diagnosis. These cards are an attractive addition to clinical diagnostic protocols as RNA is extracted from punches made in the sample area leaving the unpunched area for replicate testing. Although RNA yield from a single 9mm FTA card punch was significantly less than the gold standard of RNA extracted directly from a contrived specimen, pooling RNA extracted from two FTA punches improved the limit of detection to a level comparable to that of the gold standard. In experiments addressing the ability of FTA cards to stabilize YFV RNA over time, RNA was stable on cards held at room temperature for up to four weeks. Even more promising, YFV RNA was detectable on cards held at 37°C from 2.1 days to over three weeks depending on viral input. These results support that utilization of FTA cards, which are cost effective and easy to use, could improve YFV molecular diagnostics by preserving viral RNA in situations where cold chain is not available.

0618

HEMOGLOBINOPATHIES AND MEROZOITE SURFACE PROTEIN-2 GENE POLYMORPHISMS ARE NOT ASSOCIATED WITH ACQUISITION OF EPSTEIN BARR VIRUS IN CHILDREN BELOW THREE YEARS OF AGE IN WESTERN KENYA

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Endemic Burkitt's Lymphoma (eBL) is a pediatric cancer associated with morbidity and mortality among children resident in holoendemic *Plasmodium falciparum* regions, such as western Kenya. *P. falciparum* infections share a causal link with Epstein Barr virus (EBV) infection. Moreover, *P. falciparum* has exerted a strong selection pressure on sickle cell trait, alpha (α)-thalassemia, glucose-6-phosphate dehydrogenase (G6PD), and merozoite surface protein 2 (MSP-2) genes to confer reduced malarial disease severity. The current study investigated the impact of polymorphisms in those genes on susceptibility to EBV in children (aged 0-36 months, n=81) resident in western Kenya enrolled into a longitudinal study. Clinical, demographic, and sample collection was performed at enrolment and 6 mos. post-enrollment. Bivariate regression analyses revealed that carriage of sickle cell trait (SCT, HbAA), α-thalassemia heterozygosity, and G6PD mutations [Mahidol (487G>A)/Coimbr (C592T), the Viangchan (871G>A)/Chinese (1024C>T) and the Canton (1376G>T)/Kaiping (1388G>A)] were not associated with acquisition of EBV either before or after 6 mos. of age. Additional bivariate analysis of infants <6 mos. of age illustrated that exposure to either 3D7 (p=0.921) or FC27 (p=0.914) MSP-2 alleles was not protective against EBV acquisition. Exposure to the MSP-2 alleles (3D7, FC27, or both) in children aged ≥6 months also had no impact on EBV acquisition (p=0.108; p=0.754; p=0.357, respectively). In conclusion, results presented here show that variation in SCT, α-thalassemia, G6PD variants, and exposure to MSP-2 (FC27, 3D7) had no impact on susceptibility to EBV in children from this holoendemic region of western Kenya.

0619

DIAGNOSIS OF MALARIA IN CAMEROON: A NEED FOR ADDRESSING NON-FALCIPARUM MALARIA!

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Plasmodium falciparum (*Pf*) is commonly thought as the dominant malaria species in sub-Saharan Africa (sSA) countries. However, recent studies reported high prevalence of yet neglected non-falciparum species in sSA. In this study, we demonstrated the importance to address non-falciparum species in Cameroon, which are still largely understudied in the country. The performances of a commonly used *Pf*-detecting rapid diagnostic test (RDT) were determined in 355 symptomatic individuals aged 1-65 years living in the town of Douala. The RDT reliability was evaluated using light microscopy (LM) as gold standard method. Polymerase chain reaction (PCR) of the *Plasmodium* 18S gene was performed for samples for which results between LM and RDT were discordant (i.e., False negative-RDT-/LM+, and False positive-RDT+/LM-). The PCR amplicons of non-falciparum species were sequenced and BLASTed. The sensitivity and specificity of *Pf*-detecting RDT was 94.0 % and 66.7 %, respectively. Thirty discordant results between LM and RDT were found *viz.* 25 LM+/RDT-, and 5 LM- / RDT+. The 18S PCR analysis for the 25 RDT-/LM+ samples revealed that 5 and 20 were positive for *P. ovale curtisi* (*PoC*) and *Pf*, respectively. All *PoC* cases were found in children below five years as mono-infections. Regarding the five RDT+/LM- samples, PCR was negative for all human malaria species. *PoC* sequences were found to be phylogenetically closer to sequences reported from China-Myanmar border and Malaysia. This is the first report on molecular characterization of *P. ovale* subspecies in Cameroon. The study also outlines the good diagnostic performances of the *Pf*-detecting RDT. However, the presence of *PoC* in false negative RDT results highlights a need to pay attention non-falciparum species for a better management of malarious patients in Cameroon.

0620

HIGH PREVALENCE OF SUBMICROSCOPIC MALARIA INFECTION IN PEOPLE LIVING WITH HIV/AIDS ON ART AND COTRIMOXAZOLE PROPHYLAXIS IN FAKO DIVISION, SOUTH WEST REGION OF CAMEROON

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Malaria and HIV coinfection is common in sub-Saharan Africa (SSA). However, epidemiological surveys using light microscopic detection of the malaria parasites frequently report prevalence lower than that of the general population. Studies suggest that the lower prevalence of malaria among people living with HIV/AIDS (PLHIV) could be attributed to the ART and cotrimoxazole prophylaxis they are routinely on. However, studies using molecular diagnostic methods to detect malaria parasites in PLHIV are also not readily available. This study was designed to compare light microscopy and PCR in the detection of malaria parasites in PLHIV and on ART and cotrimoxazole prophylaxis in Fako Division of Cameroon. PLHIV were enrolled from selected HIV treatment centers in Fako Division, Southwest region of Cameroon. Screening for malaria parasites was done using microscopic examination of Giemsa-stained blood films. And the results were confirmed by multiplex PCR. In all, 404 samples were analyzed, from 315 (78.0%) females and 89 (22.0%) males. A majority (84.2%) of the participant were on first line ART and all (100%) were on cotrimazole prophylaxis. Of the 404 participants, 7 (1.7%) and 82 (20.3%) were positive for malaria parasites by light microscopy and multiplex PCR respectively. The mean (±SD) parasite density was 191.57±159.51 parasites/µl. The prevalence of submicroscopic malaria parasites was therefore 19.1% (77/404). All (100%) the positive cases by light microscopy and confirmed by PCR, were *Plasmodium falciparum*. Meanwhile PCR detected 51 (62.2%) *P. falciparum*, 9 (11.0%) P. malariae and 13 (15.9%) *P. falciparum* + P. malariae coinfection. This study revealed a high prevalence of submicroscopic malaria parasites in the target population. The findings have significant epidemiological implications and warrants the need for the evaluation of current malaria diagnostic protocols in PLHIV and on treatment

0621

DETECTION OF MALARIA AS A MAJOR CAUSE OF ACUTE FEBRILE ILLNESS: FINDINGS FROM AN ACUTE FEBRILE ILLNESS SURVEILLANCE ACTIVITY IN TWO URBAN HEALTH FACILITIES IN LIBERIA

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Malaria is endemic in Liberia and transmission occurs year-round. We sought to understand the contribution of malaria as a cause of Acute Febrile Illness (AFI) through expanded laboratory testing for *Plasmodium* species in an AFI surveillance program at two health facilities in Monrovia, Liberia. We recruited patients with measured temperature of ≥37.5°C or self-reported fever within the past seven days. Malaria Rapid Diagnostic Tests (RDTs) or microscopy were performed on suspicion of malaria. We collected blood samples for analysis using real-time polymerase chain reactions in the TaqMan Array Card (TAC) format at Liberia's National Public Health Reference Laboratory. We enrolled 1,506 patients (12/2018-3/2020), including 398 (26%) pregnant women. 1,039 (69%) patients were assessed by RDT, 276 (18%) by microscopy, and 191 (13%) were not tested for malaria at the clinical site. No patients received both RDT and microscopy. All patients' samples were tested via TAC. In total, 987 (65%) patients were positive for malaria by any test (RDT, microscopy, or TAC): 602 had a positive RDT, 178 had positive microscopy results, and TAC detected *Plasmodium* in 670 patients. Among the TAC-detected cases, *P. falciparum* represented 93% (622/670). TAC detected 95 cases of *Plasmodium* that were RDT negative, 35 cases that were microscopy negative, and 68 cases among patients who did not receive RDT or microscopy. 338 (85%) pregnant patients were positive for malaria by any test. In conclusion, malaria was a common cause of AFI in two urban health facilities in Monrovia, Liberia. The use of TAC led to the identification of an additional 198 cases of malaria, which were missed in clinical care. The high detection of malaria among pregnant women points to the need for additional prevention efforts, especially given the potential adverse effect of infection on pregnancy and outcomes of pregnancy. Finally, in areas with known malaria circulation, implementation of an AFI surveillance program with PCR testing of all acutely febrile patients can complement ongoing surveillance efforts and clinical testing to provide a better understanding of the full burden of malaria.

0622

MORPHOLOGY-BASED AUTOMATED MICROSCOPY MALARIA TEST TO OVERCOME THE LIMITATION OF HRP2 RDT IN THE REGION OF A HIGH PREVALENCE OF HRP2 DELETION IN ETHIOPIA, GONDAR

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HRP2-based malaria rapid diagnostics tests (mRDTs) are widely used due to their accessibility, short time consuming, and straightforward interpretation in resource-poor settings. On the other hand, microscopic diagnosis has been relatively less used than mRDTs in these settings mostly because it requires skillful technicians, laboratory facility and local quality control. A high prevalence of parasites with *pfhrp2/3* deletion has been observed in many malaria-endemic countries leading to false-negative test results when using HRP2 based RDTs. These deletions raise concerns that patients will not be given appropriate medication and treatment on time. According to the world malaria report 2020, *Pfhrp2/3* deletions were confirmed in 11 countries, including Ethiopia. Particularly in these regions, mRDTs may have limitations to detect *Plasmodium falciparum* with the mutation. Therefore, examination of morphology is needed for accurate diagnosis and appropriate medication. Here, we suggest miLab, an automated sample preparation with an artificial intelligence image analyzing platform, with the result of the study conducted in Ethiopia. We recently (December 2019 to January 2020) undertook a malaria diagnostic study comparing different diagnostic methods in an area of a high risk of malaria transmission in Gondar, Ethiopia. Samples were collected at Maraki Health Clinic. A total of 157 patients were screened, and 104 were enrolled during the given period of time. 18 samples were *P. falciparum* positive, and 86 were negative. As a result, mRDTs showed 61.11% of sensitivity, while miLab showed 94.44%. This result illustrates the possibility of a high prevalence of *Pfhrp2/3* deletion in Gondar region. Thus, there is a limitation of using mRDTs in these settings. Unlike mRDTs, miLab diagnostic platform identifies *Plasmodium* species based on cell morphology, just like standard microscopy which is the gold standard. Therefore, It allows accurate diagnosis of P.*falciparum* parasite regardless genetic mutation such as *Pfhrp2/3* deletion. This research provide a useful insight into the paradigm shift in our understanding of malaria elimination.

0623

CASE REPORT: IDENTIFICATION OF PLASMODIUM VIVAX INFECTION IN RWANDA

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Although all four human malaria *Plasmodium* species are present in Africa, the World Health Organization estimates that *Plasmodium falciparum* accounts for approximately 99% of symptomatic infections on the continent. *Plasmodium vivax* is commonly found in the Horn of Africa and Madagascar but has not been reported in Rwanda since a single case was confirmed in a traveler returning to Japan in 2005. This current study reports a PCR-confirmed case of *P. vivax* in a 49-month old boy from Masaka, Rwanda. The child was initially recruited as part of an antimalarial therapeutic efficacy study conducted in 2018 after presenting to his community health center with symptomatic malaria. He had a hemoglobin level of 10.2 mg/dL and was diagnosed with *P. falciparum* malaria with a parasite density of 14,200 p/µl by microscopy. He was administered antimalarial treatment with artemether-lumefantrine, and a dried blood spot was collected at enrollment and later sent to the US Centers for Disease Control and Prevention for laboratory analysis. Multiplex malaria antigen detection assay revealed the sample to have no HRP2 present but a positive signal for *P. vivax* lactate dehydrogenase. DNA was extracted from this sample and subjected to photo-induced electron transfer-PCR and Snounou nested PCR assays to verify *Plasmodium* species. Both PCR assays revealed the sample to be positive for *Plasmodium* genus and *P. vivax*, but no amplification was observed for *P. falciparum*, *P. malariae*, or *P. ovale*. Current malaria surveillance efforts in Rwanda are mainly focused on detecting *P. falciparum*, *P. malariae,* and *P. ovale* since *P. vivax* has not been considered a possibility due to the high proportion of Duffy negative individuals in the country. Because *P. vivax* infection requires radical cure with an 8-aminoquinoline such as primaquine to prevent relapse, additional evaluations to determine if *P. vivax* is being transmitted in Rwanda should be considered.

0624

PRELIMINARY VALIDATION OF A NOVEL POINT-OF-CARE HAEMOZOIN ASSAY (GAZELLE™) FOR RAPID DETECTION OF PLASMODIUM KNOWLESI MALARIA IN SABAH, EAST MALAYSIA

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The zoonotic parasite, *Plasmodium knowlesi,* is the dominant cause of human malaria in Sabah, Malaysia. Rapid and accurate diagnosis allows for effective clinical management. In Malaysia, microscopy is the first-line point-of-care (POC) malaria detection method, however misidentification

of *P. knowlesi* with other endemic species occurs. The Gazelle™ (Hemex Health, USA) is a rapid and innovative POC malaria diagnostic device utilising Magneto-Optical Detection for hemozoin, a pigment by-product found in all *Plasmodium* species infections. A pilot phase enrolled 40 patients with positive *P. knowlesi* microscopy to refine the Gazelle™ detection algorithm. The main analysis of diagnostic accuracy will include a further 100 cases and 50 febrile malaria-negative controls. All samples will have *Plasmodium* species PCR conducted. A limit of detection (LoD) analysis will be conducted on a subset of samples serially diluted with non-infected whole blood. The completed pilot phase demonstrated a sensitivity of 95% (38/40; 95% CI 83.1 - 99.4). Ongoing main analysis has enrolled 58 *P. knowlesi* microscopy-positive patients and 16 febrile controls. The median age is 43 years (range 4 - 87) with a geometric mean parasitaemia of 1078/µL (95% CI 694 - 1673). The sensitivity and specificity of the Gazelle™ against reference microscopy was 94.8% (55/58; 95% CI 85.6 - 98.9) and 100% (95% CI 81.5 - 100) respectively. Overall test accuracy (area under receiver operating curve) is 97.4% (95% CI 94.5 - 100). Positive and negative predictive values were 100% (95% CI 93.5 - 100), and 85.7% (95% CI 63.7 - 97.0) respectively. Of those tested prior to antimalarial treatment (*n*=31; 53%), test sensitivity was 100% (95% CI 88.8 - 100). Sensitivity for samples tested with parasitaemia of <500/µL (*n*=16) was 81.3 % (95% CI 54.4 - 96.0), with the lowest parasitaemia detected of 34/µL. The average calculated LoD is 77 parasites/µl (*n*=7; range 4 - 267). Updated results from main analysis will be presented at the ASTMH meeting. The Gazelle™ is a potential *in situ* alternative tool for rapid and sensitive detection of *P. knowlesi* by healthcare workers in remote endemic areas.

0625

DELETIONS IN HRP2 GENE IN PLASMODIUM FALCIPARUM ISOLATES IN BENIN

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Malaria Rapid Diagnosis Tests (RDTs) are specific, sensitive, easy to use and affordable. As a result, RDTs became a major tool for detection of *P. falciparum* infections. Most of the RDTs are based on the immune-detection of *Pf*HRP2, a parasitic protein detectable in host's blood samples. If any deletion appears in *pfhrp2* gene, then, *Pf*HRP2 protein is not expressed and so the infection is not detected using *Pf*HRP2 based RDTs (false negative). To our knowledge, no data have been published on *pfhrp2* deletions in Benin, a West African country where malaria accounts for 40% of outpatient consultations and 25% of all hospital admissions. In this study, we have investigated *pfhrp2* deletions in isolates from a rural village in the south of Benin collected from February to March 2020. Out of 471 samples positive by PCR for *P. falciparum*, 241 have shown discordances between RDTs and PCR results (RDT-/PCR+). Discordance can be explained by a higher sensitivity of the PCR compare to RDT or by the absence of *Pf*HRP2 expression by the parasites. Twenty-one of the 471 *P. falciparum* isolates (4.5%) had at least one deletion in *pfhrp2* gene representing 8.7% of the discordant samples. A majority (95.2% (20/21)) of the deletions were detected during submicroscopic infections whereas submicroscopic infections represented only 55% (259/471) among all infections. This study describes, for the first time, the presence of *pfhrp2* deletions in *P. falciparum* isolates in Benin. Not detected, those infections would not be treated and probably contribute to a large dissemination of the deletion. Isolates presenting deletions in *pfhrp2* gene may therefore represent a reservoir of infection not documented up to now in Benin. It has been described in the literature that the prevalence of *pfhrp2* deletions can vary depending on the geographical area. We will complete this work performing similar studies in other locations in Benin. Documenting the distribution of *pfhrp2* deletions and the dynamic of its expansion is a priority until new tools for *P. falciparum* diagnostic are accessible in the field.

0626

DELETIONS OF THE PFHRP2 AND PFHRP3 GENES IN A HIGH PERCENTAGE OF PLASMODIUM FALCIPARUM INFECTIONS: DJIBOUTI CITY, DECEMBER 2019 TO MARCH 2020

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Malaria rapid diagnostic tests (RDTs) detecting the *Plasmodium falciparum* histidine-rich protein 2 (HRP2) antigen have been instrumental in expanding access to appropriate malaria diagnosis in many countries throughout the world. Reports on deletions of the *pfhrp2 (*and paralogue *pfhrp3*) genes have caused great concern in the African continent where *P. falciparum* causes significant morbidity and mortality. Recently, *pfhrp2* deletions have been reported in the horn of Africa including Eritrea, Ethiopia, Somalia, Sudan, and Djibouti. This current study examined 1,002 dried blood spot (DBS) samples collected as part of WHO investigation of possible *pfhrp2* deleted parasites in Djibouti city. DBS from persons presenting with malaria-like illness to Hôptial Général Peltier in Djibouti City from December 2019-March 2020 were evaluated for *Plasmodium* antigens by multiplex bead assay and for presence of *P. falciparum* infection by PCRs. Of all DBS, 44 (4.4%) were found to be *P. vivax* single-species infections, and 311 (31.0%) were from persons infected with *P. falciparum*. All 311 *P. falciparum* DNA positive (Pf+) samples had *pfhrp2* and *pfhrp3* (*pfhrp2/3*) genotyping performed to identify presence or absence of these two genes. The *pfmsp1* and *pfmsp2* single-copy control genes were amplified from 291 (95.7%) of the Pf+ samples and *pfhrp2/3* genotypes reported: 37 (12.5%) a genotype of *pfhrp2+/pfhrp3+*, 51 (17.2%) a genotype of *pfhrp2+/pfhrp3-*, 5 (1.7%) a genotype of *pfhrp2-/pfhrp3+*, and 203 (68.6%) a double-deletion genotype of *pfhrp2-/pfhrp3-*. Reductions in concentration of HRP2/3 antigens were seen in DBS samples with corresponding deletions in the *pfhrp2* and/or *pfhrp3* genes. Analysis of microsatellite markers to determine parasite relatedness with *P. falciparum* in surrounding countries is ongoing. *P. falciparum* with deletions in both *pfhrp2* and *pfhrp3* genes are highly prevalent in Djibouti City; this finding has recently led to a change in national malaria diagnostic strategy.

0627

VALIDATION STUDY OF A NEW POINT-OF-CARE HEMOZOIN BASED MALARIA DIAGNOSTIC DEVICE GAZELLE™ IN INDIA

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Malaria diagnosis is a challenge in resource poor settings. The current field diagnostic tools such as microscopy and RDT have limitations. Although inexpensive, microscopy is labor intensive, time consuming, requires skilled personnel and has poor sensitivity and specificity in routine use, especially for low parasitemia. While RDTs provide quick results, easy handling and storage, they cannot reliably detect low density parasitemia and sensitivity is variable by malaria species and *hrp2* deletion. Previously, we tested the performance of a hemozoin based point-of-care malaria diagnostic device Gazelle, in comparison to microscopy, RDT and PCR. The performance of the device was on par with microscopy and showed higher specificity than RDTs. The device is rapid, rugged, battery operated, easy-to-use and can be employed in regions with malaria parasites with hrp2/3 deletion. However, it has been limited by its ability to detect only

malaria positive versus malaria negative cases. This creates an extra burden wherein both *Plasmodium falciparum* and *P. vivax* are prevalent and require different treatment regimens. To further improve and validate the algorithm to identify the presence of *P. falciparum* and *P. vivax* species, we are conducting a validation study at three study sites in India: Sheopur (Madhya Pradesh), Jagdalpur (Chhattisgarh) and Udaipur (Rajasthan). To date, screening of 11,322 febrile patients has resulted in enrolment of 1,699 patients. Of the 1,699 febrile patients, a total of 345 patients were malaria positive by both microscopy and RDT. The PCR analysis of 1,373 samples yielded 509 malaria positive cases. Most of the malaria cases were caused by *P. falciparum* (63%) followed by *P.vivax* (18%), *P. falciparum* + *P.vivax* (14.5%), *P. falciparum* + *P. malariae* (2%), other mixed (1.7%) and *P. malariae* (0.4%). The comparative analysis with PCR as gold standard and improvisation in algorithm is currently ongoing, and the results will be presented at the conference.

0628

IMPROVING THE COMPETENCE OF MALARIA MICROSCOPISTS THROUGH BASIC MALARIA DIAGNOSTIC REFRESHER TRAINING, MADAGASCAR, 2019-2021

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High quality microscopy remains the reference method for malaria diagnosis and allows for parasite detection (PD), parasite species identification (ID), and parasite count (PC). In Madagascar, where *Plasmodium falciparum*, *vivax, ovale* and *malariae* all exist, microscopists have limited skills due to a lack of malaria microscopy (MM) training, validated malaria species slides for practicing, and adequate supervision. To improve MM quality, PMI Impact Malaria supported the Government of Madagascar to conduct ten, five-day basic malaria diagnostic refresher trainings (bMDRT) from August 2019 to March 2021. Trainings included didactic and practice sessions on microscope maintenance, slide preparation, staining and reading, and quality assurance. Improvement was measured by comparing pre- and post-test scores on a written exam and by assessing competency in reading validated malaria slides. In line with WHO-defined proficiency levels, we aimed for participants to achieve scores of at least 80% for PD and ID and at least 40% for PC. A total of 188 microscopists completed one five-day training; microscopists from all 22 regions of the country were represented. The mean written test score increased pre to post from 33% (range, 8-100%) to 57% (range, 8-100%). Slide reading average scores increased from 70% (range, 0-100%) to 88% (range, 29-100%) for PD, 43 (range, 0-91%) to 60% (range, 9-100%) for ID, and 15% (range, 0-100%) to 30% (range, 0-100%) for PC. A total of 149 (79%), 39 (21%), and 67 (36%) participants achieved a WHO proficiency score of at least level B in PD, ID and PC, respectively. Malagasy microscopists' diagnostic skills improved after a five-day training; nearly 80% demonstrated competence in PD; however, ID and PC will require additional practice and training. Cyclical training and supervision will be important to improve overall microscopy skill levels to meet WHO proficiency level B. In addition, supplying microscopists with validated slides of the four malaria species would allow them to continuously practice their MM skills.

0629

SERIOUS HAEMOLYSIS DURING PRIMAQUINE RADICAL CURE TREATMENT OF PLASMODIUM VIVAX MALARIA: A SYSTEMATIC REVIEW AND DESCRIPTIVE ANALYSIS

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Primaquine (PQ) is the only widely available drug that kills *Plasmodium vivax* hypnozoites. It can cause serious drug induced haemolysis in individuals with glucose-6 phosphate dehydrogenase (G6PD) deficiency. We conducted a systematic review of all clinical trials, prospective cohorts, case-control studies and case reports published between 1900 and 2020 that included individual patient data reporting serious haemolysis associated with PQ radical cure of vivax malaria. Serious haemolysis was defined as a requirement for hospitalization, blood transfusion, renal replacement therapy or death thought to have been attributable to either haemolysis or anaemia. A total of 17 eligible studies with 157 patients from 11 countries were identified. Overall 79.6% (121/152) of cases were prescribed with a high dose of PQ (>0.5 mg/kg/day) for radical cure and, at the time of hospitalization, the median total dose of PQ received was 2 mg/kg (IQR 1.5-2.5; range 0.5-5). The most common clinical presentations were pallor (92.7%, 102/110), jaundice (90%, 126/140) and dark urine (89.1%, 98/110). Of 153 patients with a documented G6PD activity result, 94.7% (n=145) had G6PD deficiency. The first symptoms of haemolysis were reported within 5 days of initiation of treatment in 92.3% (96/104) of cases, with most reported on 3rd (24%, 25/104) and 4th day (24%, 25/104). All patients were hospitalized for serious haemolysis within 7 days of the first dose of PQ. Overall 57.8% (74/128) of patients required transfusion and 6.7% (8/119) were dialyzed. Of 157 patients, seven (4.5%) died. The remaining patients were discharged from hospital after a median of 4 days (IQR: 3-6). Even with G6PD testing, enhanced monitoring for severe haemolysis is warranted following PQ radical cure. Our findings suggest that clinical review within the first 5 days of treatment may facilitate early detection of haemolysis, reduce hospitalization and improve outcomes of patients who are prone to serious PQ-associated haemolysis.

0630

OPTIMIZATION OF OUTREACH TRAINING AND SUPPORTIVE SUPERVISION PLUS (OTSS+) TO IMPROVE MALARIA DIAGNOSTICS SERVICE DELIVERY IN SIERRA LEONE

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The WHO recommends that malaria treatment be based on a parasitological test (either microscopy or Rapid Diagnostic Tests) for rational use of drugs. Quality assured malaria microscopy (MM) is the gold standard used for the management of severe malaria. The President's Malaria Initiative Impact Malaria (IM) project supports the Sierra Leone National Malaria Control Program (NMCP) in the implementation of Outreach Training and Supportive Supervision Plus (OTSS+), an approach in which government laboratory (lab) supervisors use an electronic supervisory checklist to monitor and improve provider performance through quality supportive supervision at the health facility (HF) level. The first round of lab OTSS+ in Sierra Leone occurred Oct- Dec 2020

and reached a total of 53 health facilities. A total of 202 lab staff were mentored during this round of OTSS+. The number of lab staff in facilities ranged from 1 to 47, depending on level of HF. Although 78% of HFs had at least one functional microscope, none had a system in place for the repair and maintenance of microscopes nor had standard operating procedures available. Regarding key MM skills required for parasite detection, species identification and counting; 15%, 4% and 0% of HF met the WHO MM standards, respectively. None of the HFs had an internal or external quality control system. While OTSS+ results indicated that malaria lab diagnosis was available in all assessed HFs, the identified gaps jeopardize the quality and accessibility of malaria diagnostic services. Strong MM capacity of lab staff and quality assured diagnostic services along with access to needed equipment leads to improved quality malaria services. IM will work with the Ministry of Health and Sanitation through the NMCP to convert these findings into activities aimed at building in-country malaria diagnostic human resource capacity; expanding OTSS+; supporting malaria diagnostic refresher trainings; and liaising with supply chain partners to ensure the availability of commodities, such as buffer tablets of pH 7.2 and stock Giemsa staining solution as well as equipment such as functioning microscopes and pH meters.

0631

DETECTION OF NUCLEIC ACIDS EXTRACTED FROM RAPID DIAGNOSTIC TESTS REVEALS A SIGNIFICANT PROPORTION OF FALSE POSITIVE TEST RESULTS ASSOCIATED WITH RECENT MALARIA TREATMENT

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The diagnostic performance of field-deployed rapid diagnostic tests (RDTs) used for malaria surveys can be assessed by retrospective molecular analysis of the blood retained on the tests. Of the 2,865 RDTs that were collected in 2018 on Bioko Island and analysed in our study, 4.7% had a false-negative result. These false-negative RDTs were associated with low parasite density infections. No pfhrp2 gene deletions and four pfhrp3 gene deletions (11.1%) were found among 36 investigated RDTs. Masked pfhrp2 and pfhrp3 gene deletions due to a high proportion of multiclonal infections were found in 30.6% and 50.0% of samples, respectively. A proportion of 28.4% of positive RDTs were tested negative by qPCR and therefore considered to be false-positive. This could be explained by PfHRP2 antigen persistence after malaria treatment by analysing the questionnaire data collected from the participants. RDTs recording a false-positive result are likely to cause an underestimation of the potentially negative impact of asymptomatic malaria infections on the anaemia status of children under five years of age. Malaria surveillance depending solely on RDTs need well-integrated quality control procedures assessing the impact of reduced sensitivity and specificity of RDTs on the malaria control programs.

0632

REINFORCING MICROSCOPY SKILLS DURING MALARIA DIAGNOSIS REFRESHER TRAININGS TO IMPROVE THE QUALITY OF MALARIA DIAGNOSIS IN THE DEMOCRATIC REPUBLIC OF CONGO (DRC)

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Similar to many malaria endemic countries in sub-Saharan Africa, practicing laboratory (lab) technicians in the DRC often lack adequate malaria diagnostic skills, particularly in malaria microscopy (MM), due to limited training and reinforcement of skills following initial education. Malaria Diagnosis Refresher Trainings (MDRT) and Outreach Training and Supportive Supervision (OTSS+) aim to reinforce basic lab competencies with an emphasis on updated MM techniques through practical modules for technicians who have received pre-service malaria diagnostic training. MDRT is a five-day in person training that includes theory and practical microscopy modules. OTSS+ is a quality improvement approach aimed at enhancing the quality of malaria clinical and diagnostic service delivery during visits to health facilities (HF). A checklist together with on-site training are used to ensure that procedures follow national guidelines. PMI Impact Malaria supported nine provinces in the DRC to lead refresher trainings in 2019 and 2020 in order to improve the quality of MM among lab technicians in 51 HF. The aim of these trainings was to increase the percentage of HFs with lab technicians meeting the minimum WHO competency level at 90% for MM parasite detection (PD). The 2020 MDRT included 32 returning lab technicians from the 2019 training, along with 14 new participants. During the training, returning lab technicians improved their competencies in PD, in species identification, and parasite counting. Returning participants had an average PD score of 75% in pre-test and 86% in post-test during the first MDRT, followed by 81% in the pre-test and 90% in post-test in their second MDRT. During the 2020 MDRT training, new participants had an average PD score of 64% in the pre-test and 82% in their post-test. Results show that the continuity of training of lab technicians helps to improve MM competencies. We plan to measure the correlation between PD scores during MDRTs and OTSS+ in order assess if MDRTs contributed to an increase in MM competencies. These skills are essential for lab technicians to diagnose malaria, and for clinicians to provide adequate treatment.

0633

A PHASE IB CLINICAL TRIAL OF BLOOD STAGE MALARIA INFECTION AFTER DIRECT VENOUS INOCULATION OF PLASMODIUM FALCIPARUM SPOROZOITES (PFSPZ-DVI) FOR EVALUATING NEW ANTIMALARIAL DRUGS

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Malaria Volunteer Infection Studies (VIS) accelerate antimalarial drug development. The *Plasmodium falciparum* Sporozoite Direct Venous Injection (*Pf*SPZ-DVI) VIS model is established for evaluating new antimalarials for chemoprotection but is not currently utilized for evaluating new antimalarials with blood stage activity. This single-center, open-label, phase Ib study in malaria-naive healthy adult volunteers evaluated if direct venous injection of 3200 *P. falciparum* (NF54 strain) sporozoites was safe and feasible for evaluating new antimalarials with blood stage activity. Sixteen healthy males and females with no prior history of malaria were enrolled in two sequential cohorts of 8 participants per cohort. Following *Pf*SPZ-DVI, parasitemia was assessed from day 7 using quantitative PCR with artemether-lumefantrine administration (regimen approved for the treatment of acute uncomplicated *P. falciparum* malaria in adults) planned to commence at a target threshold of ≥5000 parasites/mL. A total of 31 adverse events (AEs) occurred in 15 participants with 29 AEs grade 1 (mild) or 2 (moderate), and 2 AEs (both transient neutropenia) grade 3 (severe). All 16 participants developed parasitemia ≥250 parasites/mL after a geometric mean of 9.68 days (95%CI: 9.06; 10.35). The target threshold of ≥5000 parasites/mL was reached in a median of 11.46 days (95%CI: 10.42; 12.42) when the geometric mean pre-treatment parasitemia was 15530 parasites/mL (95%CI: 10268; 23488). The pre-treatment parasitemia profile was characterized by an oscillating log-linear growth mixed effect model with an estimated parasite multiplication rate per 48 hours ($log_{10}PMR_{48}$) of 1.25 (95%CI: 1.25; 1.26). Artemether-lumefantrine rapidly cleared parasites with a geometric mean time to parasite clearance of 1.34 days (95%CI: 0.86; 2.09) and mean parasite reduction ratio per 48 hours ($log_{10}PRR_{48}$) of 3.56 (95%CI: 3.38; 3.74). The findings of our study successfully demonstrated the safety/tolerability and feasibility of *Pf*SPZ-DVI VIS model for evaluating new antimalarials with blood stage activity.

0634

COMPARISON OF DIFFERENT GENOTYPING TECHNIQUES TO DIFFERENTIATE PLASMODIUM FALCIPARUM RECRUDESCENCE FROM NEW INFECTIONS IN ANTIMALARIAL DRUG EFFICACY STUDIES

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The assessment of antimalarial drug efficacy for *Plasmodium falciparum* requires "PCR correction" to distinguish a true failure due to recrudescent parasites or a failure due to a new infection. New infections are defined as the presence of solely novel *P. falciparum* clones in post treatment samples compared to the pre-treatment samples. A recrudescent is present if the follow-up sample contains one or multiple identical *P. falciparum* clones as in the pre-treatment sample. The World Health Organization (WHO) recommends the genotyping of three highly polymorphic genes: merozoite surface protein 1 (*msp1*), merozoite surface protein 2 (*msp2*), and glutamate rich protein (*glurp*) by capillary electrophoresis for PCR correction. However, in practice, different genotyping methods are used, making it difficult to compare results from different studies. This work aimed at comparing different techniques, to assess their sensitivity in detecting minority clones, their robustness, workload and cost. The methods were assessed using four well-characterized *P. falciparum* lab-strains (3D7, K1, HB3 and FCB1), which were mixed in different ratios. The following techniques were compared: fast capillary electrophoresis, and high-resolution capillary electrophoresis using polymorphic length markers including *msp1*, *msp2*, *glurp* and four microsatellites. Targeted amplicon deep sequencing using single nucleotide polymorphism markers (*ama1-D3*, *cpmp*, *cpp*, *csp*, *msp7*), and high-resolution melting analysis using either length-polymorphic markers (*msp1* and *msp2*), or 24-SNP barcode were also assessed. Overall, minority clones detection and robustness varied greatly between the different techniques, with high-resolution capillary electrophoresis using length-polymorphic markers and targeted amplicon deep sequencing using SNP-rich markers performing the best. However, they were associated with increased cost and workload. The two methods are more appropriate for genotyping in high-transmission settings; but would require substantial investment to establish reference laboratories in malaria endemic settings.

0635

MALARIA DURING PREGNANCY: EFFECTS OF INTERMITTENT PREVENTIVE TREATMENT (IPTP) WITH DP, DPAZ AND SP ON MATERNAL IMMUNE ACTIVATION AND PLASMODIUM FALCIPARUM CLEARANCE

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Intermittent Preventive Treatment (IPTP)) is an important component of malaria prevention inpregnant women in malaria-endemic areas. But the emergence of resistance to sulphadoxine-pyrimethamine (SP), which is recommended by WHO, is compromising the efficacy of IPTp incountries like Malawi and alternative agents are needed. One such, DP (dihydroartemisinin-piperaquine), has entered human clinical trials. The IMPROVE* trial (NCT03208179), hasexplored the feasibility of replacing SP with DP for IPTp, alone or combined with the antibioticazithromycin. Our lab is currently exploring *in vitro* the effect of the different IPTp drugs (SP, DPand AZ) on the maternal immune response. We are measuring cytokine responses to *Plasmodiumfalciparum*-infected red blood cells (iRBCs) and other stimuli, and the ability of maternal immunecells to take up iRBCs. To do that, we are using PBMCs collected at three-time points fromMalawian women enrolled in the IMPROVE trial (who received different IPTp regimes duringtheir pregnancy). We will examine paired cytokine responses and PBMCs activity using samplescollected from the same patient before and after receiving IPTp, and/or at study enrolment andatdelivery, using parametric and non-parametric tests as appropriate. Additionally, we are usingPBMCs from Australian donors who have never been exposed to *P. falciparum* to examinewhether *in vitro* exposure to these drugs alters their immune responses. We hypothesize that AZwill decrease cytokine secretion and that compared to SP, DP will be superior at inducingmonocyte clearance of *P. falciparum* iRBCs.

0636

MALARIA POSITIVITY FOLLOWING A SINGLE ORAL DOSE OF AZITHROMYCIN AMONG CHILDREN IN BURKINA FASO: A RANDOMIZED CONTROLLED TRIAL

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Azithromycin is a broad-spectrum antibiotic that has moderate antimalarial activity and has been shown to reduce all-cause mortality when biannually administered to children under five in high mortality settings in sub-Saharan Africa. One potential mechanism for this observed reduction in mortality is via a reduction in malaria transmission. We evaluated whether a single oral dose of azithromycin reduces malaria positivity by rapid diagnostic test (RDT). We conducted an individually randomized placebo-controlled trial in Burkina Faso during the high malaria transmission season

in August 2020. Children aged 8 days to 59 months old were randomized to a single oral dose of azithromycin (20 mg/kg) or matching placebo. At baseline and 14 days following treatment, we administered an OnSite™ rapid diagnostic test to detect *P.falciparum* and measured tympanic temperature for all children. Caregiver-reported adverse events and clinic visits were also recorded. We enrolled 449 children with 221 randomized to azithromycin and 228 to placebo. The median age was 32 months and 48% were female. A total of 8% of children had a positive RDT for malaria at baseline and 11% had a fever (tympanic temperature ≥37.5°C). In the azithromycin arm, 8% of children had a positive RDT for malaria at 14 days compared to 7% in the placebo arm (*P*=0.65). Fifteen percent of children in the azithromycin arm had a fever compared to 21% in the placebo arm (*P*=0.12). The day 14 positivity rate among those who had a fever was 3% in both arms (*P*=0.96). However, caregivers of children in the azithromycin group had lower odds of reporting fever as an adverse event compared to children in the placebo group (OR 0.41, 95% CI 0.18-0.96, *P*=0.04). Caregiver-reported clinic visits were uncommon, and there were no observed differences between arms (*P*=0.32). We did not find evidence that a single oral dose of azithromycin reduced malaria positivity during the high transmission season. Caregiver-reported fever occurred less often in children receiving azithromycin compared to placebo, indicating that azithromycin may have some effect on non-malarial infections.

0637

A DIVERSE GLOBAL FUNGAL LIBRARY FOR DRUG DISCOVERY

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Secondary fungal metabolites are important sources for new drugs against infectious diseases and cancers. To obtain a library with enough diversity, we collected about 2,395 soil samples and 2,324 plant samples from 36 regions in Africa, Asia, and North America. The collection areas covered various climate zones in the world. We examined the usability of the global fungal extract library (GFEL) against parasitic malaria transmission, Gram-positive and negative bacterial pathogens, and leukemia cells. Nearly ten thousand fungal strains were isolated. Sequences of nuclear ribosomal internal transcribed spacer (ITS) from 40 randomly selected strains showed that over 80% were unique. Screening GFEL, we found that the fungal extract from *Penicillium thomii* was able to block *Plasmodium falciparum* transmission to *Anopheles gambiae*, and the fungal extract from *Tolypocladium album* was able to kill myelogenous leukemia cell line K562. We also identified a set of candidate fungal extracts against bacterial pathogens.

0638

POST-TREATMENT TRANSAMINASE ELEVATIONS IN MALARIA VOLUNTEER INFECTION STUDIES: DETERMINING WHAT IS CONSIDERED SIGNIFICANT

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Transaminase elevations encountered during malaria volunteer infection studies (VISs) where investigational antimalarials are being evaluated raise concerns for the safety of healthy volunteers in these studies, and may lead to cessation of drug development due to concerns of drug induced liver injury (DILI). Hence, incorrect assignment, or lack of correct assignment, of causality of these adverse events to a new clinical entity has serious implications. The threshold for grading severity of transaminase levels are typically assigned by WHO or by the Common Terminology Criteria for Adverse Events. To explore the effect of setting the thresholds in transaminase levels we investigated transaminase elevations from two human *Plasmodium vivax* VIS (n=32). An Alanine transaminase (ALT) cut off of ≥2 times × upper limit of normal (ULN) was compared to that of ≥2.5 × ULN. Reducing the ALT cut off from ≥2.5 × ULN to ≥2.0 × ULN increased the number of volunteers with significant transaminase elevations from 11/32 (34%) to 14/32 (44%) and strengthened the associations between transaminase elevations and CRP (OR 1.43 [95% CI 1.10-1.86; p=0.008] to 1.81 [1.21-2.71; p=0.004] for every 1 × ULN increase in maximum CRP) and parasite clearance burden, defined as the log-fold reduction in parasitemia 24-hours post-treatment (OR 4.28 [95% CI 1.26-14.59; p=0.020] to 5.09 [1.40-18.48; p=0.013]). Peak parasitemia and maximum temperature were associated with an increased risk of having a peak ALT ≥2.0 × ULN (OR 5.86 [95% CI 1.17-29.43; p=0.032] and OR 3.13 [95% CI 1.14-8.61; p=0.027] respectively) but not ALT ≥2.5 × ULN (OR 3.72 [95% CI 0.77-18.03; p=0.10] and OR 1.92 [95% CI 0.78-4.69; p=0.15] respectively). We believe a peak ALT ≥2.0 × ULN is significant in healthy volunteers experimentally infected with *P. vivax* and should be used when assessing for associations. The mechanisms underlying these elevations remain unclear. We suggest that similar analyses should be conducted for *P. falciparum* VIS and our data illustrate the importance of further studies of mechanisms and time course of liver function abnormalities in antimalarial clinical trials where DILI is a concern.

0639

FIRST-IN-HUMAN EVALUATION OF A PLASMODIUM FALCIPARUM TRANSMISSION-BLOCKING MONOCLONAL ANTIBODY

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Novel transmission-blocking interventions (TBIs) are urgently required to reduce the global malaria burden. TB31F is a humanised version of monoclonal antibody (mAb) 45.1 that targets the Pfs48/45 protein on gametocytes and gametes of *Plasmodium falciparum* (*Pf*) and is the most potent *Pf* transmission-blocking mAb described to date. We here present the final results of the first-in-human trial assessing the safety, pharmacokinetics and transmission-reducing activity (TRA) of TB31F in adult malaria-naive volunteers. Four groups of five healthy F/M subjects were administered a single intravenous (i.v.) dose of respectively 0.1, 1, 3 and 10 mg/kg TB31F and monitored until day (D) 84 post-administration. A fifth group of 5 volunteers was administered a single subcutaneous (s.c.) dose of 100mg mAb TB31F to explore bioavailability. Solicited local, solicited systemic and unsolicited adverse events were recorded until D7, D28, and D84, respectively. Titres of circulating TB31F mAb were measured by ELISA and TRA was assessed by standard membrane feeding assays using laboratory-reared *Anopheles stephensi* mosquitoes, cultured *Pf* gametocytes (NF54) and subjects' serum. Anti-drug antibodies were measured by sandwich ELISA using anti-id mAbs. TB31F administration was safe and well-tolerated at all administered i.v. and s.c. doses, including the highest dose of 10mg/kg i.v.. With a single dose of 1, 3 or 10 mg/kg i.v. or 100mg s.c., sufficient serum TB31F concentrations were obtained to achieve >80% TRA in 19/20 volunteers, which was maintained up to D28, D56, D84 and D28 after administration respectively. With an estimated TB31F half-life of 20.5 days, a single dose of 10mg/kg i.v. is predicted to

maintain >80% TRA for over four months. This makes TB31F a potent tool for deployment in areas of seasonal malaria transmission where effective transmission blockade may cover the entire season after a single administration. Future work will include testing TB31F in field settings to inform the potential of mAbs as a TBI and SMFA as a tool for efficacy assessment, and exploring Fc-modification of TB31F mAb to extend serum half-life.

0640

IMMUNIZATION OF WOMEN OF CHILDBEARING POTENTIAL WITH PFSPZ VACCINE PRIOR TO PREGNANCY IN OUÉLESSÉBOUGOU, MALI: SAFETY DURING SUBSEQUENT PREGNANCY, POSTPARTUM AND INFANCY PERIODS

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Sanaria® PfSPZ Vaccine (radiation-attenuated, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ)) has been shown to be efficacious using condensed (4-week) regimens suitable for pregnancy. This double-blind, normal saline (NS) placebo-controlled trial in women of child-bearing potential (WOCBP) investigated safety and vaccine efficacy (VE) against naturally transmitted Pf malaria of two dosage regimens, 9x105 and 1.8x106 PfSPZ administered on days 1, 8 and 29, to women who planned to become pregnant after vaccination. An important objective was to evaluate safety during post-vaccination pregnancy, post-partum, and infancy follow up. 300 women were randomized to receive 9 x105 or 1.8x106 PfSPZ or NS via direct venous inoculation (DVI) (n=100/arm) and were followed for two years to assess safety by recording adverse events (AEs) and pregnancy/infancy outcomes. All received artemether lumefantrine 2 weeks prior to 1st and 3rd vaccination to clear any existing parasitemia. 180 pregnancies occurred post vaccination in 163 women, with 29 pregnancy losses in 27 women, rates comparable to those recorded in an independent Pregnancy Registry Study in the same community. The majority of AEs during the intrapartum and postpartum periods were Grade 1. Nine subjects experienced intrapartum or postpartum SAEs, 4 in the 1.8x106 arm, 1 in the 9x105 arm and 4 in the NS arm. Eight resolved without sequelae but 1 (puerperal sepsis) resulted in death 5 days post- partum. 104 infants were born, with the majority of AEs Grade 1 and related to neonatal infections; 22 SAEs occurred in infants, all considered unrelated to vaccination and the majority being hospitalization for neonatal infection. Two stillbirths occurred, one each in the 1.8x106 PfSPZ and NS arms. Two pre-term twin deliveries (1.8x106 PfSPZ Vaccine group) resulted in 4 early neonatal deaths. One infant death (1.8x106 PfSPZ Vaccine) occurred, at 1 month of life secondary to malaria and anemia. Detailed safety results in pregnant women (intrapartum and postpartum) as well as in their offspring will be presented.

0641

EXTENDED DURATION ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF PLASMODIUM FALCIPARUM MALARIA IN HIV-UNINFECTED CHILDREN IN UGANDA: A RANDOMIZED PK/PD CLINICAL TRIAL

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Artemether-lumefantrine (AL) is the most widely used artemisinin-based combination therapy in sub-Saharan Africa. Lumefantrine (LUM) exposure is lower in young children and is associated with differences in treatment outcomes. Dose-limited absorption requires extending AL duration to improve LUM exposure. We conducted a prospective PK/PD study of extended duration AL in children ages 6 months to 17 years in a high transmission setting in Uganda. Children with uncomplicated *P. falciparum* malaria were randomized to 3-day (standard 6-dose) or 5-day (extended 10-dose) AL with intensive or population PK sampling for artemether, dihydroartemisinin (DHA), and LUM for 21 days and clinical follow-up for 42 days. 228 children were enrolled into 3-day (n=115) or 5-day (n=113) AL. Day 28 microscopy determined treatment outcomes for 3-day versus 5-day AL were: ACPR (48% vs 60%), LPF (40% vs 32%), and LCF (12% vs 8%). Day 42 microscopy determined treatment outcomes were: ACPR (25% vs 31%), LPF (55% vs 51%), and LCF (19% vs 18%). PCR genotyping of six microsatellites suggests that 10 microscopic recurrences represented recrudescence, with four in the 3-day arm and six in the 5-day arm. 100 children were enrolled into intensive 3-day (n=50) or 5-day (n=50) PK sampling. Terminal LUM concentrations were significantly increased in the 5-day versus 3-day regimen on days 8, 14, and 21 (822, 184, 80.7 ng/mL vs 330, 131, 58.6 ng/mL, respectively; all p-values <0.005). Total LUM exposure (area under the concentration versus time curve) measured from the time of the last dose over 21 days indicates a geometric mean $AUC_{0\text{-}21d}$ of 288 and 252 ug·hr/mL for the 5-day and 3-day AL regimens, respectively. Compared to 3-day AL, 5-day AL significantly enhances terminal LUM concentrations out to day 21. Increased exposure in the 5-day AL regimen appears to coincide with a modest reduction in the risk of recurrent malaria at 28 days, with risk reduction diminishing by 42 days in this high transmission setting. Data is preliminary but final statistical analysis of treatment outcomes with incorporated population PK data will be presented.

0642

AN ADAPTIVE, RANDOMIZED, ACTIVE-CONTROLLED, OPEN-LABEL, SEQUENTIAL COHORT, MULTICENTER STUDY TO EVALUATE THE EFFICACY, SAFETY, TOLERABILITY, AND PHARMACOKINETICS OF INTRAVENOUS CIPARGAMIN (KAE609) IN ADULT AND PEDIATRIC PARTICIPANTS WITH SEVERE PLASMODIUM FALCIPARUM MALARIA (KARISMA - KAE609'S ROLE IN SEVERE MALARIA)

PAMAFRICA KAE609 Consortium

Novartis Pharma AG, MMV, University of Tubingen, Basel, Geneva, Tubingen, Switzerland

Severe malaria is a life-threatening disease, which predominantly affects children under 5 years in Africa. To date, intravenous artesunate is the recommended first-line parenteral therapy. However, increasing resistance threatens its efficacy. KAE609 is a PfATP4 inhibitor with potent and fast-acting schizonticidal activity. Oral KAE609 has been evaluated in ~190 patients with uncomplicated malaria. Rapid parasite clearance properties of KAE609 suggest a promising role in severe malaria. KARISMA is a Phase 2 adaptive, multicenter, randomized, open label, sequential cohort study to evaluate the efficacy, safety and PK of two different IV dose regimens (20mg once a day and 40 mg once a day) of KAE609. About 250 patients will be enrolled in an age descending (≥12 years old in Cohorts 1-2 and patients between <12 years to ≥6 months of age in Cohorts 3-5) and severity ascending manner (moderately severe malaria in Cohort 1 and severe malaria patients in Cohorts 2-5). Upon completion of Cohort 2, dose for patients <12 years old will be selected based on interim assessment of data from Cohorts 1-2. Traditionally, severe malaria studies used mortality as primary endpoint in thousands of patients. KARISMA will use proportion of patients with ≥90% parasite clearance as primary endpoint to enable dose selection and will also evaluate a novel composite clinical success endpoint (based on survival, absence of key symptoms of severe malaria and parasite clearance) as a key secondary endpoint. Clinical success after 48 hours will function as a surrogate for the survival and speed of recovery and is defined by absence of parasites, absence of key severe malaria symptoms (i.e. altered consciousness, renal impairment, lactic acidosis and respiratory distress) and death. Data collected from this study is expected to enable the use of clinical success as the primary endpoint for pivotal regulatory trials in severe malaria and thereby enable

an efficient development program. This program is an important step in the direction of finding an alternative to IV artesunate for management of severe malaria in the near future.

0643

MECHANISM OF ACTION STUDIES OF PHENOTYPIC WHOLE CELL-ACTIVE ANTIMALARIAL LEADS; IDENTIFYING NOVEL TARGETS FOR THE TREATMENT OF MALARIA

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The 2020 WHO Malaria Report estimates 229 million cases of malaria worldwide with over 409,000 deaths. The disease continues to cause significant morbidity and mortality, globally, with children under five and pregnant women being the most groups affected. Contrary to popular belief, recent malaria figures suggest that the infection decline rate has stalled since 2014 and has even reversed in certain areas. The recrudescence in the disease burden may be due to factors, including the emergence of resistant parasite mutants to frontline treatments as observed in South-East Asia, as well as the development of resistance to insecticides from the mosquito vectors. Also, due to the recent impact of the deadly coronavirus, malaria morbidity is expected to be on the rise as most health facilities and health care systems in malaria-endemic regions are severely overwhelmed. These among other factors have made malaria elimination a more challenging venture than otherwise anticipated and thus calls for accelerated research efforts to identify safe and efficacious agents with novel modes of action, with activity across multiple stages of the parasite lifecycle, for the treatment of malaria. In this regard, we previously reported the antimalarial properties of pyrido[1,2-a]benzimidazole (PBI) compounds, identified via a phenotypic whole-cell screening approach. Although this approach has been successful in identifying new molecule entities to feed the drug discovery pipeline, mechanistic deconvolution of hits to leads has remained a very challenging aspect. Herein, we report a resistance selection, fluorescent drug localization, and chemical proteomics approaches to studying the mechanism of action of the PBIs and identifying their molecular targets.

0644

NOVEL MULTIPLE-STAGE ACTIVE ANTIMALARIAL TAMBJAMINES

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The impact of malaria is staggering. Each year, malaria causes about 200 million clinical cases and claims nearly half a million lives, mostly children under the age of five and pregnant women. Drug resistance to current therapies, such as quinolines and artemisinin derivatives, is growing at an alarming rate. Therefore, the novel antimalarial chemotypes that can combat multiple stages of the parasite (blood stage, liver stage and gametocyte stage) through novel mechanism(s) of action are ideal and they are urgently needed to treatment and prevention of malaria. In this context, we present a novel antimalarial tambjamine chemotype that demonstrates single oral dose efficacy against blood stage malaria, in addition to prevention of sporozoite-induced *Plasmodium* liver stage infection and potential for transmission blocking.

0645

MEASUREMENT OF OOCYST AND SPOROZOITE INFECTION RATES IN ANOPHELES GAMBIAE S.L. UNDER NATURAL CONDITIONS IN BANCOUMANA, MALI

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The dynamics of mosquito infections play a central role in *P. falciparum* transmission and human infection rates, and can be considered as an endpoint in trials of transmission-interruption interventions like vaccines. Here we measured natural oocyst and sporozoite infection rates in wild-caught *An. gambiae s.l.* Every month (Mar 2018-Jul 2019), a team collected mosquitoes in 63 households comprising 503 rooms, for 7,435 collections total (372-494 collections per month). Live mosquito collections using mouth aspiration were followed by pyrethrum spray collections of killed mosquitoes. Live *Anopheles* mosquitoes with recent bloodmeal were separated from unfed mosquitoes and kept seven days; midguts were dissected and oocyst infections were counted. Killed *Anopheles* mosquitoes were preserved in 80% ethanol and retained for ELISA-CSP (sporozoite infection rates) and PCR (*Anopheles* speciation). Collections yielded 4,089 live female *Anopheles* with an average density of 0.55 (SD = 0.84) per hut; 3,164 (77.4%) mosquitoes survived to dissection, of which 84 (2.7%) were infected with mean of 2.1 oocysts [range 1-15]. Among 3,143 killed mosquitoes identified morphologically as *An. gambiae s.l* (mean 0.42 mosquitoes per hut), 25 (0.79%) were positive for CSP infection by ELISA. As expected, the highest number of infected mosquitoes (n=21) were collected during peak transmission season (Aug-Oct). A subset of mosquitoes underwent PCR analysis that identified two predominant species, *An. coluzzii* and *An. arabiensis*, at frequencies of 61.5% and 8.6% respectively. These large-scale collections provide an estimate of the incidence of malaria infection in circulating mosquito populations and can be a valuable tool to measure the impact of any malaria control measures tested or implemented in communities.

0646

ANEMIA AND MALARIA INCIDENCE BETWEEN 2007-2018 IN UNSTABLE MALARIA TRANSMISSION SETTING OF WESTERN KENYA

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Malaria is a leading cause of anemia in many areas of sub-Saharan Africa. In high malaria transmission settings, children often have reduced hemoglobin levels, in contrast to low transmission settings where hemoglobin levels are not reduced. Our previous studies in the western Kenyan highland areas of Kipsamoite and Kapsisiywa, areas of low and unstable malaria transmission, showed that hemoglobin levels in asymptomatic individuals increased after 1 year of malaria interruption, 2007-2008. We hypothesized that during the subsequent years of low transmission, months with higher malaria incidence would be associated with a higher prevalence of anemia in the population. To test this hypothesis, we evaluated malaria incidence and hemoglobin concentrations in all individuals who presented to the local health centers with fever or headache from 2007-2018 (n=3852). We performed a time-series regression with negative binomial distribution to compare monthly malaria count to monthly anemia count, using a spline function of time with adjustments for seasonality and long-term trends. Anemia was defined as <11 g/dL in children <5 years; <11.5 g/dL in children 5-15 years; <12 gdL in females >15 years; and <13 g/dL in males >15 years.

In month-based age-and sex-stratified time-series models, malaria was significantly associated with increased anemia in all ages when individuals with clinical malaria were included in anemia counts, and in children <5 years (incidence rate ratio [95% confidence interval], 1.13 [1.05, 1.23]) and males ≥15 years (1.18 [1.07, 1.31]) when individuals with clinical malaria were excluded from anemia counts. Over the 11-year study period, the proportion of malaria cases and anemia in children <5 years decreased, while the proportions in children 5-14 years and individuals ≥15 years increased. The study findings suggest that reduction or elimination of malaria would lead to a significant population-level decrease in anemia even in areas of low and unstable malaria transmission.

0647

RISK FACTORS FOR BORDER MALARIA IN A LOW TRANSMISSION SETTING: A CASE-CONTROL STUDY IN JAZAN, SAUDI ARABIA

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Saudi Arabia has successfully reduced the malaria incidence and is considered malaria-free, apart from Jazan and Aseer region in the south. Malaria remains a public health threat in the southern border of Saudi Arabia, especially among the high-risk population communities living along the border with Yemen. An unmatched case-control study was conducted to identify risk factors for border malaria in Jazan region. A total of 261 participants were recruited for the study, including 81 cases of confirmed malaria through the RDTs and microscopy, and 180 controls were residence of the same area but not the same household in the Baish Governorate in Jazan Provinces, Saudi Arabia. Using a web-based data management system; A questionnaire was administered, and the data were captured electronically. The study aimed to evaluate the demographic characteristics, livelihood activities, travel history, and preventive measures associated with malaria infection between December 2017 to January 2019. A logistical regression model was used to investigate factors associated with infection. After adjusting for several confounding factors, persons who reported traveling away from their home village in the last 30 days OR 11.5 (95% CI 4.43 - 29.9), those who attended a seasonal night spiritual gathering OR 3.04 (95% CI 1.10 - 8.42), involved in animal husbandry OR 2.52 (95% CI 1.09 - 5.82), and identified as male OR 4.57 (95% CI 1.43 -14.7), were more likely to test positive for malaria infection. Eliminating malaria program might consider addressing social and behavioral practices within the border communities.

0648

POPULATION SIZE ESTIMATION OF SEASONAL FOREST GOING POPULATIONS IN CHAMPASAK PROVINCE, SOUTHERN LAO PDR

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Forest-going populations are key to malaria transmission in the Greater Mekong Sub-region (GMS) and are therefore targeted for elimination efforts. Estimating the size of the population of forest-goers is essential for programs to assess, track and achieve their elimination goals. Forest-goers were defined as individuals spending a night outdoors for forest or agricultural activities. Leveraging data from three cross-sectional household surveys and one survey of participants in an intervention among forest-goers, the size of this high-risk population in a southern province of Lao PDR between December 2017 and November 2018 was estimated by two methods: individual household survey estimates and capture-recapture. During the first month of the dry season, the first month of the rainy season, and the last month of the rainy season, respectively, 16.2% [14.7; 17.7], 9.3% [7.2; 11.3], and 5.3% [4.4; 6.1] of the household population 15 years or older were estimated to have engaged in forest-going activities. Cumulatively, the capture-recapture method estimated a total population size of 18,426 [16,529; 20,669] forest-goers, meaning 61.0% [54.2; 67.9] of the population 15 years or older had engaged in forest-going activities over the 12-month study period. This study in a historically epidemic-prone area demonstrates two feasible methods for developing population size estimates to inform malaria research and programming. The seasonality and turnover within this forest-going population provide unique opportunities and challenges for control programs across the GMS as they work towards malaria elimination.

0649

THE IMPACT OF THE COVID19 PANDEMIC ON CARE SEEKING AND IMPLICATIONS FOR THE PROCESS OF CERTIFYING MALARIA ELIMINATION IN CABO VERDE

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Cabo Verde, a nation with a population over 550,000 people, reported the first case of COVID-19 on March 19, 2020. Containment measures were quickly implemented and over 80,000 COVID tests were performed in 2020 with 11,840 confirmed infections (2% of the population) and 154 deaths. The COVID pandemic is expected to lead in a reduction in care-seeking for non-COVID health concerns. In a setting with the last locally acquired malaria case in January 2018, any interruptions to malaria care-seeking have the potential for transmission to re-establish. This work aims to determine whether there was any change in the number of people seeking care for malaria aligned with the progression of the COVID pandemic and determine whether the COVID interventions implemented had any impact on testing for malaria using an interrupted time series analysis. The routinely collected surveillance data for outpatient attendance, malaria, and COVID were aggregated per month for each facility (outpatient and malaria) and municipality (COVID) from 2017 to 2020. The timeline of COVID interventions was generated based on when and where official interventions were implemented. Preliminary results suggest that there was a marked shift in care-seeking in Cabo Verde. The mean outpatient attendance dropped from 1777 visits per month between January 2017 and March 2020 to 813 between April to December 2020. Malaria testing in Cabo Verde has generally declined since 2017, consistent with the reduction in malaria transmission. However, from April 2019 to March 2020 (pre-COVID, post malaria interruption) facilities tested a mean of 25 people per month compared to 15 tests per month between April to December 2020 (p=0.206). Therefore, results suggest that the pandemic has impacted both care-seeking and testing for malaria. However, with the cessation of international travel, the risk of imported infections seeding new transmission also declined likely contributing to the reduction in testing. It is important for countries to understand their specific malaria risks and vulnerabilities in order to ensure the achievements made to interrupt malaria transmission can be sustained.

0650

PREDICTORS OF HUMAN-TO-MOSQUITO PLASMODIUM FALCIPARUM TRANSMISSION IN A HIGH TRANSMISSION AREA OF WESTERN KENYA

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With malaria case reductions stalling under current interventions, efficient reduction of *Plasmodium falciparum* human-to-mosquito transmission may be enhanced by identifying clinical and parasitologic factors associated with successful transmission to mosquitoes. Previous work has measured predictors through direct or membrane feeding of mosquitoes, which fail to fully capture transmission dynamics in a natural setting. We captured *P. falciparum* human-to-mosquito transmission using longitudinal sampling of people as well as naturally-collected mosquitoes from their households over 14 months in Western Kenya. We matched parasites in human and mosquito infections using amplicon deep sequencing and haplotype inference of the parasite gene *pfcsp,* and adapted a previously-published probabilistic modelling approach to identify predictors of successful transmission. Univariate multi-level logistic regression models compared the probability of transmission across people's sex, age, infection parasite density, infection type (asymptomatic or symptomatic), bed net usage, and transmission season. Covariates with *p*-values < 0.2 in univariate models were included in a multivariate model to estimate adjusted Odds Ratios (aORs). We identified 1,039 human and 203 mosquito infections, which comprised 229 unique parasite *pfcsp* haplotypes. We used these haplotypes to estimate transmission across 3727 human-mosquito pairs from 198 people and 182 mosquitoes collected in 37 households. A higher likelihood of successful transmission to caught mosquitoes was associated with lower parasite densities [aOR 1-200 p/µL *vs.* <1 p/µL: 0.63, 95% CI: 0.49, 0.82; aOR >200 p/µL *vs.* <1 p/µL: 0.23, 95% CI: 0.17, 0.31], irregular bed net usage [aOR 0.50, 95% CI: 0.38, 0.66], or occurrence in the high transmission season [aOR 1.46, 95% CI: 1.22, 1.74]. Further analyses investigated the influence of parasite density on transmission. These results suggest that future elimination efforts focus on reducing low density infections and increasing usage of insecticide-treated bed nets during the high transmission season to decrease malaria transmission.

0651

DECLINES IN MODERATE AND SEVERE ANEMIA DURING A LONGITUDINAL STUDY IN HIGH MALARIA TRANSMISSION REGIONS OF KINSHASA, DEMOCRATIC REPUBLIC OF THE CONGO

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Malaria-related anemia is a major public health concern in Sub-Saharan Africa. We aimed to quantify anemia burden during a longitudinal surveillance study across seven villages in Kinshasa Province, Democratic Republic of the Congo, from 2015-2017. Active malaria surveillance was performed at enrollment and biannual follow-ups using rapid diagnostic tests (RDTs), and detected cases were referred to study clinics for antimalarial treatment. Passive surveillance was carried out through symptomatic subject presentation to clinics, where RDTs and hemoglobin levels were measured and treatment was administered according to national guidelines. Anemia prevalence at passive clinic visits was estimated overall and by season (rainy/dry), and factors associated with anemia were evaluated. In total, 1,033 (64.9%) symptomatic subjects presented to clinics (2,991 visits). Anemia period prevalence was 62.2% (n=643), ranging from 47.4% (n=230) among subjects 15+ years to 82.4% (n=164) among subjects less than five. Overall, 38.7% (n=400), 36.4% (n=376), and 7.1% (n=73) of subjects presented to clinics at least once with mild, moderate, and severe anemia, respectively, according to WHO age/sex strata. While total anemia prevalence remained stable during the study (season 1: 48.4% [95% CI: 34.2-62.5%] to season 7: 42.6% [95% CI: 33.3-51.9%], p_{trend}=0.0496), moderate/severe anemia declined across seasons (36.6% [95% CI: 24.3-48.8%] to 18.4% [95% CI: 12.3-24.5%], p_{trend}=0.0001), both overall and among subjects with concurrent RDT+ malaria (34.4% [95% CI: 22.5-46.3%] to 14.8% [95% CI: 9.3-20.3%], p_{trend}<0.0001). Unadjusted odds of anemia were higher in subjects with ages <15 years, male gender, poorer wealth scores, and PCR+ and RDT+ malaria at visits. While results confirm anemia was common in this setting, a decreasing trend of moderate/severe anemia was observed over the course of the study. Key study interventions - routine surveillance, community sensitization and improved access to malaria diagnostic testing and treatment - deserve further evaluation in high-transmission settings.

0652

INFECTIVITY OF CLINICAL AND ASYMPTOMATIC MALARIA CASES TO ANOPHELES FARAUTI S. S.MOSQUITOES IN PAPUA NEW GUINEA

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Transmission of malaria parasites can arise from asymptomatic parasite carriers as well as from individuals with clinical symptoms. Understanding the infectiousness of these different sub-populations is important to target malaria control focusing on interrupting transmission. Using an *Anopheles farauti* colony, we performed direct skin feeding assays on 11 asymptomatic parasite carriers identified in a population of 103 individuals from a highly endemic village in Madang Province, Papua New Guinea using rapid diagnostic tests (RDT) and qPCR techniques. We also conducted membrane feeding assays with samples collected from 182 clinical patients from two local health facilities enrolled on the basis of positive rapid diagnostic tests. All samples were further characterized retrospectively using expert light microscopy and qPCR. In the population of asymptomatic individuals, 2/11 were diagnosed by qPCR as *Plasmodium vivax* carriers while 3/11 were diagnosed with *P. falciparum*. Only the two *P. vivax* positive individuals successfully infected *Anopheles farauti* colony mosquitoes. Microscopy diagnosis done retrospectively also confirmed that the two participants were *P. vivax* positive with one having detectable gametocyte stages. The remaining 6/11 were qPCR negative but RDT positive. Of the 182 clinical malaria samples 38 (21%) resulted in successful mosquito infections. While according to qPCR only 10% (8/80) of *P. falciparum* gave rise to mosquito infections, 44% (24/55) of *P. vivax* cases were infectious to mosquitoes. Interestingly, 7.4% (2/27) of samples from patients who were negative by qPCR resulted in successful mosquito infection. Similar results were obtained for light microscopy for *P. falciparum*, 11% (10/88) and for *P. vivax* 44% (23/52) with an increase in mosquito infection rate to 58% (14/24) for *P. vivax* gametocyte carriers. Overall, *P. vivax* seemed to be more infectious in both, clinical and asymptomatic populations. In further studies, we plan to test more asymptomatic parasite carriers in order to clearly understand the differences in infectiousness of clinical versus asymptomatic malaria parasite carriers in PNG.

0653

MALARIA SURVEILLANCE AND RESISTANCE PATTERNS IN THE ENDEMIC BORDER REGIONS OF THAILAND, 2019-2021

MALARIA SURVEILLANCE AND RESISTANCE PATTERNS IN THE ENDEMIC BORDER REGIONS OF THAILAND, 2019-2021

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In 2019, AFRMS began conducting a malaria surveillance study in collaboration with Thai MoPH and the Royal Thai Army to characterize malaria isolates for in-depth *in vitro* and genetic studies of drug resistance. Data was analyzed from 129 malaria patients from four provinces, each along a different international border: 56 cases from Yala Province (bordering Malaysia), 45 in Sisaket Province (bordering Cambodia), 3 cases from Ratchaburi Province (Myanmar border) with and 25 cases in Ubon Ratchathani Province (Laos border). The majority enrolled were male (86%), with 40% reporting work at rubber plantations and 25% in the military. There is approximately a 12% rate of G6PD deficiency with 71% being the Viangchan variant. Only 14 cases were *Plasmodium falciparum*, and all but one patient came from Yala Province. Initial testing of isolates from Yala by PCR demonstrated wild type for resistance markers PfCRT, plasmepsin 2, Pfmdr1 and cytochrome b. The Kelch13 mutation was wild-type by PCR, but deep amplicon sequencing called 580Y at low signals in five isolates, suggesting the presence of mixed population. This low level of molecular markers associated with anti-malarial resistance stands in stark contrast to the rest of Thailand, where numerous mutations have been reported. The drug sensitivity profiles for 21 *P. vivax* isolates showed an *ex vivo* IC50 of 47.6 nM (29.9-71) against chloroquine, demonstrating continued sensitivity to the first-line treatment in Thailand. The low number of *P. falciparum* cases demonstrate the great strides Thailand has made in the recent years, but continued efforts must be maintained in order to eliminate *P. vivax*.

0654

BURDEN, DETECTABILITY AND CLINICAL IMPACT OF PLASMODIUM FALCIPARUM INFECTION ON PREGNANT MOZAMBICAN WOMEN IN TRANSITIONING EPIDEMIOLOGIES

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Understanding how transmission intensity determines the detectability and clinical progression of malaria in pregnancy is crucial to guide the most efficient control and elimination approaches. We conducted a three-year prospective observational study (from 2016 to 2019) at antenatal care (ANC) clinics and maternity wards in three areas with different intensities of malaria transmission in southern Mozambique (at Magude and Manhiça districts). Finger-prick blood samples onto filter papers collected at first antenatal visit and delivery, were analyzed by real-time quantitative polymerase chain reaction (qPCR) to assess the presence of *Plasmodium falciparum* (*Pf*). The *Pf* positivity rate by qPCR (PfPRqPCR), which was higher in Ilha Josina (19% ([255/1329]) than in Magude (4% [175/4907]) and Manhiça (4% [262/6199], p<0.001) and at first ANC visit (7% [483/6471]) than at delivery (4% [209/5964], p<0.001), decreased with time in the 3 sites. Parasite densities increased during the study period in Magude while remained constant in Manhiça. In Ilha Josina, densities decreased and increasied with time in primigravidae and multigravidae women, respectively. Overall, 39% (271/692) of the qPCR detected infections were above 100 parasites/µL and therefore considered as detectable by conventional RDTs. The proportion of detectable infections was higher in first ANC visit than delivery for Magude (43% vs 27%; p=0.029) and Manhiça (47% vs 27%; p=0.002). While hemoglobin levels decreased with increases in parasite densities in women from the 3 areas, the newborn weight was affected by *Pf* infection only in women from Manhiça. These results provide evidence of the value of pregnant women to identify changes in transmission even in low transmission settings, as well as malaria hotspots. Increases in the densities and detectability of *Pf* infections in Magude suggest the potential adaptation of malaria parasites to elimination efforts. Finally, declines in malaria transmission in high transmission settings are associated with significant epidemiological changes that indicate the rapid loss of herd immunity during pregnancy.

0655

IDENTIFYING MALARIA ASSOCIATED RISK FACTORS AMONG ADOLESCENTS LIVING IN AREAS WITH RESIDUAL MALARIA TRANSMISSION IN SENEGAL: A CASE CONTROL STUDY

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In Senegal, malaria morbidity has decreased over these past years but malaria epidemiology remains heterogeneous with persisting transmission in the southeastern part of the country and more important cases among older children and adolescents. However, little is known about factors associated with clinical malaria among this group. A better understanding of malaria transmission will guide future interventions targeting these key populations. This study aimed at identifying factors associated with clinical malaria among adolescents in Senegal. A case-control study was conducted from November to December 2020 in four health posts, in the Saraya health district. The case was adolescent of 10-19 years with uncomplicated malaria (fever, Temperature>37.5° or history of fever, with positive malaria RDT) according to the National Malaria Control Program (NMCP) guidelines. Control was a same age group person, living in the neighborhood of the case, with negative mRDT. A questionnaire was administered to each participant followed by home visit to assess the living conditions. Factors associated with clinical malaria was assessed using a Stepwise Logistic regression analysis. In total, 492 individuals were recruited; 246 cases and 246 controls. In a multivariate analysis, factors associated with clinical malaria included non-use of bed net (aOR=2.65; 95% CI=1.58-4.45), non-use of other preventive measures (aOR=2.51; 95% CI=1.53-4.11) and indoor sleeping (aOR=3.22; 95%CI=1.66-6.23). Protective factors included age of 15-19 years (aOR=0.38; 95% CI 0.23-0.62), absence of stagnant water around the house (aOR=0.27; 95% CI=0.16-0.44), having a female household head (aOR=0.47; 95% CI=0.25-0.90), occupation such as apprentice (OR=0.24; 95%CI=0.11-0.52).The study revealed that environmental factors and non-use of preventive measures are the main determinants of malaria among adolescents living in areas with persisting transmission in Senegal. Strategies aiming at improving disease awareness and access to health care interventions such as LLIN are thus needed to improve malaria control and prevention among these vulnerable groups.

0656

MALARIA DEATHS EVOLUTION FROM 2015 TO 2019 IN THE DEMOCRATIC REPUBLIC OF CONGO (DRC)

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Malaria remains one of the most widespread and deadly parasitic diseases in the world and constitutes a real public health problem in the Democratic Republic of Congo (DRC), it is the leading cause of morbidity and mortality for which children under 5 years of age pay the heavy price. Deaths from malaria are often the result of no treatment, late treatment, or inappropriate treatment. The objective is to determine the evolution of deaths due to malaria from 2015 to 2019. Data were collected from DHIS2 software and from the National Malaria Control Program (NMCP) database. Analyzes were performed with Excel software. Between 2015 to 2019, a gradual decrease in deaths due to malaria was observed, 39,054 deaths were recorded in 2015, 33,997 deaths in 2016, 27,458 deaths in 2017, 18,030 deaths in 2018 and 13,072 deaths in 2019. The proportion of malaria deaths in children under 5 represents 77% of all malaria deaths in 2015, 73% in 2016, 69% in 2017, 75% in 2018 and 69 % in 2019. It is noticed an average of 73% over the 5 years considered, so children under 5 die more from malaria. The proportion of severe malaria out of all malaria cases varied between 12% and 14% with an average of 12% from 2015 to 2019. The good results obtained in relation to the decline in deaths caused by malaria between 2015 and 2019, would probably be due to the combination of several interventions including the providers training in case management, the regular supervision of care providers and the availability of drugs for the management of severe cases of malaria in the health facilities, as well as free malaria treatment practiced in DRC. These gains should be preserved by strengthening the management of malaria cases, by improving the supply chain for antimalarial drugs and other commodities ,also by stepping up training supervision in health facilities. Sustained and special efforts should be directed towards the cases management in children under 5 who are much more affected by deaths due to this terrible endemic.

0657

BOTTLENECKS TO INTERVENTION SCALE UP: SUPPLY AND DEMAND SIDE PERSPECTIVES FROM A LARGE, COMMUNITY-BASED TRIAL OF MALARIA TESTING IN WESTERN KENYA

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Prompt malaria diagnosis either by microscopy or rapid diagnostic tests is recommended for all patients with suspected malaria before treatment. In the Kenyan retail sector, RDTs are not always available, and most patients who purchase antimalarials are not diagnosed before treatment. We tested an intervention where CHWs provided free RDTs in the community. In this analysis, we sought to identify factors that influenced the demand for malaria testing in the community and supply of testing by CHWs. A community-based survey was conducted to collect individual study outcomes based on a population-based survey sampling strategy at 12- and 18-months post-implementation. Testing data was collected from all 244 CHWs and in-depth interviews conducted with a random sample of 70. Of all survey participants, 55% (n=948/1738) reported having a malaria test for their recent illness where 38% of those were tested by a CHW. Delayed seeking care beyond the first 24 hours (AoR=1.23, 95%CI:1.06-1.43), being aware of your local CHW (AoR=1.50, 95%CI: 1.10-2.04) and belonging to a wealthy household (AoR=1.53 95%CI: 1.14-2.06) was associated with high malaria testing uptake. Wealthier families (AoR=0.32, 95%CI:0.17-0.60), and delaying testing beyond two days reduced the odds of testing with a CHW (AoR=0.41, 95%CI:0.23-0.73). School-aged children between 5-17 years were more than twice as likely to be tested by a CHW (AoR =2.47, 95%CI: 1.47-4.14). Both confidence in AL treatment (AoR =3.27, 95% CI: 1.71-5.25) and accuracy of an RDT performed by a CHW (AoR =2.29, 95% CI: 1.01-5.22) were strongly and positively associated with testing at a CHW. CHWs who were formally employed performed on average 1.37 more tests per month (Adj coeff=1.37,95% CI: 0.05,2.70) and those serving areas with a high proportion of positive tests (proportion positive >25%) tested on average 2 more clients per month than those in lower prevalence areas (Adj coefficient= 2.14, 95%CI: 0.63,3.64). These results show that there is a high demand for malaria testing at the community and provision of rapid test by CHW is feasible.

0658

HIGH-RESOLUTION SUB-DISTRICT STRATIFICATION OF MALARIA RISK IN ZANZIBAR

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To achieve their malaria elimination target, Zanzibar has decided to implement an intervention approach based on risk strata. This study describes a novel approach to create a shehia (sub-district) malaria stratification map for Zanzibar. Case-level malaria data were obtained from the Malaria Case Notification system from January 2017 to December 2019. Data were aggregated and used to estimate the number of cases, incidence, fraction of imported cases, and probability of finding new cases during follow-up investigation of primary index cases for all 388 shehias. For each selected malaria indicator, pre-determined thresholds were assigned to determine risk levels of each shehia. Malaria indicator risk levels were analyzed using a multiple correspondence analysis (MCA) to assign a score for each shehia. The quartiles of the scores were then used to divide 333 shehias in four strata (1st-4th stratum, from high to low burden, with approx. 80 shehia per stratum). The performance of the MCA in creating distinct groups was evaluated comparing the distribution of the indicators among the strata (Wilcoxon signed-rank test). An additional fifth stratum was also created for 55 shehias in Mjini (urban) district due to different approaches for targeted interventions. Two stratification maps were created by varying indicator thresholds, one for the whole archipelago and one for each district. Malaria indicators showed a significant difference among the 1st-4th strata ($p<0.05$, Wilcoxon's test), highlighting good performance of grouping shehias using MCA. The maps showed that most of the high malaria burden strata aggregated in southern Unguja and low burden strata aggregated in southern Pemba. The resulting stratification will rapidly be implemented in Zanzibar. Intervention packages for shehias in the 2nd, 3rd, and 4th, strata will be based on community-level interventions while the 1st stratum will adopt a shehia-level approach. Specific interventions will be implemented per each urban shehia. This stratified approach will improve intervention effectiveness and programmatic resource allocation to support Zanzibar's path to elimination.

0659

SYMPTOMATIC MALARIA IS ASSOCIATED WITH REDUCED RISK OF REINFECTION WITH PLASMODIUM FALCIPARUM PARASITES HARBORING HOMOLOGOUS CIRCUMSPOROZOITE PROTEIN EPITOPES

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The antigenic diversity of *Plasmodium falciparum* is a major hurdle in understanding antiparasite immunity and developing efficacious vaccines against malaria. Even for well described parasite proteins, such as Circumsporozoite protein (CSP) and Apical Membrane Antigen 1 (AMA1), the effect of antigenic diversity on naturally acquired protection against infection remains incompletely understood. Using a 14-month longitudinal cohort of 239 people in a high-transmission setting in Western Kenya from 2017 to 2018, we analyzed the influence of CSP and AMA1 diversity on the risk of re-infection. Using reported CSP and AMA1 variants cataloged by amplicon deep sequencing, we analyzed 296 unique CSP and 449 unique AMA1 amino acid sequences from 861 *P. falciparum* positive samples. We used a random forest algorithm to identify the most important amino acid positions for predicting overall fragment diversity, and then used these informative positions to examine the risk of reinfection with similar CSP or AMA1 proteins with mixed effect cox proportional hazard models. For CSP, the most predictive amino acids were clustered in previously identified T-cell epitopes. To further investigate this, we grouped CSP into 27 types, which despite the lower number (27 types vs. 298 haplotypes), were closely correlated with overall CSP haplotype multiplicity of infection (MOI) in individual infections (slope = 0.95, R^2 = 0.99). Compared to asymptomatic infections, exposure to a CSP variant in a symptomatic infection was significantly associated with a prolonged time to reinfection by parasites with homologous CSP type (Adjusted hazard ratio = 0.6, p = 0.0036). For AMA1, defining types based on the most predictive amino acid positions was highly correlated with MOI (slope = 0.9, R^2 = 99); however, there was no significant effect of symptomaticity on time to homologous reinfection based on AMA1 types. These findings highlight the importance of immune responses to specific CSP epitopes in conferring protection against *P. falciparum* infection, as people with symptomatic compared to asymptomatic infections were less likely to be reinfected with homologous CSP types.

0660

IMPACT OF PROACTIVE COMMUNITY CASE MANAGEMENT ON MALARIA AND ANEMIA PREVALENCE AMONG PREGNANT WOMEN IN BANKASS, MALI

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Malaria and anaemia in pregnancy remain common in Mali despite the availability of proven interventions for their control. We conducted a study to assess whether proactive community case management (ProCCM) is more effective than the conventional, passive, fixed site approach to integrated community case management (iCCM) in reducing the prevalence of malaria and anaemia among pregnant women in a rural area of Bankass, Mali, in 2020. This study was nested in a three-year cluster randomized controlled trial assessing the impact of proactive case detection in reducing under-5 mortality. The study was conducted in the catchment areas of seven primary health facilities in which all 137 village-clusters were randomized to ProCCM or control arms. In clusters assigned to the ProCCM arm, community health workers (CHWs) were trained and deployed to conduct at least two hours daily of door-to-door case-finding home visits with the goal of visiting each household at least two times a month. CHWs provided health services in the home including screening for recent illness or symptoms and follow-up for sick patients. CHWs in the control arm provided the same services in their communities at a fixed location. CHWs in both arms offered: malaria rapid diagnostic tests (RDT); antimalarial treatment for those with positive tests; pregnancy testing; referral to facility-based ANC; and follow-up maternal and newborn services. Baseline and annual household surveys were conducted. At the endline survey, all pregnant women were administered RDTs for malaria and hemoglobin levels for assessment of anaemia. The endline survey prevalence of malaria among pregnant women (N= 1,848) was 16.8% (14.9-19.7) and that of mild, moderate, and severe anaemia was 27.2% (95% CI 24.9-29.4), 37.1% (95% CI 34.4-39.8), and 2.7% (95% CI 0.4-3.5), respectively. The study will be unblinded in June 2021, and results will be presented by treatment arm to assess whether the prevalence of these outcomes varied by treatment arm to inform community health system design and malaria control programs.

0661

PEDIATRIC MALARIA DETERMINANTS AND RISK MAPPING WITHOUT CLINICAL DATA IN SUSSUNDENGA MUNICIPALITY, MOZAMBIQUE

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Malaria is a parasitic borne disease representing a large burden in poor countries' economies such, as Mozambique. In Mozambique malaria is the main public health concern. Children and pregnant women are at higher risk for morbidity and mortality due to decreased acquired immunity to clinical symptoms. Malaria transmission has a strong association with weather, socio-demographic determinants and age. Few studies on pediatric malaria have been done in Mozambique. Malaria risk is rarely uniform between places and the knowledge of malaria determinants and the evaluation of local heterogeneity can improve the understanding of pediatric malaria incidence and spatial distribution. Two consecutive years (2018 and 2019) of pediatric malaria case data (N = 42,248, n = 21,663) and, climatic (precipitation, mean temperature and Relative humidity) monthly data for 2019 were extracted from Sussundenga Rural Hospital and, world weather online records respectively. Attributable factor of the disease, incidence, sex, pediatric age category and patient origin were investigated. The relationship between the malaria cases and climate was tested using regression and Pearson correlation. For risk mapping, ten malaria factors (sociodemographic, climatic and clinic) were used to produce two maps one using malaria incidence data and, other without. Bioclimatic, Diva GIS 7.4.0 and, Landsat 8 image were used to produce the maps. From the visiting patients, 51.2% tested positive for malaria with an incidence of 45.7%. There is a difference between neighborhood in malaria incidence ($p<0.05$). As per gender, the malaria positive patients were equally distributed. Age groups 5 to 14 years presented 45.6 % of the malaria cases. R^2 was implying that, 74.5 % of the malaria cases can be explained by rainfall, temperature and humidity. Both maps showed a malaria risk in the northeast and southeast areas. The malaria high risk areas seem to be located in high populated areas and, close to water bodies. Relevant information is provided for effective planning in malaria intervention for pediatric malaria patients.

0662

EXPLORING DISCREPANCIES BETWEEN MALARIA TEST POSITIVITY RATES FROM AUTOMATED READERS AND ROUTINE SURVEILLANCE DATA IN THE DEMOCRATIC REPUBLIC OF CONGO

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Test positivity rate (TPR) can be a strong predictor of malaria transmission, provide estimates of temporal changes in malaria burden and display seasonal fluctuations, particularly in areas with unimodal rainfall patterns. TPR has a direct relationship with incidence but is comparatively less influenced by reporting rates or care seeking. TPR is important in assessing true burden of malaria, but it is vulnerable to provider misreporting of test results, which warrants exploration. From early 2016 to mid-2019 automated malaria rapid diagnostic test (RDT) readers, Deki Readers, were deployed in health facilities across two provinces in southern Democratic Republic of Congo (DRC). Previous research has shown that Deki Readers are as good at interpreting RDTs as trained healthcare workers. A retrospective review of malaria data was conducted, comparing TPRs reported from the Deki Readers against TPRs reported from the same facilities in the DRC Health Management Information System (HMIS). Data from 102 health facilities that regularly used the Deki Readers in 2017 and 2018 were extracted. Paired t-testing showed a statistically significant ($p<0.001$) difference in TPRs of 30.2 percentage points by source, with an average annual Deki Reader TPR of 23.6% (CI: 22.7 - 24.6), compared to 53.8% (CI: 52.6 - 55.0) from HMIS. TPRs from the Deki Readers correlated strongly with climate trends, whereas data from HMIS largely lacked the expected seasonality. The difference was more extreme during the low malaria transmission season (May - September), during which the Deki Readers reported a TPR of 16.3% (CI: 15.1 - 17.5) compared to 51.1% from DHIS2 (CI: 49.1 - 53.1). For the two-year period these data show the TPR from HMIS is 128% higher than the TPR from Deki Readers. In DRC, the majority of suspect malaria cases are tested by RDT and present at peripheral facilities where diagnostic capacity is limited, and healthcare workers may have less support and training than staff at larger hospitals. These data suggest that potential reporting biases in HMIS data may be substantial and have considerable implications for true measures of cases, incidence and treatments.

0663

SPATIO-TEMPORAL DYNAMICS OF MALARIA IN ZANZIBAR

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Since 2017, malaria incidence in Zanzibar has gradually been increasing, undermining efforts to reach malaria elimination. This study describes the spatio-temporal dynamics and rainfall effect on malaria cases in Zanzibar. Case-level malaria data were obtained from Zanzibar's Malaria Case Notification (MCN) system from January 2015 to April 2020. For each primary (index) case attending public or private health facilities and reported through the MCN system, the following additional data were recorded at index case households: sex, age, other household members tested and treated for malaria, and self-reported travel history outside or within Zanzibar during the previous month. Using data aggregated at shehia (subdistrict) level, we performed a statistical hot-spot analysis, spatio-temporal regression and Kendall's concordance test to describe the spatial distribution of cases and its correlation with seasonal rainfall. During the study period, 27,046 index cases were reported through the MCN system, of which, 40% had a travel history outside of Zanzibar. The total number of index cases ranged from 3,745 in 2015 to 4,252 in 2017, but steadily increased after 2017 to 5,494 in 2018 and 6,766 in 2019. From 2017 to April 2020, precipitation increased in both the short (Nov-Jan) and long (March-May) rainy seasons. The number of reported cases was significantly correlated with the precipitation of the previous three months. The percentage of tested household members who were malaria positive (secondary cases) showed a declining trend from 2015 (5.1%) to 2018 (4.1%) and an increase in 2019 (5.8%). The identified hot spots of index cases showed similar spatial distribution over the years. In 2019 and 2020, the number of cases and their spatial distribution during both rainy seasons were similar (Kendall's $W= 0.76$, $p<0.05$). Results show a correlation between the recent increase in malaria cases and precipitation in Zanzibar. Identified malaria hotpots will be used by the Zanzibar Malaria Elimination Program for more effective targeting of interventions and allocation of programmatic resources.

0664

COMPREHENSIVE ASSESSMENT OF HETEROGENEITY OF MALARIA PREVALENCE AMONG CHILDREN UNDER FIVE YEARS IN THREE MALARIA ENDEMIC SUB-REGIONS OF UGANDA

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The current national malaria indicator surveys are conducted at regional level thus they don't provide district and sub-district malaria prevalence data. This study was conducted to provide district and sub-district specific malaria prevalence for timely localized malaria control decision making. A total of 6,350 children under five years of age (U5) from 7,684 Households with the consent of their caregivers/parents were tested for malaria using both microscopy and malaria Rapid Diagnostic Tests (mRDTs). Microscopy results were used to compare the heterogeneity of malaria prevalence. Analysis was conducted using STATA version 12 and significance levels done using Fisher's test at 5%. The overall U5 malaria prevalence in the three sub regions was 17.1%. Karamoja sub-region had the highest malaria prevalence of 36% followed by Acholi (9.8%) and Lango (7.9%). In Karamoja sub-region, Moroto and Amudat districts had the lowest prevalence of 1.9% and 2.3% respectively, while Kotido, Nabilatuk and Nakapiripirit had the highest at 55.4%, 50% and 42.1% respectively. In Acholi sub-region, Amuru and Lamwo districts had the lowest prevalence at 2.1% and 3.2%, while Nwoya, Kitgum and Gulu had the highest at 22.8%, 10.7% and 10.6% respectively. In Lango, Amolator and Alebtong had the lowest prevalence at 2.4% and 2.9% respectively, while Kole, Kwania and Otuke had the highest at 17.0%, 15.9% and 14.9% respectively. Malaria prevalence significantly (Fisher's value=0.005<5%) increased with age ranging from 8.4% for children 0-11 months to 22.1% for children 48-59 months. Study findings also showed that malaria prevalence significantly (Fisher's value=0.000<5%) decreased with increasing wealth status of the individuals from 31.6% (lowest quintile) to 15.2% (second lowest quintile), 14.8% (middle quintile), 11.3% (fourth quintile) and 10.1% (highest quintile). However, the prevalence did not significantly (p-0.425>5%) change with gender. i.e. 17.7% for males and 16.4% for females. The study confirmed that malaria prevalence across the three sub-regions is highly heterogeneous in terms of geographical, age and wealth quintile.

0665

MULTIPLE ARTEMISININ-BASED COMBINATION THERAPIES REMAIN EFFECTIVE FOR PLASMODIUM FALCIPARUM MALARIA IN MALI: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Artemisinin-based combination therapies (ACTs) were deployed in 2005 as an alternative to chloroquine and are considered the most effective treatments for uncomplicated *Plasmodium* (P.) falciparum malaria. While widespread artemisinin resistance has not been reported to date in Africa, recent studies have reported artemisinin resistance in Southeast Asia. Review studies have recently been performed on a country-specific basis for Sudan, Ethiopia, and Cameroon. The purpose of this study is to complement these studies with a current review of ACT efficacy based on trials occurring at Mali study sites, where *P. falciparum* malaria is highly endemic. A systematic review of the literature was performed to identify randomized clinical trials on ACTs at Mali study sites. Selected studies included clinical trials occurring at Mali study sites with reported polymerase chain reaction (PCR)-corrected adequate clinical and parasite response rates (ACPRcs) at 28 days. Data were pooled and analyzed using random effects meta-analysis approaches. ACPRcs were pooled and compared between artemether-lumefantrine (AL; the first-line treatment for *P. falciparum* malaria in Mali) and non-AL treatment arms. Results A total of 10 studies met the inclusion criteria, and a risk of bias assessment carried out by two independent reviewers determined low risk of bias among all assessed criteria. The overall ACPRc for the first line AL ACT at Mali sites was 97.3% (95% CI: [95.9%, 98.8%]), while the ACPRc among non-AL treatment arms was 98.2% (95% CI: [97.2%, 99.3%]). The difference in ACPRcs between non-AL treatment arms and AL treatment arms statistically significant (p = .016) suggesting that there are potential treatment alternatives beyond the first-line of AL in Mali. ACT remains highly effective in treating uncomplicated *P. falciparum* malaria in Mali. Country-specific meta-analyses on ACTs are needed on an ongoing basis for monitoring and evaluating drug efficacy patterns to detect drug resistance and guide local malaria treatment policies, particularly in the wake of observed artemisinin resistance in Southeast Asia.

0666

IMPACTS OF SEVERE CYCLONES ON MALARIA RISK ALONG THE MOZAMBIQUE AND ZIMBABWE BORDER

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Mozambique currently has the 5th highest malaria prevalence globally. While there is within country heterogeneity, the central region has high malaria incidence and prevalence. The central region is also prone to seasonal cyclones which have the potential to impact malaria transmission through increased rains and altered water tables. These cyclones also cause damage to property and infrastructure which can increase the risk of malaria and impact the effectiveness of intervention programs. Sussundenga District in Manica Province lies directly on the Zimbabwe border. A cross-sectional community survey was conducted from December 2019-February 2020 to determine the malaria prevalence, risk factors, and impacts of severe weather events. This study was conducted one year after Cyclone Idai, which was devastating to the central region of Mozambique and eastern Zimbabwe. *Plasmodium falciparum* community prevalence was documented as 31% in 2019. The primary intervention available to the community is limited to insecticide treated nets (ITN), and coverage was 65%. 59% of households reported structural household damage related to the cyclone, with 21% reporting that their home was completely destroyed. Household damage done during the cyclone was also strongly associated with malaria positivity during the survey. Additionally, 21% of households reported that all of their ITNs were destroyed or lost during the cyclone. While ITN coverage is moderate in this area, they are protective against infection. Destruction of ITNs during the cyclone was also associated with malaria infection at the time of the survey. The impacts of this cyclone are still contributing to malaria risk in the long-term. With increased frequency of cyclones in this region, it is imperative that these long-term impacts are well understood to respond accordingly.

0667

BULK SEGREGANT APPROACHES TO FINDING DRUG RESISTANCE LOCI IN PLASMODIUM FALCIPARUM

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Understanding the genetic architecture of complex phenotypes, such as drug resistance, is critical to developing effective interventions. Resistance to the antimalarial artemisinin (associated with mutations in the *kelch13* gene), along with resistance to other partner drugs (such as piperaquine), has emerged and is spreading in Southeast Asia. We demonstrate that bulk selection of uncloned progeny from *Plasmodium falciparum* genetic crosses cause allele frequency shifts at drug resistance loci and these selection effects can depend on genetic background. Our human liver-chimeric mouse model was used to generate four genetic crosses: i) multi-drug resistant Cambodian (KH004-2-019) and drug-sensitive Malawian (Mal31), ii) drug-sensitive lab-line (NF54) and an artemisinin-resistant C580Y *kelch13* mutant from the Thai-Myanmar border (NHP1337), iii) drug-sensitive from the Thai-Myanmar border (MKK2835) and NHP1337, and iv) drug-sensitive from the Thai-Myanmar border (MKK2835) and NHP1337 Y580C (the natural C580Y *kelch13* was CRISPR-edited to Y580C and is artemisinin-sensitive). The bulk recombinant populations from these four crosses were cultured *in vitro* and treated with antimalarial drugs at various concentrations. To optimize selection protocols, we first determined IC_{50} drug-dose responses and ring-stage survival (for DHA) for the six individual parents of these crosses and four of the bulk populations. Treatment of bulk populations with 100 nM of chloroquine identified a locus at *pfcrt*, confirming the ability of this approach to pinpoint resistance loci. Using a synchronized bulk population treated with 50 nM of DHA, we selected a strong allele frequency shift at the *kelch13* locus, extending our approach to drugs with strong stage specificity. Bulk segregant analysis allows us to initially screen phenotypes in thousands of progeny to identify candidate loci to be further investigated by individual clonal phenotyping and/or CRISPR validation.

0668

WHOLE GENOME ANALYSIS OF FOUR KENYAN PLASMODIUM FALCIPARUM STRAINS AS A PROOF OF CONCEPT OF DOWNSELECTION FOR CONTROLLED HUMAN MALARIA INFECTION

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Given its morbidity, mortality, and global distribution, malaria is a significant risk to human health worldwide. Despite efforts with chemotherapy, bed nets, and insecticides, additional drugs and a vaccine are sorely needed. The WRAIR model of Controlled Human Malaria Infection (CHMI) is an invaluable tool to test vaccine and drug efficacy in healthy volunteers. However, as of 2021, only 4 *Plasmodium falciparum (Pf)* strains have been optimized for use in CHMI studies, and they do not represent genetic diversity of *Pf* strains in the field. To help to fulfill this gap, identified by FDA as one of the major gaps preventing CHMI studies from aiding licensure of a malaria vaccine for immune-naïve adults, we recently started the project to select and develop a suite of GMP-vialed *Pf* that represents geographic, genomic, and antigenic diversity for use in pre-clinical and clinical experiments, particularly CHMI. As a proof of concept, we have developed and optimized the pipeline to downselect novel *Pf* strains using culture-adapted *Pf* strains previously collected in Kenya. We demonstrated that 4 out of 32 strains can be successfully propagated *in vitro*, produce healthy gametocytes and sporozoites that are able to infect primary human hepatocytes. In order to evaluate genetic diversity, we sequenced the genomes of 4 strains. An average of ~43,000 SNPs and ~44,000 indels were observed across the 4 strains. All 4 strains contained a substantial number of coding variants in a panel of 16+ immunologically relevant regions for *Pf* vaccine development such as CSP, AMA1, TRAP, and LSA1. To assess a full genomic profile of 4 strains, we compared SNP calls against publically available *Pf* genomes revealing a strong representation to other Kenyan parasites despite rigorous culture adaptation. All 4 strains were also genetically distant of laboratory strains currently used for CHMI. The genomic characterization of *P. falciparum* strains provides invaluable information critical to development of CHMI strains to support vaccine and drug strategies against malaria.

0669

DEVELOPMENT AND EVALUATION OF NEW MULTIPLEXED AMPLICON SEQUENCING ASSAYS FOR PLASMODIUM FALCIPARUM MOLECULAR SURVEILLANCE

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Molecular surveillance of *Plasmodium falciparum* can aid in tracking drug resistance mutations, transmission intensity, and connectivity of parasite populations. Multiplexed amplicon sequencing of small, highly diverse genomic regions balances the information content of targeted deep sequencing with the feasibility necessary for surveillance at scale in disease-endemic regions. While the use and availability of *Plasmodium* amplicon panels are increasing, no previous study has rigorously assessed the need for customization based on geographic region and expected diversity. Here we describe two new amplicon panels and evaluate their performance across a range of geographies: a four-amplicon panel composed of highly heterozygous antigen targets that works on samples with parasitemia as low as 10 parasites/ul without a pre-amplification step, and a 128-amplicon panel that incorporates drug markers and benefits from selective whole genome amplification (sWGA) for samples with parasitemia below 1000 parasites/ul. We evaluated the panels on mock samples of varying parasitemia level and polyclonality as well as clinical samples from a range of geographies. We find that the four-amplicon panel improves upon the current standard of AMA1 sequencing for estimating complexity of infection (COI), with no additional protocol complexity or cost, and can reliably distinguish clonal vs. unrelated parasites. The larger 128-amplicon panel performs well in detecting intermediate levels of relatedness, and supports geographic attribution at a level comparable to two other recently published amplicon panels of similar size. We demonstrate that panels designed to target highly diverse populations are also informative in low diversity populations, suggesting that custom panel design will not be needed in most cases. Our findings suggest that choice of panel should depend on the specific study questions, and support the use of amplicon panels as information-dense, cost-effective methods for COI estimation, geographic attribution, and relatedness inference.

0670

TRACKING PLASMODIUM VIVAX THROUGH THE GREATER MEKONG SUBREGION USING GENOMES

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The Greater Mekong Subregion (GMS) intends to eliminate malaria by 2030. Malaria incidence in the GMS is heterogeneous; some countries, such as China, are approaching elimination status, while others, such as Myanmar, still have a high malaria burden. Additionally, multiple species of *Plasmodium* cause malaria in the GMS, though *Plasmodium vivax* is present within the majority of the region's malaria infections. *P. vivax* predominance in recent years has become more staggering as t *P. falciparum* incidence has decreased. Large asymptomatic reservoirs and a dormant hypnozoite stage that can re-emerge months or years later make elimination of *P. vivax* especially challenging. Crossing this hurdle towards elimination will require targeted studies of *P. vivax* within the GMS.Genomics can provide a wealth of information for understanding the evolution and transmission of *P. vivax* through the GMS. In our studies, *P. vivax* whole genome sequences from across the GMS revealed notable structure between the east and west. While the China-Myanmar border was quite distinct from its neighbors, substantial genetic similarity existed between samples from Thailand and Cambodia. Despite the high incidence and a unique genetic profile, obtaining high-quality parasite samples from Myanmar is difficult, and little information exists from this part of the region. Genetic barcodes can help discern genetic differentiation information from relatively low amounts of parasite DNA and increase our available data. Therefore, we tested differentiation with existing genetic barcodes in this region and found that many of the variables in existing barcodes are not differentiated enough between populations to be informative here. Using our prior genetic differentiation results, we propose a new barcode. Whole-genome quality parasite sample size from Myanmar was small and whole-genome variant calling may not fully predict the capability of genetic barcodes, so follow-up analysis with existing barcodes in the northern part of the GMS is underway to determine if existing barcodes are capable of predicting structure from practical field data.

0671

PARASITE DETERMINANTS OF DISEASE SEVERITY FROM INFECTION WITH PLASMODIUM FALCIPARUM

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We present a statistical analysis of deep meta-transcriptomic sequencing directly from whole blood of 220 samples obtained from pediatric malaria patients admitted to the Siaya County Reference Hospital in Siaya, Kenya. Our study design enabled the correlation of genetic features (expression profiles, genome inventory, complexity of infection, and mutational patterns) with various disease attributes, including parasite level, hemoglobin level, day zero vs. day 14 changes, and white blood cell counts. Transcriptomic data were analyzed on a by-read basis using peptide signatures and the Sequedex metagenomics analysis software, and by read-mapping to the KE01 completed genome from the MalariaGen project with bowtie2. Mapped reads were visualized with the Integrated Genome Browser, with gene expression quantified with htseq and variants identified with samtools. We observed numerous highly expressed parasite genes significantly associated with severe infections, including numerous surface antigens, histidine-rich proteins, and a cluster of exported proteins at the end of chromosome 14. Proteins with expression levels associated with less severe disease were also observed, including a lactate dehydrogenase. Functional characterization with metagenomic signatures showed considerable variation of *Plasmodium* heat shock proteins and those associated with glycolysis, redox metabolism, and vitamin B6 biosynthesis. Examination of patterns of polymorphisms associated with highly expressed genes provided evidence for both multiple infections within specific individuals and the same strains infecting multiple individuals in our sample set.

0672

FREQUENCY AND EVOLUTION OF HISTIDINE-RICH PROTEIN 2 AND 3 DELETION AMONG GLOBAL PLASMODIUM FALCIPARUM STRAINS

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Rapid diagnostics tests (RDTs) are a user-friendly diagnostic tool for suspected malaria infections in clinical and epidemiological settings. The majority of RDTs detect for presence of circulating *Plasmodium falciparum* (Pf) histidine-rich protein 2 (HRP2) in peripheral blood. HRP2-detecting RDTs can also cross-react with histidine-rich protein 3 (HRP3) in high-density infections (>1000 p/ml). Of concern is an increase in the frequency of global Pf isolates with HRP2/3 deletions, compromising our capabilities to monitor malaria in limited resource settings. We developed a computational pipeline to analyze whole genome sequence (WGS) datasets, generated using the Illumina platform, of field isolates to detect for the presence of deletions in the loci region encoding HRP2 and HRP3. Present deletions are characterized by start and end coordinates, the extent of the deletion (whole *vs*. partial coding sequence deletion), and whether it involved flanking intergenic regions and flanking genes. We are currently analyzing WGS data of ~1400 Pf isolates, collected in 24 countries in South America, Africa, Southeast Asia and Oceania. Samples identified as having deletions at either *hrp2* or *hrp*3 will be stratified by deletion characteristics, to determine the minimum number of independent deletion events per country. When applicable, we will perform a longitudinal analysis of HRP2/3 gene characteristics between samples from the same region collected at different time points. The historical use of RDTs will be examined in countries with increase deletion events over time. Within the partial deletion group, further investigation will identify the extent of the deletion (exon 1, exon 2, and intron). Results will be validated using a HRP2/3 gene-specific deletion assay that is effective in single or mixed genotype infections to ensure the reliability of pipeline results. Our study will demonstrate the capability and feasibility to accurately investigate *hrp2*/*hrp3* gene modifications and its frequency, characteristics and evolution to better understand potential drivers of these modifications over time and on a global scale.

0673

PLASMODIUM FALCIPARUM POPULATION STRUCTURE IN SOUTHWESTERN AFRICA, BASED ON NEWLY GENERATED GENOME-WIDE SEQUENCE DATA

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Angola, seventh in Africa both in area and in estimated number of malaria deaths in 2020, encompasses the southwestern-most edge of the distribution of *Plasmodium falciparum* in the continent. It is surrounded by distinct *P. falciparum* subpopulations, one in central Africa (Cameroon/Gabon) to the north, and another in south-central Africa (Democratic Republic of the Congo) to the northeast. Due to the lack of *P. falciparum* population genomics studies in Angola, it is unknown how genetically distinct its parasite population is from those of its neighbours, or whether molecular markers specific to the Angolan *P. falciparum* population(s) can be identified, to inform on parasite migration within and between countries. To address these knowledge gaps, we will generate and analyze whole-genome sequence (WGS) data for ~150 *P. falciparum* samples from children ≤ 5 years old, obtained from several Angolan provinces. Blood samples collected between 2006-2009, by fingerprick on filter paper, will be used. For each sample, DNA was extracted within one year of collection and stored at -20ºC. Initially, to determine if the DNA was still viable for genome-wide analysis, we used *P. falciparum* selective whole genome amplification on four samples. Genomic libraries were sequenced in an Illumina NovaSeq sequencer, to generate paired-end 150 bp-long reads, and average genome coverage >35X. The sequencing data was mapped against the canonical *P. falciparum* 3D7 reference genome, single nucleotide polymorphisms (SNPs) identified according to best practices, and a principal component analysis was done using the recovered high confidence SNP calls, together with SNP calls from ~400 publicly available *P. falciparum* samples from East, West and Central Africa. Preliminary results show that Angolan samples group with others from Central Africa, indicating that these decade-old samples have adequate quality for WGS studies. WGS data currently being generated for the remaining >100 samples will be used to identify Angola-specific molecular markers and to initiate a study of *P. falciparum* demography in southwestern Africa, with a focus in Angola.

0674

USING BULK SEGREGANT ANALYSIS TO PROBE THE NUTRITIONAL REQUIREMENTS OF PLASMODIUM FALCIPARUM BLOOD STAGE GROWTH

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In efforts to find novel targets to combat the malaria causing parasite *Plasmodium falciparum*, scavenging of nutrients and metabolic biosynthetic pathways can be potent targets for drug design. To study the roles of genes involved in parasite fitness in different nutrient conditions, we conducted an allopatric experimental genetic cross between a parasite of African origin, NF54 and a parasite of Asian origin, NHP4026. After we transitioned the recombinant parasites from liver stage-to-blood stage, we used bulk segregant analysis to compare genome-wide allele frequency changes in biologically independent progeny populations. Populations were grown in human serum, mirroring *in vivo* conditions, or AlbuMAX, a commercial bovine serum formulation commonly used to maintain asexual blood stage parasites in the laboratory. Genome wide changes in allele frequency were then measured over 15 asexual cycles. This bulk segregant approach *detected* three quantitative trait loci linked with differential growth conditions that contained strong candidate genes: aspartate transaminase, *AST (chromosome 2); cysteine protease, ATG4 (chromosome 14)* and *EBA-140 (chromosome 13).* Alleles inherited from NF54 (chromosomes 2 and 14) and from NHP4026 (chromosome 13) were positively selected for in AlbuMAX, while the same alleles were selected against in serum. Selection driving differential growth was strong (the selection coefficient, s = 0.10-0.23 per 48-hour lifecycle) and was observed in all biological replicates. These results demonstrate the effectiveness of using bulk segregant approaches to reveal nutritional polymorphisms in *Plasmodium falciparum*. In turn, this will allow for a systematic dissection of nutrient acquisition and metabolic pathways that are potential targets for intervention against *P. falciparum*. Ultimately, this technique is suitable, alongside classical progeny analyses for the genetic mapping of a wide range of selectable traits including host specificity, immune evasion, and drug resistance.

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COMPLEXITY OF INFECTION ESTIMATION WITH ALLELE FREQUENCIES

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Polyclonal or complex malarial infections can provide insight into the genetic diversity and population structure of parasites as well as their transmission and within-host dynamics. The proportion of complex infections and the complexity of infection (COI—the number of strains infecting an individual) have been shown to be the most informative for inferring transmission intensity. Current state-of-the-art methods to estimate the COI such as THE REAL McCOIL are computationally intensive when working with a large number of samples or numerous genetic loci. In this study, we determined two relationships that tie the population-level minor allele frequency and the within-sample frequency of the minor allele to estimate the COI. We derived easily calculable measures to directly estimate the COI from these parameters that are easily obtained from sequencing read depth data. Our methods are computationally efficient, estimating the COI of samples in less than a second, and perform well on simulated data. Furthermore, we demonstrate that our methods are comparably accurate to current methods in the literature. We apply both THE REAL McCOIL and our new methods to estimate the COI for 5,970 *P. falciparum* samples from 28 malaria-endemic countries collected as part of Pf3k (release 6). We explore the global heterogeneity of the COI and characterize the relationship between malaria prevalence at the time of sample collection and the COI. Lastly, we detail how our methods can be used to explore further within-sample parasite information, including the relatedness of parasites within mixed infections.

0676

NEXT-GENERATION-SEQUENCING-BASED ANALYSIS OF THE POLYMORPHISM OF MALARIA GENES IN AN ENDOGENOUS POPULATION OF PALAWAN ISLAND

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Genetic polymorphism of *Plasmodium falciparum* gene is a major concern for malaria control strategy. Host immune evasion, as well as genemutation, are known to be two important factors underlying the mechanism of resistance. Genetic analysis is one of the best ways used to identify mutant alleles in a local population parasite. In this study, we applied Next Generation Sequencing method to analyze 11 nominal genes obtained from an endogenous population of Palawan Island for their genetic polymorphisms. This study revealed the habitation of a variety of *P.f* strains in this region. 72 peripheral blood samples were collected from confirmed malaria cases. After DNA extraction and amplification by PCR, the PCR product was sequenced following Illumina miseq NGS protocol. GENETYX VER.15 application was utilized to make a multiple sequence alignment of those consensus sequences in each gene. The phylogenic tree helped to categorize the different alleles present in those genes. NGS succeeded in sequence around 87.8% of the sample. 46.38% of successful sequences showed high-quality coverage. Newly identified SNPs were obtained from 5 vaccine candidate genes and 3 antimalarial drug target genes. The identified alleles varied between examined. B-cell epitope and T-cell epitope areas were analyzed for their SNPs distribution. SNPs in all the immunogenic genes were mainly targeted to the B-cell areas. 40% of our sample presented *PfDHFR* a resistant gene associated with a mutation at C59R. We didn't get enough PCR product explaining the low number of sequences observed in certain genes. *PfDHFR* and *GLURP* show very much different allele frequency due to the functional difference Conclusion NGS-based analysis can be applicable to population genetics study.

0677

ELUCIDATING GENETIC DIVERSITY IN MALARIA DRUG RESISTANCE GENES IN AFRICA FOR THEIR IMPACT ON DRUG DEPLOYMENT AND TO MODEL EFFICACIOUS DRUGS

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Malaria continues to plague the world, particularly sub-Saharan Africa. The gradual spread of resistance to artemisinin-based combination therapies in South East Asia is a clarion call for malaria-endemic regions, including Ghana. Heterogeneity in artemisinin resistance markers in Africa most likely. A prememptive approach to drug resistance monitoring will ensure a better control than how chloroquine and sulphadoxine-pyrimethamine resistance were handled. Additionally, the mechanism of action of resistant drugs is fully not understood. We, therefore, hypothesized that new mutations could be one of the driving forces of resistance across

Africa. The aim of this study is to identify putative and novel resistant variants, estimate their prevalence across Africa and the impact a variant or haplotype may have on protein structure, function and phenotypic associations of proteins encoded by *Pfcrt*, *Pfdhfr and Pfmdr1*. This study was carried out using 2596 African genome variations in *Pfcrt*, *Pfmdr1* and *Pfdhfr* from the MalariaGEN database. The retrieved variants comprise samples from Ethiopia, Gambia, Ghana, Kenya, Malawi, Mali, and Tanzania per each gene. Data pre-processing, sequence translation, sequence alignment and phylogenetic analyses were done. Mutability and high-quality structures of *Pf*crt, *Pf*mdr1 and *Pf*dhfr were generated for downstream analyses. Mapping of mutations and their effect on structural properties were done via Chimera. We expect to identify a novel set of mutations and haplotypes in *Pfcrt* and *Pfmdr1, Pfdhfr and Pfmdr1* across the African continents which could potentially drive ACT partner and SP drug resistance respectivelyin Africa, and relate these mutations to protein structure and function to elucidate the possible mechanism of action. This may form the basis for designing better novel antimalarial drugs.

0678

AGE-RELATED DIFFERENCES IN MONOCYTE DNA METHYLATION AND IMMUNE FUNCTION IN HEALTHY ADULTS AND CHILDREN LIVING IN AN AREA WITH INTENSE MALARIA TRANSMISSION

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Age-related changes in adaptive and innate immune cells have been associated with a decline in effective immunity and chronic, low-grade inflammation. Epigenetic, transcriptional, and functional changes in monocytes occur with aging, though most studies to date have focused on differences between young adults and the elderly in populations with European ancestry; few data exist regarding changes that occur in circulating monocytes during the first few decades of life or in African populations. Such changes in innate immune cells early in life are likely to have a substantial impact on immunity to pathogens that disproportionately affect young children, most notably malaria. We analyzed DNA methylation profiles, cytokine production, and inflammatory gene expression profiles in negatively selected monocytes from healthy young adults (aged 19-35 years) and children (aged 1-9 years) from Kisumu County, Kenya, an area with perennially high malaria transmission. We identified several hypo- and hyper-methylated CpG sites in monocytes from young adults vs. children that replicated findings in the current literature of differential DNA methylation in monocytes from elderly persons vs. young adults across diverse populations. Differentially methylated CpG sites were also noted in gene regions important to inflammation and innate immune responses. Monocytes from young adults vs. children displayed increased production of IL-8, IL-10, and IL-12p70 in response to TLR4 and TLR2/1 stimulation as well as distinct inflammatory gene expression profiles. These findings complement previous reports of age-related methylation changes in isolated monocytes and provide novel insights into the role of age-associated changes in innate immune functions, which may be relevant to the acquisition and maintenance of clinical immunity to malaria.

0679

DISSECTING ANTIBODY MEDIATED FUNCTIONAL RESPONSES ASSOCIATED WITH PROTECTIVE IMMUNITY IN MALARIA INFECTED INDIVIDUALS USING A RAPID, WHOLE BLOOD FLOW CYTOMETRY ASSAY

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Naturally acquired antibodies against *Plasmodium falciparum* malaria are key to protection, but their functional mechanisms remain poorly understood. Antibody dependent phagocytosis (ADP) of *P. falciparum*-infected erythrocytes (IEs) by neutrophils and monocytes is an important potential mechanism of pathogen clearance. To produce a more physiological model to measure ADP of *P. falciparum*-IEs, we devised a rapid flow cytometry assay using human whole blood without cell isolation. IEs opsonised with heat-inactivated plasma from malaria-exposed pregnant women were subjected to phagocytosis by neutrophils and monocytes in prediluted whole blood from malaria naïve volunteers. Fluorescently labelled antibodies against CD16 and CD66b, CD14, and CD45 were used to identify neutrophils, monocytes, and all leukocytes, respectively. Neutrophil and monocyte ADP of opsonised IEs is exposure-specific, and lysis of erythrocytes followed by fixation increase assay throughput without altering ADP of opsonised IEs. We observed good intra-donor and inter-donor correlation when assays were repeated among different donors (n=3) for both neutrophils (Spearman r=0.69-0.92) and monocytes (Spearman r=0.6-0.69) representing high reproducibility. In plasma collected in mid pregnancy from 77 women, we observed significantly more phagocytosis of IEs opsonised with plasma from pregnant women who progressed to placental infection at delivery compared to women who did not have placental infection, by both neutrophils (Mann-Whitney U test, P<0.0001) and monocytes (P=0.0007). A comparison ADP assay conducted with isolated neutrophils using IEs opsonised with the 77 plasma samples and correlated with neutrophil phagocytosis in whole blood showed a moderate correlation (Spearman r=0.5) between the assays. This assay can simultaneously quantitate exposure-specific opsonic phagocytosis of IEs by neutrophils and monocytes in human malaria. ADP was increased in pregnant women protected from placental malaria compared to those with placental malaria, indicating the assay's potential to measure protective immunity in malaria.

0680

EXPANDED NATURAL REGULATORY T CELLS IN PREGNANCY-ASSOCIATED MALARIA ALTER DENDRITIC CELL FUNCTION INDIRECTLY THROUGH EFFECTOR T CELLS

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In malaria endemic area where pregnancy modulates malaria-specific responses in women, we sought to determine whether expanded populations of natural regulatory T cells (nTregs) directly modulate the production of malaria-induced cytokines by antigen presenting cells (APCs). We first used flow cytometry to phenotype nTregs in malaria-infected and malaria-uninfected pregnant women. We found that the frequencies of nTreg expressing CTLA-4, GITR, LAG-3, and IL-10 were significantly higher in malaria positive compared to malaria negative pregnant women. Depletion of CD25+ cells from PBMCs or antibody blockade of GITR, LAG3, PD1, CTLA-4 and IL-10R in PBMCs from malaria positive women significantly increased the production of malaria-specific IL-12p70 (p = 0.02), CXCL-10 (p=0.04) and CXCL-9 (p = 0.02) by APCs. However, co-culturing nTregs and APCs purified from malaria positive women did not affect malaria-specific cytokine production compared to APCs alone (p > 0.05), nor did blockade of GITR, LAG3, PD1, and CTLA-4. Antibody blockade of IL-10R, in contrast, did up-regulate APC production of malaria-specific CXCL-9 (p= 0.02), CXCL-10 (p = 0.0078) and IL-12p70 (p= 0.0049) when APCs were co-cultured with nTregs

0681

MALIAN CHILDREN DEVELOP ACUTE BUT NOT DURABLE ANTI-PFEMP1 SEROLOGIC RESPONSES TO CLINICAL MALARIA INFECTION

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Plasmodium falciparum erythrocyte membrane protein-1s (PfEMP1s) comprise a diverse family of proteins expressed on the surface of infected red blood cells. Each *P. falciparum* genome encodes dozens of antigenically distinct PfEMP1s. These parasite proteins adhere to host vasculature, aiding in replication and immune evasion. Host anti-PfEMP1 immune responses may play a role in protecting against clinical illness, particularly severe malaria. Using a custom protein microarray, we measured the serologic responses of children (n=40, 1-6 years old) in Bandiagara, a rural Malian town, over the course of a malaria transmission season. We hypothesized that at the start of the malaria transmission season, children who did not develop clinical infection have greater serologic responses to extracellular PfEMP1 fragments than children who developed clinical infection, that children who developed clinical infection have an increase in responses at peak transmission season compared to season start, and that these responses persist through end of season. We defined a fragment as serorecognized if a group's mean fluorescence intensity (MFI) to the fragment was significantly greater (Kolmogorov-Smirnov test, $p<0.05$) than naïve North American control's. At the start of the season, children who did not develop clinical infection serorecognized a similar number of fragments as children who developed clinical infection (28/138 extracellular PfEMP1 fragments and 25/138, respectively). Also at the start, they had similar (115/138) and lower (22/138) MFIs than children who developed clinical infection. Children who developed clinical infection gained serorecognition (40/138) and had higher MFIs (18/138) at peak-season than they did at the start. These increased responses waned quickly: serorecognition was lost (18/138) between peak and end of season, and MFIs to all PfEMP1 fragments did not differ between season start and end. Further studies of PfEMP1 serologic responses in a wider age range of Malian children over multiple malaria transmission seasons may provide insight into their role in protective immunity.

0682

CYTOKINE PROFILE IN PREGNANT WOMEN WITH PREECLAMPSIA AND PLACENTAL MALARIA

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Placental malaria and preeclampsia are independently known to alter the inflammatory/angiogenic pathways and are associated with poor pregnancy outcomes. However, it is unknown if the inflammatory/angiogenic pathways during PM precipitate or aggravate the syndrome of PE. We studied how markers of inflammation and angiogenesis compare in cases of preeclampsia comorbid with placental malaria. Our study sampled peripheral and placental plasma from 74 pregnant women (30 with Normal pregnancy and 44 diagnosed with preeclampsia) at delivery. We measured levels of twenty-six inflammatory and angiogenic markers using a multiplex bead-based ELISA assay. Placental malaria was diagnosed by the histological examination of placental biopsies. Biomarker levels were mostly elevated in placental plasma compared to the periphery except C-Reactive Protein (CRP) which showed an inverse trend. In the peripheral plasma of preeclampsia diagnosed women with placental malaria, most biomarkers associated with inflammation/anti-angiogenesis such as CRP, CXCL8 showed higher concentrations. Preeclampsia diagnosed women with past placental malaria had lower concentrations of regulatory markers such as Il10 compared to normal pregnant women. The pattern was quite similar in the placental plasma. Correlation analysis was used to explore association between same markers from peripheral and placental plasma. The markers in peripheral plasma were distinct from those from placental plasma across the groups with and without placental malaria or preeclampsia. This study has generally shown that placental malaria further dysregulates inflammation/angiogenesis in preeclampsia. This knowledge should be expounded to inform clinical management.

0683

ANTIBODIES TO PFEMP1 EPCR-BINDING ANTIGENS BUT NOT CD36-BINDING ANTIGENS REMAIN ELEVATED FOR TWELVE MONTHS IN CHILDREN HOSPITALIZED WITH SEVERE MALARIA

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Antibodies to *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) are naturally acquired over time in people living in malaria-endemic countries and contribute to malaria immunity. Antibodies to endothelial protein C receptor (EPCR)-binding domains, which are frequently expressed in children with severe malaria (SM), are typically acquired before antibodies to other PfEMP1 domains, such as CD36-binding domains, which are more often expressed in uncomplicated malaria. The ordered acquisition of antibodies is believed to contribute to clinical immunity to severe malaria in early childhood in highly malaria endemic areas. To understand the role of PfEMP1 antibody mediated protection in SM, we measured IgG antibodies to 18 PfEMP1 domains in cohort of Ugandan children with SM (n=116) or asymptomatic community control children (n=22, 8 of whom had *P. falciparum* parasitemia) at enrollment and 12-month follow-up, using a multiplex cytometric bead assay. 83.6% of children with SM had antibodies to ≥1 of nine EPCR-binding antigens tested and 82.8% had antibodies to ≥1 of 6 CD36-binding antigens. 68.2% of CC group had antibodies to EPCR-binding antigens ($p=0.09$ compared to SM), and 68.2% of CC group had antibodies to CD36 antigens ($p=0.11$ compared to SM). At 12-month follow-up, prevalence of antibodies to EPCR-binding antigens among children with SM was similar to baseline (87.9%), but the prevalence of antibodies to CD36 antigens decreased significantly (58.6%, $p<0.001$ compared to enrollment). However, among CC, there was no significant reduction in prevalence of antibodies to EPCR- or CD36-binding antigens from enrollment to 12-month follow-up. In this study, children with SM had persistent antibodies to PfEMP1 EPCR-binding antigens, but decreased antibodies to CD36-binding antigens over time, while asymptomatic community control children had persistence of antibodies to both antigens over time. Ongoing analysis is being performed to determine if antibodies to either PfEMP1 antigen family were associated with protection from recurrent severe or uncomplicated malaria.

0684

CELLULAR AND HUMORAL RESPONSES TO THE HIGHLY IMMUNOGENIC PLASMODIUM VIVAX PROTEIN VIR14

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While *Plasmodium vivax* is the most widely distributed malaria parasite, tools to prevent, diagnose, and treat *vivax* infections are lacking. The *P. vivax* superfamily of VIR proteins can localize to the surface of infected red blood cells and is targeted by naturally acquired immunity which could aid in the development of new tools. In a proteomic survey, we identified a peptide identified to be from VIR14 in the urine of 2/4 infected Brazilian subjects. We then assessed VIR14 immune responses in diverse malaria-endemic populations, including residents of *vivax*-endemic Rondonia (Brazil) and Pursat province (Cambodia) and *falciparum*-endemic Mali. By ELISA, we detected VIR14-IgG in 74/121 (61%) adults in Brazil and 19/55 (34.5%) adults and children in Cambodia during vivax infections. IgG subclass profiling of Brazilian subjects revealed the response is dominated by IgG1 (detected in 42.9%) and IgG3 (in 36.1%). None of the 28 Malian adult *falciparum*-infected subjects showed detectable VIR14 reactivity, implying the absence of a cross-reactive *P. falciparum* antigen. Immune cell profiling via flow cytometry revealed that subjects with the highest VIR14-IgG levels had higher proportions of monocytes in PBMCs, as well as higher CD8+ T cells and lower CD4+ T cells measured as percent of CD3+ T cells. Considering the prevalence of VIR14 antigen and antibody in affected populations, further studies of VIR14 as a target for vaccines or diagnostic tools for *P. vivax* are warranted.

0685

CELLULAR CORRELATES FOR PROTECTION AGAINST MALARIA ACQUIRED ACROSS MULTIPLE PREGNANCIES

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Malaria during pregnancy remains a significant cause of morbidity and mortality for both pregnant women and their infants, although with successive pregnancies, women eventually develop immunity against these adverse outcomes. To identify cellular correlations of malaria protection in pregnancy, we obtained cryopreserved peripheral blood mononuclear cells both at enrollment (2nd trimester) and delivery among 100 pregnant women. Cellular phenotypes and intracellular cytokine production following stimulation with 3D7 and CS2-infected red blood cells were assessed by flow cytometry. We found that women in their first pregnancies have a malaria-specific CD4+ T cell response characterized by high IL-10, and that multigravida women have a malaria-specific response that is more TNF-producing (P=0.009). Higher malaria-specific IL-10 producing CD4+ T cells at enrolment were associated with an increased likelihood of malaria parasitemia during pregnancy (P&lt0.0001). Furthermore, women with placental malaria had a malaria-specific CD4+ T cell response characterized by increased IL-10, whereas women without placental malaria had a malaria-specific response characterized by increased TNF. To further characterize these cytokine-producing populations we are performing RNA sequencing on sort-purified cells expressing TNFA or IL10/IFNg. Also, correlations between cellular responses and malaria-specific antibody repertoires are ongoing. Identification of correlates of protection against malaria in pregnancy will guide mechanistic studies and assist in prevention efforts, including chemoprevention and vaccines.

0686

INDUCTION OF HIGH ANTIBODY LEVELS AND PROTECTIVE IMMUNITY BY MRNA-LNP VACCINES BASED ON PRE-ERYTHROCYTIC AND TRANSMISSION-BLOCKING ANTIGENS IN PLASMODIUM FALCIPARUM

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The mRNA based vaccination represents a new promising alternative to conventional vaccine approaches against infectious diseases because of its high potency and rapid development. Whether mRNA vaccines may elicit potent immune responses and effective protection against human malaria parasites is still elusive. In this study, mRNAs encoding *P. falciparum* circumsporozoite protein (PfCSP) and P25 (Pfs25) were optimized with appropriate UTRs and nucleoside modifications and complexed with lipid nanoparticles (LNPs). mRNA-LNPs were administered intramuscularly to female Balb/c mice (three doses at 4 week interval). Groups of mice (N=5) received various doses (3, 10, 30 ug) of either PfCSP or Pfs25 mRNA-LNPs as well as combination of both mRNAs (10ug each). In parallel, groups of mice were immunized with DNA vaccines encoding PfCSP, Pfs25 or a combination of both via *in vivo* electroporation for comparison of immunogenicity differences. Antigen specific antibodies were analyzed by ELISA. Immune protection against sporozoite challenge was evaluated by microscopic evaluation of blood stage parasitemia, and transmission blocking activity was evaluated in standard membrane feeding assays. All the mRNA-LNP immunized mice generated much higher antigen specific antibody titers than those in DNA vaccine immune mice. Elevated specific antibodies were detected after the primary dose of mRNA-LNPs, increasing dramatically to much higher levels after subsequent immunizations in all mRNA-LNP groups. Similar antibody levels were observed for both PfCSP and Pfs25 between the single mRNA groups (10ug/dose) and combined mRNA group (10ug each/dose), suggesting no interference of immunogenicity. After sporozoite challenge, all the mice in the groups receiving 10 and 30 ug PfCSP mRNA-LNPs were fully protected, whereas in the group receiving 3 ug mRNA-LNP only 1 out of 5 mice was fully protected. Also, 4 out of 5 mice in the combined mRNA-LNP group were fully protected. Our studies strongly support further studies on the development of mRNA-LNP formulations for vaccines targeting multiple stages in multiple *Plasmodium* species.

0687

ANTIBODIES TO PLASMODIUM FALCIPARUM CIRCUMSPOROZOITE PROTEIN: A NON-MECHANISTIC CORRELATE OF PROTECTIVE EFFICACY OF PFSPZ VACCINE IN THE FIELD

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Plasmodium falciparum (Pf) sporozoite (SPZ) vaccine (PfSPZ Vaccine) has been assessed in 1,726 5-month to 61-year-olds in Africa, Europe, and

the US. Vaccine efficacy (VE) has been assessed against controlled human malaria infection (CHMI) and field exposure to heterogeneous, intensely transmitted Pf. VE against CHMI has been > 90% in 5 trials in the US, Europe, Tanzania, and Mali, and durable for at least 14 months. Best VEs against naturally transmitted Pf infection in Mali and Burkina Faso have ranged from 48% to 56% and been shown to last for at least 18 months. Vaccine development and implementation would be enhanced by having an assay that predicts protection, a correlate. VE is thought to be mediated by tissue (liver) resident CD8+ cells, which cannot be assayed, and assessment of peripheral blood mononuclear cells does not correlate with protection. We have extensively studied antibodies to PfSPZ and the major surface protein on PfSPZ, the Pf circumsporozoite protein (CSP) for an association with protection in the field. The results of 3 studies in 18-50 year olds (MLSPZV1, N=42 vaccinees, 5 doses of $2.7x10^5$ PfSPZ), (MLSPZV2, N=54, 3 doses of $1.8x10^6$ PfSPZ), (BFSPZV1, N=39, 3 doses of $2.7x10^6$ PfSPZ) and one study in 5-12 month olds (KSPV1, N=67, 3 doses of $1.8x10^6$ PfSPZ) show that IgG antibodies to PfCSP 2 weeks after last vaccine dose were significantly higher in subjects who did not become infected (protected) as compared to those who became infected (unprotected) in follow up; serum dilution at which the OD was 1.0: 1,742 vs 231 (p=0.044), 3,790 vs 978 (p=0.001), 5,550 vs 1,410 (p=0.01), and 10,138 vs 3,724 (p=0.007) in the 4 trials respectively. Assessment of IgM antibodies to PfCSP and IgG antibodies to PfSPZ by IFA showed similar results. However, results of a functional assay, inhibition of sporozoite invasion assay, did not show an association with VE. These data suggest that the antibodies are not mediating VE, but are a non-mechanistic correlate (biomarker) of VE of PfSPZ Vaccine in the field at the group, but, because of extensive overlap in the ranges, not at the individual level. These data as well as data from CHMI studies will be presented.

0688

MAXIMIZING THE POTENTIAL WINDOW OF OPPORTUNITY FOR MALARIA ELIMINATION VIA GENE DRIVES AND CHEMICAL VECTOR CONTROL

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Gene drives result in select genes spreading through a population at a higher than normal inheritance rate leading to phenotypes associated with these genes dominating a population faster than random genetic drift. Phenotypes that have been considered for mosquitoes carrying a gene drive include among others mosquitoes that are rendered refractory to malaria transmission as well as ones that distort the sex of the progeny to bias the male-female ratio to suppress a population. Given rising levels of insecticide resistance across sub-Saharan Africa, gene drives are being developed as a solution to decreased chemical intervention efficacy. However, populations of mosquitoes that experience gene drive introductions eventually develop resistance to the drives as well either through natural mutations or due to incorrect cleaving and copying of the drive components at the target site. This would eventually lead to drive failure in the population and the loss of desirable phenotypes. Additionally, uncertainty in future climate cycles as well as heterogeneity of mosquito migration and behavior patterns over large geographies would necessitate continued chemical insecticide pressure on regional vector populations to drive transmission to zero within a specific window of opportunity. Here, we investigate which gene drive strategies offer the most optimal path to malaria elimination in a range of transmission settings by leveraging a large scale individual-based model of malaria transmission, EMOD, in combination with a multi-locus, agent-based model of vector genetics that accounts for mutations and many-to-many mappings of genotypes to phenotypes. We also evaluate release conditions that will maximize the efficacy of gene drive propagation as well as chemical intervention rotation strategies that offer the largest window of opportunity for elimination than either method deployed individually in a range of transmission settings.

0689

EVALUATING THE ROLE OF COVERAGE, CAMPAIGN TIMING, AND ADHERENCE ON THE PROGRAMMATIC IMPACT OF SEASONAL MALARIA CHEMOPREVENTION: A MODELING STUDY

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The WHO recommends seasonal malaria chemoprevention (SMC) with sulfadoxine-pyrimethamine plus amodiaquine (SP+AQ) for children in regions where malaria is highly seasonal and drug resistance is low. While SP+AQ is highly effective at reducing uncomplicated malaria incidence in clinical trials, its effectiveness declines during programmatic SMC deployment. This study implemented simulation modeling to identify and quantify factors that affect the programmatic performance of SMC. We modeled malaria transmission with EMOD, an established individual-based simulation modeling software, and extended the model to include the pharmacokinetics and pharmacodynamics of SP and AQ. We incorporated an SMC campaign by distributing four cycles of SMC monthly throughout the rainy season. The population of eligible children was divided into two groups: high or low SMC accessibility. Children in the high-access group received all four rounds of SMC while those in the low-access group had variable chance of receiving SMC each cycle. We validated the model against study data from a programmatic SMC deployment in Kaya District, Burkina Faso then simulated the effect of SMC after varying coverage level, campaign timing relative to the transmission season, or adherence to doses 2 and 3 of AQ. We found that coverage has the greatest effect on reducing clinical incidence followed by timing. Increasing coverage to 95% of children resulted in a 77% greater reduction in clinical incidence compared to the baseline coverage level of 58% of children and starting the first SMC cycle any time in the month before peak transmission season decreased clinical incidence 10% more than starting SMC during peak transmission season. We find no evidence that adherence rate affects clinical incidence. Our results suggest that the decay in SMC impact during programmatic deployment is more likely attributable to insufficient coverage and suboptimal timing rather than poor adherence. We plan explore the role of drug resistance in future model iterations. The results of our model provide supportive data for optimizing future SMC deployments.

0690

MODELING PLASMODIUM FALCIPARUM PARASITE GENETICS INSIDE AN AGENT-BASED, SPATIAL MODEL

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The genetics of *Plasmodium falciparum* is playing an increasingly important role in the understanding of malaria transmission and guiding the use of interventions. A model of the parasite's genetics has been integrated into IDM's open source Epidemiological MODeling software. EMOD is a stochastic, mechanistic, agent-based model that simulates the actions and interactions of individuals within geographic areas in order to understand the disease dynamics in a population over time. The new parasite genetics module takes the existing model from sporozoites to gametocytes in the human and extends it with a detailed model in the mosquito of the parasite progressing from gametocyte to oocyst to sporozoite. This enables us to more easily model parasite genome changes due to mutation and recombination. We will explain how this is modeled as well as demonstrate how different intervention strategies - such as ITN, IRS, and MDA - impact the complexity of infection as a metric of changing transmission intensity.

0691

EVALUATING THERAPEUTIC EFFICACY STUDY METHODOLOGIES WITHIN AN AGENT-BASED MODEL OF MALARIA WITH EXPLICIT PARASITE GENETICS

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Therapeutic Efficacy Studies (TES) are a critical tool in the surveillance for drug resistance to antimalarials. The ability to genotype parasites observed in treatment failures during these drug trials is necessary to distinguish true drug failures (recrudescences) from reinfections as individuals in endemic settings continue to be exposed to infectious bites. As WHO guidelines for molecular corrected of TES have not been revisited in over a decade, and diverse approaches are used to evaluate parasite genotypes, mathematical models of malaria offer the capability to test the robustness of both molecular techniques and algorithms against simulations of drug treatment trials. Here we demonstrate the accuracy of novel and traditional methods for molecular correction of TES results within the context of EMOD, a calibrated individual based model of malaria epidemiology with explicit parasite genetics. Using simulation, we explore the sensitivity of molecular correction methods as a function of drug type and failure rate, sequencing technology, ranges of transmission intensity, seasonality, and within-host immunity. We demonstrate the relative impact of these sensitivities on the resolving power of commonly used genotyping methodologies and classification algorithms. These results add to a growing body of evidence that updated methods are needed for obtaining accurate failure rate estimates that can be compared across transmission settings.

0692

A SYSTEMS-LEVEL GENE REGULATORY NETWORK MODEL FOR PLASMODIUM FALCIPARUM REVEALS ELEVATED TRANSCRIPTIONAL VARIATION IS ASSOCIATED WITH REDUCED ARTEMISININ SENSITIVITY

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Malaria is a life-threatening disease caused by *Plasmodium* parasites that are transmitted to humans through the bites of infected female *Anopheles* mosquitoes. Unfortunately, reduced sensitivity of even highly effective artemisinin-based combination therapies has been observed in the field. Here, we aim to understand the gene regulatory programs that govern the sensitivity of *P. falciparum* to artemisinin. We built a systems-level gene regulatory network for *P. falciparum* upon the transcriptomic data of parasite isolates from patients with acute malaria, using a well-validated, machine-learning approach. To build the inference network, co-regulated genes were first identified based on the coherence of their transcriptomic profiles across multiple samples and the presence of common upstream binding motifs. With a compiled list of transcriptional regulators, we then implemented a linear regression-based statistical modeling method to determine the directed and weighted gene regulatory interactions. The resulting network accurately predicts expression levels of transcriptionally coherent gene regulatory programs in independent transcriptomic datasets generated in geographically diverse settings by multiple research groups. The model serves as a hypothesis-generating tool for illuminating clinically relevant gene regulatory mechanisms. We found that reduced artemisinin sensitivity is associated with incoherent gene expression across many regulatory programs, including antigenic variation and erythrocyte-host interactions. The association between increased transcriptional variation and reduced artemisinin sensitivity could be explained by the parasite utilizing bet-hedging strategies to diversify the population and help ensure the survival of the population as a whole under drug pressure. Our work demonstrates the importance of transcriptional heterogeneity in promoting the likelihood of survival for certain subpopulations of *P. falciparum* in the context of environmental stresses.

0693

IMPROVING MALARIA SURVEILLANCE THROUGH ROBUST FACILITY CATCHMENT POPULATION ESTIMATION

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Malaria surveillance systems are critically important for local-scale monitoring and evaluation, supporting evidence-based decision making, and ultimately pushing towards elimination. However, their overall utility is undermined when facility catchment populations are either unknown or poorly described. Without accurate population denominators, it is difficult or impossible to determine comparable rates of malaria incidence or intervention and commodity coverage. In the Zambian HMIS DHIS2, 818 out of 2,338 gelocated health facilities are missing population denominators. We developed a geospatial modelling framework for estimating catchment populations, which integrates patient travel times and attributes from individual facilities (e.g., facility type, total outpatient records). This approach produces catchments that account for inherent differences between facilities, incorporate meaningful distance measures, and overlap geographically. This method was used to estimate catchment populations for all geolocated malaria-treating health facilities in Zambia. Population estimates were validated using facility headcount (where available) and household field surveys, and compared to "nearest facility" Voronoi estimates. The catchment model had a higher overall correlation ($R = 0.39$) with the HMIS populations compared to the Voronoi estimates ($R = 0.30$), and had equal or higher correlations in 9 of the 10 individual provinces. The purpose of this model is to support national monitoring, intervention targeting and commodity distribution. We also developed tools to apply this framework to other geographies, and incorporated additional information such as treatment seeking rates and seasonal dynamics. These robust estimates for catchment populations will fill existing gaps and improve the utility of malaria surveillance systems.

0694

USING ROUTINE CASE DATA TO QUANTIFY THE EFFECTIVENESS OF SEASONAL MALARIA CHEMOPREVENTION UNDER PROGRAMMATIC IMPLEMENTATION: A QUASI-EXPERIMENTAL STUDY IN BURKINA FASO

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Seasonal malaria chemoprevention (SMC) was first recommended by the WHO in 2012 to prevent uncomplicated malaria in children, with 21.5 million children under 5 years old receiving at least one dose in 2019. Systematic assessment of the impact of national SMC campaigns requires data with high temporal resolution and spatial coverage over several years. Using routine health facility data from 2015 to 2018 from Burkina Faso, we quantify the effectiveness of SMC against symptomatic malaria with

generalized linear mixed models in a difference-in-differences framework. We group health districts into SMC rollout groups, defined as the group of districts receiving SMC for the first time in the same year. We quantify the effectiveness of SMC in reducing the incidence of confirmed malaria cases, hospitalizations, and the malaria proportion of outpatient visits for each group. SMC showed a weak protective effect on reducing the malaria proportion of outpatient visits in children under 5: 7.13% [95%CI 1.99%, 12.00%] reduction for the 2016 SMC rollout group, 16.94% [10.69%, 22.75%] reduction for the 2017 group, and no significant effect for the 2018 group. SMC reduced reported incidence in children under 5 by 38.53% [32.31%, 44.18%], 45.20% [37.05%, 52.30%], and 25.36% [13.12%, 35.87%] in the 2016-2018 SMC groups, respectively. SMC was also associated with a reduction in malaria hospitalizations in children under 5 by 36.68% [26.97%, 45.10%] in the 2016 SMC rollout group and 23.51% [4.31%, 38.85%] in the 2017 group. SMC was not associated with a significant reduction in any of the three indicators for individuals over 5. Reporting quality and treatment-seeking behavior increased in Burkina Faso concurrent with SMC rollout, potentially introducing trends in routine surveillance data independent of changes in the underlying risk of clinical disease. Analyses of crude incidence can be complemented by analyses on other variables such as the malaria proportion of outpatient visits, which can control for changing surveillance quality, to create a more complete understanding of intervention efficacy.

0695

CONVERSION RULES BETWEEN HAEMOGLOBIN AND HAEMATOCRIT IN PATIENTS WITH UNCOMPLICATED PLASMODIUM FALCIPARUM OR PLASMODIUM VIVAX MALARIA: INDIVIDUAL PATIENT DATA META-ANALYSIS USING WORLDWIDE ANTIMALARIAL RESISTANCE NETWORK DATA REPOSITORY (WWARN)

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Worldwide Antimalarial Resistance Network (WWARN) promotes data sharing and data re-use for generating new evidence to improve health and understanding of the diseases. We used data from the WWARN repository to assess the accuracy of conversion algorithms for haemoglobin (Hb) and haematocrit (Hct) measurements in patients with uncomplicated *Plasmodium falciparum* (Pf) and *P. vivax* (Pv) malaria. Studies in the WWARN repository with concurrent measurements of Hb and Hct at enrolment into clinical trials were identified and individual patient data was requested through WWARNs Independent Data Access Committee. The accuracy of two conversion rules were assessed: Rule 1 - Hct (%) = 3 × Hb (g/dL) and Rule 2 - Hct (%) = 5.62 + 2.60 × Hb (g/dL). The proportion of derived Hb values that fell within ±1 g/dL of the true measurements and the sensitivity [95% confidence interval (CI)] of the conversion rules in identifying anaemic patients (Hb <11 g/dL) were presented separately for Pf and Pv malaria. Forty-five studies enrolling 11,680 Pf patients and 13 studies enrolling 1,588 Pv patients contributed to the analysis. In Pf patients, Rule 1 led to derived Hb values within ± 1 g/dL of true Hb in 72% (2604/3624) of children <5 years, 82% (3780/4640) among aged ≥15 years, 81% (4704/5838) of non-anaemic and 74% (341/463) of severely anaemic (Hb<7 g/dL) patients. The corresponding estimates using Rule 2 were 62%, 76%, 75% and 58%. In Pv patients, Rule 1 led to derived Hb within ± 1 g/dL of true Hb in 53% (42/80) of children <5 years, 85% (1096/1297) of ≥15 years, 85% (1067/1254) of non-anaemic and 69% (229/330) of mild/moderate anaemic (Hb≥7 and Hb<11 g/dl) patients. The corresponding estimates for Rule 2 were 66%, 82%, 83% and 81%. The sensitivity of Rule 1 in identifying anaemic patients was 86% [95% CI: 82-89] in Pf and 77% [95% CI: 65-86] in Pv; the corresponding estimates for Rule 2 were 94% [95% CI: 91-95] and 88% [95% CI: 80-94]. Overall Rule 2 appeared better for identifying anaemic patients, but both rules had lower accuracy in young children and anaemic patients. Modelling is ongoing to explore alternative rules.

0696

IMPROVEMENTS TO GENEPI: INCREASING THE BIOLOGICAL REALISM AND COMPUTATIONAL EFFICIENCY FOR SIMULATING MALARIA PARASITE GENETICS FROM A TRANSMISSION HISTORY

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As malaria control programs expand their genomic surveillance efforts with new collection methods and sequencing technologies, genetic models with flexible internal representations can be valuable for analyzing the wide variety of data. We present recent extensions to GenEpi, a layered and modular framework for interfacing simulations of parasite genetics with transmission records from epidemiological models. GenEpi has been developed as a Python package and is part of comprehensive effort to build an end-to-end pipeline that can connect detailed simulations to genomic data and support the application of the data towards genomic use-cases. Here, we describe updates to the model designed to improve its mechanistic accuracy for evaluating parasite genetic diversity and multiplicity of infection in a population. We incorporate the recombination of gametocytes as they are transmitted from the mosquito vector and construct the evolving population of parasites through a series of transmission chains. With these updates, the model has a self-consistent internal representation that is easy to interpret and can be used to distinguish between the effects of superinfection or cotransmission. We discuss some simplifying reductions in the model's representation of parasites, which make simulations tractable while maintaining the fidelity of critical mechanistic components. GenEpi is intended to be an open source tool that can be adopted by collaborators and the broader scientific community. We demonstrate simulation outputs that can in turn be processed by a sampling and sequencing model, and we conclude with current and future use cases of the model for furthering our understanding and increasing the interpretability of parasite sequences.

0697

IMPROVED ESTIMATES OF MULTIPLICITY OF INFECTION IN MALARIA AND RELATED INFECTIOUS DISEASES

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The UN's Sustainable Development Goals commit to eradicate several infectious diseases, including malaria, to achieve global well-being. These efforts require monitoring disease transmission at a level that differentiates between pathogen variants at the genetic/molecular level. In fact, the advantages of genetic (molecular) measures like multiplicity of infection (MOI) over traditional metrics, e.g., basic reproduction number, are being increasingly recognized. MOI refers to the presence of multiple pathogen variants within an infection due to multiple infective contacts. Maximum-likelihood (ML) methods have been proposed to derive MOI and pathogen-lineage frequency estimates from molecular data. These estimates can be substantially biased for small sample sizes, as they often occur in practice. Considering a single molecular marker, we derive a bias-corrected ML estimator for MOI and pathogen-lineage frequencies. The bias of the estimates substantially reduced. A further bias reduction can be achieved by heuristical adjustments. This results in (almost) unbiased estimates, whose variance coincides with the theoretical minimum among all unbiased estimators (Cramér-Rao lower bound). This suggests that no further improvements are possible unless additional information is provided. Such information can be obtained by combining data from several molecular markers. By averaging MOI estimates from several molecular markers (with desirable properties), the variance of the MOI estimates can be substantially reduced. This heuristic variance reduction is a potent alternative to deriving MOI estimates from combined information

of several molecular markers if missing values are common in the data. Based on a simulation study, we investigate under which situations the heuristic variance reduction should be applied. As an example, we apply and compare different estimates, including the bias-corrected and variance-reduced ones, to several malaria molecular datasets from Africa and South America.

0698

MATHEMATICAL MODELING TRANSMISSION OF WOLBACHIA AMONG ANOPHELES ALBIMANUS MOSQUITO POPULATIONS IN HAITI

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Malaria elimination is possible in Haiti. Preliminary studies of *Anopheles (An.)* mosquitoes infected with *wAlbB Wolbachia* bacterium have shown that the infected mosquitoes are less capable of spreading malaria. The infection induces a cytoplasmic incompatibility that disrupts the infection cycle through population suppression, inhibiting within-vector replication of the *Plasmodium falciparum* parasite, and reducing vector competence. *Wolbachia* is widespread among arthropods, and there are ongoing trials for sustaining wild populations of *Wolbachia*-infected *Aedes aegypti* to prevent the spread of dengue fever. We create and analyze a model to evaluate different approaches for maintaining *wAlbB* infection within *An. albimanus* mosquitoes and apply it to assess its potential as a malaria control strategy in Haiti. Our nine non-linear differential equation model divides the population based on the mosquito's sex, infection status, pregnancy status and includes an aquatic stage. The model simulates the combination of traditional malaria vector control strategies of insecticide-treated net (ITN) distribution and indoor residual spraying (IRS) with different release scenarios of *wAlbB*-infected mosquitoes, and it evaluates the impact that seasonality can have on the releasing time and strategies for reaching a stable high-infection (90%) among wild *An. albimanus* mosquitoes. Our preliminary results indicate that the use of IRS at baseline and release of infected pregnant female mosquitoes during the rainy season lead to the fastest establishment of endemic *wAlbB* transmission. Consequently, acquiring endemic *wAlbB* infection can reduce malaria transmission in *Anopheles* mosquitoes and thereby be a potentially useful measure for preventing and controlling malaria transmission.

0699

A MICROPLANNING MODEL TO IMPROVE DOOR-TO-DOOR HEALTH SERVICE DELIVERY: THE CASE OF SEASONAL MALARIA CHEMOPREVENTION IN SUB-SAHARAN AFRICAN VILLAGES

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Malaria incidence has plateaued in Sub-Saharan Africa despite Seasonal Malaria Chemoprevention's (SMC) introduction. Community health workers (CHW) use a door-to-door delivery strategy to treat children with SMC drugs, but for SMC to be as effective as in clinical trials, coverage must be high over successive seasons. We developed and used a microplanning model that utilizes population raster to estimate population size, generates optimal households visit itinerary, and quantifies SMC coverage based on CHWs' time investment for treatment and walking. CHWs' performance under current SMC deployment mode was assessed using CHWs' tracking data and compared to microplanning in villages with varying demographics and geographies. Estimates showed that microplanning significantly reduces CHWs' walking distance by 25%, increases the number of visited households by 36% ($p < 0.001$) and increases SMC coverage by 21% from 37.3% under current SMC deployment mode up to 58.3% under microplanning ($p < 0.001$). Optimal visit itinerary alone increased SMC coverage up to 100% in small villages whereas in larger or hard-to-reach villages, filling the gap additionally needed an optimization of the CHW ratio. We estimate that for a pair of CHWs, the daily optimal number of visited children (assuming 8.5mn spent per child) and walking distance should not exceed 45 (95% CI 27-62) and 5 km (95% CI 3.2-6.2) respectively. Our work contributes to extend SMC coverage by 21-63% and may have broader applicability for other community health programs.

0700

ASSESSMENT OF INTEGRATED COMMUNITY MALARIA VOLUNTEER KNOWLEDGE AND JOB PERFORMANCE IN MYANMAR, 2018-2020

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The PMI-funded Defeat Malaria Project contributes to Myanmar's goal of eliminating malaria by 2030. As an implementing partner, Jhpiego supports capacity development for the National Malaria Control Program (NMCP) and health providers at all levels of service provision. As malaria cases are decreasing, NMCP adopted a policy on integrated community malaria volunteers (ICMV) in 2017 and trained village malaria workers (VMW) as ICMVs to sustain their knowledge and skills in malaria and 5 other infectious diseases to augment their impact in communities and to provide health education and referral of suspected cases among patients with negative mRDTs. From October 2018 to March 2020, 400 ICMVs in 12 States/Regions received a 5-day training followed by refresher training and regular supportive supervision. Defeat Malaria Project partners and the NMCP collaborated to assess the knowledge and skills of 384 ICMVs of the 400 trained from June - December 2020 (at 5 - 6 months and 9 - 12 months post-training). Due to COVID19 restrictions, assessments were conducted via phone interview using standardized knowledge questionnaires and skills assessment forms. 90% of ICMVs achieved scores of ≥80% on knowledge of malaria and 64% on non-malaria diseases (passing score is ≥80%). 73% knew mRDT testing criteria and 99% correctly performed mRDTs. 99.5% followed national treatment guidelines for malaria and 96% knew referral criteria. Due to lack of confidence only 53% correctly identified, and 34% referred, suspected cases of non-malaria diseases mentioned above to health facilities. Per self-reports, 81% supported LLIN distribution and 58% supported prevention of non-malaria diseases. 97% had stocks of mRDT and anti-malaria drugs; 99% regularly reported on malaria and 90% on non-malaria diseases. 96% advocated to village administrators and the community and 11% received support from the community for referral. ICMV knowledge and skills are influenced by regular training and follow up. The findings will be utilized by NMCP to guide further implementation to achieve malaria elimination and community health care.

0701

TRENDS IN FEVER CASE MANAGEMENT FOR FEBRILE INPATIENTS IN A LOW MALARIA INCIDENCE SETTING OF TANZANIA

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In 2010, the World Health Organization (WHO) recommended parasitological confirmation for all patients with suspected malaria prior to antimalarial initiation. We present data on changes in fever case management following the 2010 WHO policy shift among febrile inpatients in a low malaria transmission setting in northern Tanzania. We analyzed data from 2,098 children and adults enrolled in two hospital-based prospective cohort studies, cohort 1 (2011-2014) and cohort 2 (2016-2019). All patients underwent malaria blood smear microscopy. We compared diagnoses, treatments, and outcomes between the two study periods. Participants who had a negative malaria blood smear microscopy but received a diagnosis of malaria or received an antimalarial were categorized as malaria over-diagnosis and over-treatment, respectively. Of participants, the median (IQR) age was 27 (3-43) years and 1,047 (50.0%) were female. Malaria was detected by blood smear microscopy in 23 (2.3%) participants in cohort 1 and 42 (3.8%) in cohort 2, (P = 0.059). Malaria over-diagnosis occurred in 334 (35.0%) participants in cohort 1 and 190 (17.7%) in cohort 2, (P <0.001). Malaria over-treatment occurred in 528 (55.1%) participants in cohort 1 and 196 (18.3%) in cohort 2, (P <0.001). There were 30 (3.1%) deaths in cohort 1 and 60 (5.4%) in cohort 2, (P = 0.007). All deaths in both cohorts occurred among participants with a negative malaria blood smear microscopy. In summary, we observed a substantial decline in malaria over-diagnosis and over-treatment among febrile inpatients in northern Tanzania between two time periods after 2010. Despite changes, some smear-negative participants were still diagnosed and treated for malaria. Our results highlight the need for continued monitoring of fever case management across different malaria epidemiologic settings in sub-Saharan Africa and the need to concentrate efforts towards improving outcomes for patients with a negative malaria diagnostic test.

0702

IMPROVING INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY UPTAKE IN TERTIARY REFERRAL HOSPITALS IN GHANA THROUGH FACILITY-BASED PREVENTION OF MALARIA IN PREGNANCY (MIP) AND QUALITY IMPROVEMENT TRAINING

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Regional and teaching hospitals in Ghana serve as tertiary referral hospitals (RHs) with high antenatal care utilization and provide vital access to intermittent preventive treatment in pregnancy (IPTp) for pregnant women. In RHs, the National Malaria Control Programme (NMCP) supports capacity building for IPTp services through classroom-type (CT) trainings in malaria in pregnancy (MIP). Review of Health Management Information System (HMIS) data from 2015-2020 revealed low IPTp coverage and poor data quality at these RHs. In 2019, IPTp-3 coverage ranged from 0% for Komfo Anokye Teaching Hospital to a notably high coverage of 181% for Bono Regional Hospital attributable to poor data quality. To improve IPTp coverage and data quality, PMI Impact Malaria (IM) supported NMCP to implement facility-based (FB) trainings in MIP and quality improvement (QI) in six RHs. A total of 183 health workers (HWs) (about 30 per RH) were trained through the FB trainings in November 2020. Training participants included midwives, clinicians, health information officers, store managers, laboratory technologists and pharmacists involved in MIP service delivery. The training aimed to build a team with knowledge on the updated MIP guidelines, logistics and data management skills and competence for quality service delivery and QI methods. To ensure ownership, training facilitators were selected from a pool of clinicians at the RH, and all RHs developed action plans (APs) and established teams to implement them. APs listed priorities through QI to address challenges across departments in knowledge in MIP, health commodity availability and data quality. Post-training supervision occurred two months after training. The average pre-test score was 46% and average post-test score was 75%. Post-training, no RH has experienced stock outs of MIP-related commodities and all show improved MIP data quality. The average IPTp-3 coverage for 5 of the 6 RHs improved from 49% to 100% while MIP data inconsistencies decreased from 83% to 50%. Facility-based MIP training with QI methods complements CT training and improves IPTp coverage and data quality in a sustainable manner.

0703

THE ROLE OF NEEDS ASSESSMENT OF THE COMMUNITY HEALTH PROGRAM IN IMPROVING COMMUNITY MALARIA CASE MANAGEMENT, RWANDA

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Rwanda's community health program is a home-grown solution to address 2 key health challenges: access to health services and a shortage of health providers. Each village has 4 community health workers (CHWs), including a male-female pair providing basic care and the integrated community case management (iCCM) package for childhood illness. Over the past 15 years, the integrated CHW program has helped decrease all causes <5 mortality from 152 to 45 deaths/1000 live births. The Ministry of Health started a community health program needs assessment (NA) to understand program effectiveness and identify challenges. NAs provide information and guide decision-making and resource allocation. Based on community health indicators and reports, in Sept 2020, the Malaria Division and PMI Impact Malaria (IM) conducted a baseline NA on iCCM and home-based management of malaria (HBMm) packages in two new IM-supported districts served by 2360 CHWs. The NA revealed that among 2360 CHWs assessed, 82% were trained on iCCM and HBMm packages; 62.5% reported receiving supervision every quarter from the health center, and 75% from the CHW cell coordinator. The NA flagged unreported stockouts and outdated job aids and materials as challenges. Among the CHWs assessed, 81% had updated registers, 43% completed correctly the iCCM registers, 100% had a malaria treatment algorithm, while only 62% had drug storage boxes. NA results showed that 81% of CHWs were trained in the past two years but there was no plan for refresher training. Findings led to the design and implementation of a Training of Trainers program where 40 nurses and 40 community health environment officers were trained to conduct quarterly supportive supervision, enable all CHWs to provide the iCCM package and train 426 new CHWs. As a result, 83% of CHWs received supportive supervision (SS), updated materials and regular medical supplies. This study showed that NAs conducted jointly with regular SS can generate data which are critical to identifying gaps and challenges that inform the design and adaptation of high-impact solutions. NA recommendations must be well formulated and practical to be effective.

0704

SCALE UP OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY (IPTP) BY COMMUNITY HEALTH WORKERS FOLLOWING THE RESULTS OF A FEASIBILITY PILOT IN PO DISTRICT, BURKINA FASO

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Malaria in pregnancy remains a major concern in sub-Saharan Africa. Since 2012, the World Health Organization has recommended that all pregnant women receive a minimum of three doses of intermittent preventive treatment of malaria in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) starting in the 13th week of pregnancy. In Burkina Faso, the attendance rate for at least 1 antenatal care (ANC) visit is satisfactory (92%). According to the Multiple Indicator Demographic and Health Survey only 42.8% of women in rural areas attended ANC in their 1st trimester of pregnancy in 2015. In 2017, the Improving Malaria Care project (IMC) supported the Ministry of Health to carry out a feasibility study on community level provision of IPTp in three districts (Batie, Ouargaye and Po). This pilot showed that community-based health workers (CHWs) were able to work with facility-based health workers to provide IPTp at the community level (C-IPTp), thereby improving ANC attendance and IPTp coverage among pregnant women. IPTp4 coverage increased from 22% to 47% during baseline April 2017 versus end-line July 2018 in intervention sites while ANC4 attendance went from 62% to 77%. In 2019, following the successful implementation of this pilot, the NMCP—supported by IMC—extended the C-IPTp intervention from 2 to 22 health facilities (and catchment areas) in Po district. The main objective of this extension was to test the effectiveness of the intervention over a larger population. Thus, after cascade training of health care providers and their respective CHWs, the CHWs received commodities for C-IPTp delivery, and technical supervision. Due to COVID-19 restrictions, monitoring of this extension was achieved through telephone calls and analysis of Health Information Management System data. Analysis of the data from January to September 2020 showed an increase of IPTp4 from 33% in 2018 to 55% in 2020, where CHWs contributed 55% of the IPTp 4th dose administration. Additionally, ANC4 visit coverage increased from 37.5% in 2018 to 41.3% in 2020. Skilled CHWs can contribute to increasing IPTp coverage as well as ANC attendance.

0705

DOOR TO DOOR AND FOCUS GROUP COMMUNICATION BY COMMUNITY HEALTH WORKERS: FUNDAMENTAL FACTORS IN IMPROVING COMMUNITY-BASED MALARIA INTERVENTIONS IN BURKINA FASO

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The Ministry of Health in Burkina Faso provides free care for children under five and pregnant women, but attendance at health facilities by these vulnerable groups remains low. The Improving Malaria Care (IMC) project, in collaboration with the National Malaria Control Program, has developed a community-based information approach to raise malaria awareness and improve attendance at health facilities. Four regions: Centre (Ce), Centre-Ouest (CO), Hauts Bassins (HB) and South-Ouest (SO) received a pilot intervention aimed at improving attendance at health facilities through communication. IMC held meetings with 416 Community Health Workers (CHWs) from the 19 health districts covered by the project in 2018. These meetings provided an opportunity to examine sensitization activities that took place in 2018. CHWs conducted 17,872 sensitization sessions that reached 238,136 people, including 149,870 women, on the appropriate use of Insecticide Treated Nets, the importance of antenatal care for Intermittent Preventive Treatment of Malaria in Pregnancy and early consultation for early diagnosis of fever and appropriate malaria treatment. CHW-led sessions took place through educational talks, home visits, individual interviews, film screenings and theater forms. CHWs assessed referred 6,817 pregnant women to the Primary Health Care Center (PHCC); per their referral cards, 4,829 (70.8%) were specifically referred for IPTp. 16,149 children under 5 years were referred for malaria treatment. Of these, 60% were seen at the PHCC, as indicated on their referral cards. There was an overall increase in the Intermittent Preventive Treatment of malaria in pregnancy 3rd dose (IPTp3) uptake in these regions between 2017 and 2018: CO from 46.4% to 64.5; Ce from 38.9% to 43.6%; HB from 39.3% to 53.7%; SO from 38.9% to 55.7%. This intervention contributed to a decrease of under-5 malaria case fatality rate in 3 regions: CO from 1.2% to 0.8%; Ce from 1% to 0.6%; and SO from 1.2% to 1%; the rate stayed stable in the HB at 1.7%. The CHW-led pilot intervention ensured knowledge of and access to available prevention and treatment services by vulnerable groups.

0706

SURVEILLANCE, MONITORING, AND EVALUATION TRAINING IMPROVES PRACTICES: RESULTS FROM A FOLLOW-UP SURVEY OF MALARIA PROGRAM STAFF TRAINING, MADAGASCAR, 2020

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In 2018, the U.S. President's Malaria Initiative (PMI), under the MEASURE Evaluation project, supported the Ministry of Health to train 113 malaria program managers during a seven-day workshop that included content on malaria epidemiology; surveillance, monitoring, and evaluation (SME); developing district M&E plans; assuring data quality and practice with data analysis and use. Mean post-training test scores were 37% higher than pre-training scores. In March 2020, the PMI Measure Malaria, (follow-on to MEASURE Evaluation) conducted an online survey of the trainees to collect demographic information and understand their data analysis, use, dissemination and reporting practices and challenges. Of the 113 contacted, 75 (66%) completed the survey, of whom 16 (21%) were female. Respondents included physicians (49[65%]), nurses (20[27%]), and midwives (6[8%]). 47(63%) reported leading the development of their district's malaria SME plan, and 70 (93%) reported validating malaria data before entry in the District Health Information System (DHIS2) for management and analysis. 59 (79%) had developed at least one malaria bulletin over the previous 12 months, and 72 (96%) reported that their district was using data for decision making, including identifying malaria outbreaks based on weekly reports, monitoring testing and treatment supplies, and summarizing uptake of intermittent preventative treatment during pregnancy for discussions with health staff and community members. Respondents also listed factors affecting low reporting timeliness such as geographic inaccessibility to health centers (29 [39%]), health staff shortages (28 [37%]), weak internet connectivity (20 [27%]), and shortages of phone/text credit (17 [23%]). Overall, nearly all the

respondents reported validating district data, sharing malaria data via bulletins, and using data for decision making. Although the SME training contributed to improvements in data analysis and use, issues remain with staff shortages and increasing access to communication networks.

0707

STRENGTHENING MALARIA SURVEILLANCE THROUGH FRONTLINE FIELD EPIDEMIOLOGY TRAINING IN THE NORTH REGION OF CAMEROON

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To improve health care providers' capacity in malaria epidemiological surveillance, the U.S. President's Malaria Initiative Measure Malaria project supported by the National Malaria Control Program provided the Frontline Field Epidemiology Training Program (FFETP), a 3-month in-service training based on a core curriculum from the U.S. Centers for Disease Control and Prevention that aims to strengthen epidemiologic capacity at the district level of the health system by improving staff's ability to detect, investigate, and respond to diseases of public health importance. The goal was to improve malaria case detection, data collection and reporting, data analysis and interpretation, and dissemination at the operational level. The training targeted Malaria Focal Points (MFPs) working at health facilities (HF) reporting a high number of malaria cases and deaths in the North Region. Training included three weeks of didactic sessions and 9 weeks of field work. Trainees were assessed with pre and post training questionnaires; mastery was assessed using a 5-point Likert scale (no, low, fair, good, or very good knowledge). Trainees produced weekly and monthly surveillance reports, and an analysis report of an identified surveillance problem. Of 25 MFPs enrolled from five health districts, 24 successfully completed the training. Good mastery of key surveillance interventions (case detection, data collection, analysis, interpretation, and communication) increased from 2 of 24 to 21. Prior to the training, no MFP had plotted a graph of malaria cases, but after the training this increased to 22. Furthermore, trainees' report submission timeliness assessed from DHIS2 increased to 94%, compared to the previous year (74%). The training significantly improved frontline staff knowledge on malaria surveillance. Improved analytical and interpretative skills will contribute to early and effective detection of increased malaria cases at the operational level. Expanding the training to more cohorts in addition to post-training follow-up will further strengthen malaria surveillance at the service delivery point.

0708

IMPROVING HEALTH CARE WORKER COMPETENCIES AND ADHERENCE TO GUIDELINES FOR MALARIA CASE MANAGEMENT BY USE OF A BLENDED CAPACITY BUILDING MODEL: KENYA EXPERIENCE

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Current national guidelines for diagnosis, treatment, and prevention of malaria in Kenya aim at improving patient outcomes through case management (CM). Newly recruited health care workers (HCWs) have CM knowledge gaps because pre-service curricula are not always updated with current malaria CM guidelines. The 2019 PMI Impact Malaria (IM) baseline assessment conducted in 186 facilities from the malaria lake endemic counties and 2019 Quality of Care (QoC) survey indicated that many HCWs were not adhering to malaria CM guidelines. While the April 2019 cross-sectional IM baseline assessment found treatment of uncomplicated malaria cases near 100% (range 105-116%), and relatively infrequent presumptive treatment of malaria-negative diagnoses with antimalarials (8%), the QoC survey's composite malaria CM indicator demonstrated only 60% adherence to Kenya's "test and treat" policy, signaling a need for HCW training in the diagnosis and classification components of CM while continuing to promote proper treatment. IM supported 8 malaria-endemic counties to implement a cascade approach to in-service, with frequent, brief facility-based trainings, frequent mentorship, and targeted supportive supervision of new and never-trained HCWs in malaria diagnosis, classification, and treatment. From April 2019 to December 2020, IM trained 60 trainers of trainers (TOTs), supported the TOTs to train 347 subcounty mentors who trained 1,468 HCWs, and mentored 1,826 HCWs in malaria CM. Supportive supervision was conducted for 693 HCWs in 208 targeted health facilities. This blended capacity building approach was associated with an improvement of HCW competency scores in correctly diagnosing and classifying case severity from 88% to 96%, correctly managing uncomplicated malaria from 74% to 87%, correctly treating positive test results from 73% to 88%, and avoiding presumptive treatment of negative test results from 80% to 92%. This capacity building model enables counties and sub counties to have a core team of trainers, mentors, supervisors and competent HCWs who continuously address knowledge gaps for provision of quality malaria CM.

0709

ADDRESSING DATA QUALITY ISSUES OF ROUTINE HEALTH FACILITY MALARIA DATA

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The availability and access to malaria parasitological diagnosis at health facilities (HF), coupled with the adoption of the district health information system (DHIS2), has greatly strengthened the value of routine data. The emphasis in the World Health Organization's Global Technical Strategy 2016-2020 and the High Burden High Impact initiative for the increased usage of quality routine data to support tailored malaria control approach, is likely to further increase its usage. This demands the need to conduct data quality checks for a reliable analysis. In mainland Tanzania, the completeness and consistency of malaria indicators for 7,878 HF were assessed for the period 2017-2019. Essentially, the reporting rate (RR) completeness and missingness, presence of outliers and internal consistency between reports and trends were analysed. 1,208 HF with <50% RR and >5 consecutive months of missing reports were excluded. An algorithm from *anomalize* R package was used to systematically detect outliers and a further 0.1% of monthly reports were excluded. The type and extent of quality checks should be seen at two levels: (i) HF level for the purpose of improving data capturing and reporting; (ii) central level for the purpose of analysis. While detailed guidelines exist for the former, this is not the case for how to process and systematically handle aggregated routine data biases such as inconsistencies, outliers and missing values. A mini-survey was thus conducted amongst various partners between July-September 2020 to understand the current approaches taken for HF data processing and cleaning, and assess whether a harmonized approach was needed to address common challenges. The survey highlighted varying methodological approaches being undertaken, depending on the objectives of the analysis and recommended the need for developing guidelines addressing gaps in routine data and for handling such data in a systematic manner. This is essential for increasing confidence in the data, increase the use of routine data for decision making, and generally enhanced harmonization in the approaches taken.

0710

ACCURACY OF CAREGIVER RECALL OF CHILDHOOD VACCINATIONS: IMPLICATIONS FOR THE RTS,S/AS01 COVERAGE ESTIMATES IN THE CONTEXT OF THE PILOT INTRODUCTION IN WESTERN KENYA

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The RTS,S/AS01 malaria vaccine is being piloted by the Kenya Ministry of Health in 46 sub-counties of western Kenya after a recommendation by the World Health Organization. Feasibility of integrating the 4-dose malaria vaccine into the Expanded Program on Immunization (EPI) schedule is being evaluated through a series of household vaccine coverage surveys. A population representative baseline survey was conducted in July-October 2019 to assess EPI vaccine coverage in 1,338 children 12-23 months of age. Home-based vaccination records (HBR) were available for 1,151 (86%) children; caregiver recall alone was available for the remaining 14% of children. To evaluate the accuracy of recall, vaccination data were collected from HBR and recall for any vaccine dose received, and for each vaccine dose (HBR: dates; recall: number of doses). Agreement between HBR and recall was measured by Gwet's agreement coefficient (agreement). Vaccine coverage estimates from HBR for any dose were high at 95% (95% CI: 92–97) for Bacillus Calmette-Guérin (BCG), 99% (95% CI: 98–99) oral poliovirus (OPV), 98% (95% CI: 98–99) pneumococcal (PCV), 99% (95% CI: 99–100) diphtheria-tetanus-pertussis (DTP), 98% (95% CI: 97–99) rotavirus (RV), and 88% (95% CI: 85–91) measles-containing vaccine (MCV). HBR and recall agreement for any dose was good at 0.94 (95% CI: 0.93–0.96) for BCG, 0.97 (95% CI: 0.96–0.98) for OPV, 0.78 (95% CI: 0.75–0.81) for PCV, 0.95 (95% CI: 0.93–0.96) for DTP, 0.76 (95% CI: 0.73–0.79) for RV, and 0.89 (95% CI: 0.87–0.91) for MCV. Agreement was markedly lower when evaluating specific doses. For example, agreement for DTP dose-1 was 0.93 (95% CI: 0.91–9.95), but only 0.64 (95% CI: 0.59–0.68) for DTP-3. Caregiver's age, education, and number of children were not associated with level of agreement, however agreement was higher when the respondent was the child's mother compared to other caregivers (i.e., fathers, grandmothers, or other relatives, $p<0.05$). The high HBR and recall agreement for EPI vaccines has good implications for receipt of any RTS,S/AS01 doses, though recall may be less reliable for specific vaccine doses. RTS,S/AS01 results are forthcoming.

0711

ARTEMETHER-LUMEFANTRINE ANTIMALARIAL EFFICACY AMONG ADULTS ON ANTIRETROVIRAL THERAPY IN MALAWI

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We conducted a randomized, controlled trial of daily trimethoprim-sulfamethoxazole compared to weekly chloroquine or no prophylaxis among adults on antiretroviral therapy (ART) in southern Malawi. We evaluated artemether-lumefantrine (AL) treatment efficacy in this population, with 81% of participants on efavirenz (EFV)-based ART. Because EFV reduces lumefantrine levels, we hypothesized EFV use may impair treatment efficacy. We conducted a 28-day therapeutic efficacy study among participants with clinical malaria confirmed by blood smear and classified outcomes according to the World Health Organization protocol. We analyzed samples from recurrent malaria infections using PCR for three polymorphic genes: merozoite surface protein 1 and 2, and glutamate-rich protein. Pre- and post-treatment genotypes were compared via gel electrophoresis to distinguish recrudescence from reinfection. Any single difference was classified as a new infection. Plasma lumefantrine levels were determined using LC/MS/MS. From December 2012 to July 2018, 411 malaria episodes were followed with 84.2% (346/411) having an adequate clinical and parasitological response. Treatment failures included 8 early clinical failures, 35 late clinical failures (LCF) and 22 late parasitological failures (LPF). Of 57 participants with recurrent parasitemia (LCF and LPF), we successfully genotyped 42. We identified 12 recrudescent infections and 30 reinfections. PCR-corrected treatment efficacy, assuming unevaluable specimens were recrudescent, was 91.5% (376/411). Mean day 7 lumefantrine levels among 85 samples were 146 ng/mL (97), and mean day 7 desbutyl-lumefantrine levels were 23 ng/mL (9.5). AL efficacy in this population remains above the minimum 90% threshold. However, lumefantrine levels with most below 200 ng/mL, the current recommended dose, suggests increased treatment failure risk due to subtherapeutic drug concentrations. Drug-drug interactions are an important consideration for ART and other drugs with the same metabolic pathway. In addition, the possibility that HIV infection impacts antimalarial drug efficacy should be explored.

0712

PROCESS EVALUATION FOR THE IMPLEMENTATION OF ARTESUNATE RECTAL CAPSULES AS PRE-REFERRAL INTERVENTION FOR SEVERE MALARIA PATIENTS AT COMMUNITY LEVEL IN MADAGASCAR

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Artesunate rectal capsule (ARC) is a pre-referral intervention recommended by WHO since 2015 for children under 6 years of age living in remote areas, to reduce the mortality rate of severe malaria. In 2015, Madagascar National Malaria Control Programme (NMCP) conducted a training to implement this new initiative but the quality assured medicines were not in stock. In 2019, when the commodities were finally procured, a cascade training in 8 regions was coordinated by the NMCP to support the ARC introduction. A process evaluation was conducted to assess the implementation of this intervention at the community level, including the assessment of inputs, activities performed and the short-term impact, to inform the NMCP for a nationwide scaling up. The following approaches were adopted: (a) collection and analysis of existing data (b) two-pronged knowledge, attitude and practice (KAP) survey targeting healthcare providers (CHWs, CSB Heads, central level) as well as the population, to assess health care-seeking behaviour and perception of ARC. A large majority of the population (87%) was in favor of the ARC pre-referral intervention. The majority of CHWs did not have the required management tools and resources to follow the protocol and manage severe malaria cases optimally. Most CHWs (85%) declared having a problem with ARC availability, mainly due to stock out issues in ordering sites.

0713

INTRODUCTION OF RECTAL ARTESUNATE FOR PRE-REFERRAL TREATMENT OF SEVERE MALARIA AMONG CHILDREN UNDER FIVE YEARS OF AGE AT THE COMMUNITY LEVEL IN MADAGASCAR, 2019-2020

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An estimated 9% of children under five years of age (CU5) in remote Madagascar who present to community health volunteers (CHV) or health centers (CSB) with malaria have signs of severe disease. To reduce mortality in this population, the United States Agency for International Development (USAID)- and the U.S. President's Malaria Initiative (PMI)-funded Accessible Continuum of Care and Essential Services Sustained (ACCESS) and Community Capacity for Health (CCH)/Mahefa Miaraka Programs worked with the Ministry of Health to introduce pre-referral rectal artesunate (RA) treatment. For rollout, we prioritized PMI-supported communities with the highest malaria incidence in CU5 and designed a distribution and monitoring plan; developed job aids and conducted provider training; made communication tools for providers and community members; modified patient tracking tools; and analyzed preliminary data from CHVs and CSBs. From August-December 2019, 13,943 RA capsules were distributed to 44 districts (population 8.8 million, 18% of whom were CU5); 11,578 (83%) to CHVs and 2,365 (17%) to CSBs. A total of 11,672 CHVs and 1,056 CSB staff in these districts were trained in RA use. During January-December 2020, 9,535 cases of severe malaria in CU5 were reported, 3,445 (36%) of whom benefited from pre-referral RA treatment (2,067 [60%] treated by CHVs and 1,378 [40%] at CSBs). Of these, 1,360 (39%) cases had outcome data reported via project tracking tools, and all CU5 had received post-referral treatment with an injectable antimalarial. For the remaining 61% of cases, no follow-up data was available. Furthermore, of 23 deaths in CU5 reported via CSBs and the tracking tool during this time, no details regarding treatment were available. In targeted districts, about one-third of intended CU5 received RA therapy in the first year after the launch of the program. To improve, additional investigation will be necessary to describe missed opportunities for RA use in these districts, and tracking of RA recipients must be more comprehensive to understand if CU5 are properly referred and treated.

0714

DETERMINANTS OF HEALTH WORKERS' COMPLIANCE WITH OUTPATIENT MALARIA 'TEST AND TREAT' GUIDELINES DURING THE PLATEAUING PERFORMANCE PHASE IN KENYA, 2014-2016

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Health workers' compliance with outpatient malaria 'test and treat' guidelines has improved but plateaued at the highest yet suboptimal levels in Kenya. We examined the determinants of compliance at the high levels of performance. Association between 31 determinants and three 'test and treat' outcomes were examined using multilevel logistic regression models. A total of 2,752 febrile patients seen by 594 health workers at 486 health facilities were analysed. Higher odds of composite 'test and treat' performance was associated with lake endemic (aOR=8.61; 95% CI: 4.29-17.30), coast endemic (aOR=2.16; 95% CI: 1.02-4.59), highland epidemic (aOR=3.15; 95% CI: 1.81-5.47) and semi-arid seasonal (aOR=1.81; 95% CI: 1.09-3.01) compared to low risk areas; health workers' perception of malaria endemicity as high-risk (aOR=1.85; 95% CI: 1.16-2.94); in-service training (aOR=1.63; 95% CI: 1.19-2.23); correct knowledge about the 'test and treat' policy (aOR=1.64; 95% CI: 1.13-2.39); older patients compared to infants, higher temperature measurements and main complaints of fever, diarrhoea, headache, vomiting and chills. Lower odds of compliance was associated with government-owned (aOR=0.28; 95% CI: 0.17-0.46) compared to FBO/NGO-owned facilities; male (aOR=0.64; 95% CI: 0.47-0.88) compared to female health workers; and for patients having main complaints of a rash (aOR=0.46; 95% CI: 0.23-0.93) and a running nose (aOR=0.67; 95% CI: 0.47-0.94). Other factors associated with malaria testing or antimalarial compliance for test negative patients included supervision with feedback, access to guidelines, health workers age, and a cough complaint. To optimize 'test and treat' case-management, quality improvement interventions should focus on compliance within low malaria risk areas; target male, older and government health workers; disseminate updated guidelines; continue with in-service training and supportive supervision with feedback, and generally improve health workers' knowledge about malaria testing criteria considering their perceptions of endemicity.

0715

RELIABILITY OF REPORTED FEVER IN CHILDREN UNDER AGE FIVE AMONG HOUSEHOLD MEMBERS: AN EXAMINATION OF HOUSEHOLD SURVEY DATA FROM NIGERIA AND MOZAMBIQUE

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Fever among children under age five is the most common symptom household members use as criteria for initiating treatment-seeking. It also used as a screening measure in household surveys for collecting additional health-related information on children. Most surveys have relied on mothers to provide information on their child's fever. However, it is unclear if other household members could provide similarly reliable information. This analysis uses data from three Malaria Indicator Surveys (MIS) in Nigeria (2015 and 2010) and Mozambique (2018) with information on recall of fever among children under age five in the two weeks before the survey. To validate the recall of fever in children under five by household members, the recall of fever as reported by the head of household was compared to the mother's recall using sensitivity, specificity, and kappa coefficients. Across the three surveys, the sensitivity of the head of household's recall of fever ranged from 74.9% in the 2010 Nigeria MIS to 84.4% in the 2015 Nigeria MIS. The proportion of the heads of households correctly reporting that the child did not have fever as compared to the mother's report ranged from 84.7% in the 2010 Nigeria MIS to 94.8% in the 2018 Mozambique MIS. The agreement between the household respondent's report of fever and mother's report of fever, as measured by kappa scores, ranged from 0.59-0.79. Across all three surveys, if the household head was the child's father, the agreement was slightly stronger than if the child was a grandchild or other relative of the household head. This analysis shows that there is a strong collective memory of fever within a household. While a mother should always be the "gold standard" this analysis shows that other household members can potentially provide accurate responses for fostered children or others living in a household without a mother/caregiver.

0716

INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY COVERAGE ESTIMATES FROM POPULATION-BASED SURVEYS: RELIABILITY OF WOMEN'S RECALL AMONG WOMEN WITH ANC CARDS

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Large household surveys depend on recall to estimate coverage rates for various health interventions, including intermittent preventive treatment in pregnancy (IPTp). Many studies call into question the validity of recalled data, and for vaccine coverage rates it is standard practice to validate responses with medical history cards. For malaria in pregnancy interventions, large survey data is important to assess programmatic success and coverage. Sulphadoxine-Pyrimethamine (SP), is recommended by the WHO at least 3 times during pregnancy for IPTp. Key indicators for assessing optimal IPTp coverage are the percentage of women receiving at least one dose (IPTp1+) and the percentage of women receiving at least 3 doses (IPTp3+). To validate intermittent preventive treatment in pregnancy coverage rates in large household surveys, recalled coverage rates were compared to antenatal care cards using sensitivity, specificity, and kappa coefficients from four Demographic in Health data sets from the 2017 Malawi MIS, the 2017-2018 Benin DHS, the 2011-2012 Tanzania HIV and Malaria Indicator Survey (THMIS), and the 2014 Ghana DHS. Across surveys, sensitivity for women's ability to correctly report IPTp coverage ranged from 79%- 92% for IPTp1, from 79% to 96% for IPTp2, and from 73% to 100% for IPTp3+. The proportion of women correctly reporting that they did not receive IPTp (specificity) ranged from 88%-99.6% for IPTp1, from 87%-96% for IPTp2, and from 83% to 98% for IPTp3+. The results found that the recall was comparable to the coverage rates on the antenatal care cards. These findings suggest that intermittent preventive treatment coverage rates from large household surveys based on recalled data are valid without the use of the antenatal card, which is a time consuming additional step to data collection. The results of this study support the validity of recall for IPTp indicators in large household surveys suggesting that national malaria control programs can reliably assess their IPTp intervention coverage and make programmatic decisions based on survey data.

0717

GENDER DETERMINANTS OF INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY (IPTP) UPTAKE WITHIN MALARIA IN PREGNANCY (MIP) SERVICES IN CAMEROON AND KENYA

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PMI Impact Malaria (IM) conducts MiP activities in Kenya and Cameroon, where IPTp use falls from the 1st to the 3rd dose (77% to 38% in Kenya; 75% to 32% in Cameroon). This may be due to late ANC attendance: median months pregnant at ANC initiation is 5.4 in Kenya and 4.5 in Cameroon. Evidence linking gender barriers to early ANC and IPTp uptake is limited. IM examined gender determinants of IPTp use in Kenya and Cameroon using focus group discussions and interviews with parents of children under five, older women, providers, and community health workers. IM Kenya engaged 132 participants in two counties, while IM Cameroon engaged 144 participants in the North and Far North regions. Findings show that women and their partners in both countries know how, when, and why to attend ANC (and in Kenya, use IPTp). In Kenya, those with influence (e.g. friends) and decision-making power (male partners, mothers-in-law) over women determine when she will begin care, and if she will attend ANC or traditional care. Male partners are the key decision-makers in Cameroon. In both countries, men control funds for transport to ANC. Kenyan partners worry about provider abuse, while in Cameroon men hesitate to send their wives to predominantly male ANC providers. Engaging decision-makers is critical to support early ANC uptake. Once a woman begins ANC, decision-making influence shifts from the partner to the provider. If a woman starts ANC she is likely to continue, if the provider treats her with respect. (Respectful care is a gender determinant due to provider attitudes about female clients and inequitable power dynamics between providers and clients.) Cameroonian participants were generally unfamiliar with IPTp, but did note women's lack of financial control to be a barrier, as IPTp is often stocked out at facilities and must be purchased elsewhere. In Kenya, providers counsel clients on the benefits of IPTp, while women are concerned with its smell and taste. Providers can improve IPTp continuation by allowing women to voice their concerns; listening with empathy; and addressing questions. This further supports a positive experience of care, encouraging women to return to ANC.

0718

DEVELOPMENT AND EVALUATION OF VIDEO JOB-AIDS TO SUPPORT SAFE SEASONAL MALARIA CHEMOPREVENTION (SMC) DELIVERY DURING THE 2020 CAMPAIGNS

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We investigated the use of video job-aids to improve the quality of SMC delivery. The study was prompted by the need for training tools that could be used in a socially-distanced manner, and which would familiarise distributors with drug administration procedures to minimise the risk of spreading COVID-19. Videos were developed in French, English, Portuguese and Hausa, illustrating key steps to follow to administer SMC, including wearing masks, washing hands, and keeping a safe distance. A consultative group comprising representatives of Malaria Programmes of The Gambia, Guinea, Guinea Bissau, Nigeria, Senegal and Togo reviewed successive versions of the videos to ensure accurate and relevant content. A case study in Guinea to evaluate the use of the video, used focus

groups, indepth interviews, and direct observation of SMC distribution. Distributors said the video covered all steps they had been taught during their training, but they found the video easier to understand. But messages were not always followed. Though supplied with face masks, some distributors did not use them, a distributor wearing a mask was perceived as a spreader of coronavirus, and some parents refused SMC saying they did not recognise them with the mask. One distributor said "it is difficult to observe social distancing; if you distance yourself from them, they will feel offended". During the SMC campaign the video reinforces messages and can be viewed many times. Used during training, the video provides a focus of discussion and support for trainers. Audio-visuals help retain messages. Limitations were that not all distributors use android phones, and local language versions were requested. Although ownership of smartphones in sub-Saharan Africa is limited (33% in 2017, Pew Research Center 2018), it is increasing, and android devices are used by drug distributors in some countries to record SMC administration. Community health workers increasingly have access to devices that can display video job-aids that can improve quality of delivery of SMC and other primary health care interventions. The videos from the OPT-SMC project are at https://tinyurl.com/35uhf3fh.

0719

THE RELATIONSHIP BETWEEN GENDER EQUALITY AND INSECTICIDE-TREATED NET USE IN UGANDA

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Background: The Ministry of Health with support from the United States Agency for International Development (USAID) funded Social and Behavior Change Activity (SBCA) aims to use innovative social and behavior change (SBC) approaches to improve gender equality norms and healthy behaviors to prevent malaria among other diseases. This study explored insights on the effects of gender equality norms on insecticide-treated net (ITN) use in Uganda. Methodology: A nationally representative mobile phone survey was conducted among 1,400 men and women aged 18-49 years. Gender equality was assessed using four parameters of political, economic, religious, and social gender equality, which was also condensed into a composite variable. Multivariable regressions explored the relationship between ITN use and perceptions on gender equality norms, controlling for socio-demographics. Results: In this mostly rural (70%) population, most respondents slept under an ITN the previous night (83%) and supported political (94%), economic (82%), religious (83%) and social equality (64%). Overall, 80% of respondents reported having equitable gender norms, and ITN use was 5% higher among those who reported equitable gender norms. People with equitable gender norms had a 1.2 higher odds of ITN use after accounting for respondents' region, residence, age, gender, parity, and education level. Discussion: Promoting gender equality at the interpersonal and household levels may improve ITN use through use of gender transformative interventions to address gender determinants of malaria prevention and treatment behaviors. Interventions should involve communities in the co-design of SBC activities and message development, increase partner communication and dialogue about gender norms at individual, household and community levels so as to promote ITN use among all household members.

0720

EVALUATION OF A HAEMOZOIN BASED DIAGNOSTIC TEST FOR MALARIA DIAGNOSIS IN SRI LANKA

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Although malaria was eliminated from Sri Lanka in 2012, in recent years, approximately 50 imported malaria infections per year have been reported. Parasitological surveillance is one of the key strategies to prevent reintroduction of malaria in Sri Lanka. Early diagnosis and treatment of malaria infected individuals can prevent onward transmission of disease. Microscopy remains the traditional gold standard for malaria diagnosis. Rapid Diagnostic Tests (RDTs) are used as a supplementary diagnostic method. Molecular detection techniques have a much higher sensitivity and can detect malaria in cases wherein the parasite densities are below the detection threshold of either microscopy or RDTs, however, these are not cost-effective to use on a large scale. This study aimed to compare the diagnostic capability of a new haemozoin- detecting test (Gazelle™) with microscopy, Carestart RDT and PCR. Blood samples were collected from individuals referred for confirmation of malaria by a clinician (passive case detection; PCD) as well as individuals at high risk of malaria (proactive case detection; PACD). Overall, 305 individuals were screened for malaria between October 2020 and April 2021 as a part of the study. This included 27 individuals by PCD and 278 by PACD. Ten malaria positive patients (2 each of *Plasmodium vivax* and *P.ovale*, 5 *Plasmodium falciparum* and 1 *P. malariae* infections), were diagnosed by microscopy and PCR. Gazelle identified the *P. vivax, P. ovale* and *P. malariae* infections accurately. Gazelle gave false negative results in 2 patients with *P. falciparum* infection in whom the parasitaemia levels were 96/µl and 1188/µL, respectively. This is the first-of-its-kind study wherein haemozoin was tested as a biomarker for malaria diagnosis in Sri Lanka. Gazelle required low level of user training to perform the test and a large volume of samples were screened within a short period of time (~60 samples per device in a day). Use of Gazelle has the potential to benefit the malaria surveillance program and contribute to the goal of preventing reintroduction of malaria in Sri Lanka by providing rapid screening of high-risk population.

0721

ASSESSING THE ROLL OUT OF ARTESUNATE RECTAL CAPSULES AS PRE-REFERRAL INTERVENTION IN FIVE DISTRICTS IN SIERRA LEONE

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An estimated two percent of the global malaria deaths occurred in Sierra Leone in 2019. The progression of uncomplicated malaria to severe malaria is generally attributable to failures and delays in healthcare delivery and health-seeking behavior. To reduce severe malaria deaths, PMI Impact Malaria (IM) supported the National Malaria Control Program (NMCP) to roll out artesunate rectal capsules (ARC) as a pre-referral intervention to peripheral health units (PHUs) in 14 districts. In 2020, IM supported NMCP to train providers in ARC administration and referral across 14 districts reaching 2,452 PHU health providers. Average pre/post-test scores increased from 53% to 86%. In 2021, NMCP and IM conducted a follow-up record review of a convenience sample of 106 PHUs- selected by urbanicity, facility type and proximity to district capital and referral facilities in 5 districts to quantify appropriate ARC administration and referral

practices, and to document health outcomes of patients receiving ARC. All 109 patients who met severe malaria case criteria were administered ARC and were referred. However, 11% of family members/guardians refused the referral. Record review data did not indicate reasons for refusal. Of the 65 patients who were admitted to a referral facility, 5 (8%) died in hospital and 52 (80%) were discharged. Records for 7 patients were not located. Ambulances were used as the transportation method in 75% of referrals. While 72% of ARC administration took place in Community Health Posts or Maternal and Child Health Posts, which are often remote and offer basic care, 92% of patient deaths occurred in patients who were referred from these facilities. ARC rollout in Sierra Leone successfully improved quality severe malaria care to the PHU level. IM will continue to employ its Outreach, Training and Supportive Supervision Plus (OTSS+) quality improvement efforts to provide on-the-job training and mentorship at targeted facilities to maintain high quality of care. NMCP and IM will collaborate with social behavior change stakeholders to understand and address caretaker refusal of referral.

0722

FOCI INVESTIGATION OF A CLUSTER OF MALARIA CASES IN A SINGLE VILLAGE IN COASTAL MYANMAR

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Pyin Boke, the outermost village of Pumpkin Island of Rakhine State, has 55 households and a population of 232. The village is surrounded by paddy fields and streams and is close to the forested mountain. The U.S. President's Malaria Initiative funded Defeat Malaria Project supported and supervised the Village Malaria Worker (VMW) since 2017. Annual Blood Examination Rates were more than 10% for four consecutive years. There had not been any malaria cases detected since 2017 but entomological assessment revealed primary and secondary vectors including *Anopheles minimus, An. annularis, An. sundaicus* and *An. aconitus.* Although the first case had mild fever on 2nd November 2020, he did not seek care for fever from the VMW until 2nd December 2020. An additional 30 cases including his wife and neighbors were identified by reactive case detection conducted in December 2020 and January 2021. The last case was diagnosed on 25th January 2021. All cases were tested with malaria Rapid Diagnostic Test and some cases were confirmed with microscopy. All cases were treated according to the National Malaria Treatment Guideline. Case investigation results revealed that no cases had a history of travel within the previous month. The first case was diagnosed with *Plasmodium vivax* and had migrated from a higher malarious area of Rathedaung Township in the last year. However as past malaria history was unclear, it could not be conclusively classified as "relapsed" and identified it as the index case. Qualitative interviews of key informants revealed that 15 internally displaced people temporarily stayed in the village from June to August 2020. Long-lasting insecticidal treated nets (LLINs) had not been distributed as the village was classified as low risk by the national malaria risk stratification. Amalgamation of high receptivity and little or no immunity of residents against malaria triggered occurrence of cases. Socio-behavior change communication to encourage early care seeking at the onset of fever, to sleep under LLINs, and mobility monitoring are integral to prevent the reintroduction of malaria in receptive areas such as Pyin Boke.

0723

MALARIA AND INTERANNUAL CLIMATE MODES: MECHANISMS AND PREDICTABILITY

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Seasonal predictions of transmission and epidemics can allow public health officials to target malaria interventions more efficiently. Much current work on seasonal predictions of malaria utilize complicated weather forecasts, but predictions based on these detailed forecasts may be difficult and impractical for public health officials to use. Simpler and more practical predictions can potentially be generated by better understanding how interannual climate variability, as represented by large-scale climate modes such as ENSO (El Niño-Southern Oscillation) and IOD (Indian Ocean Dipole), affects malaria transmission. Climate variability is also often disregarded when evaluating the efficacy of past interventions, sometimes leading to overoptimism and a lack of preparedness for future resurgences. Better understanding the effects of different climate modes on malaria transmission can thus allow for more accurate intervention efficacy assessments. Here we compile and summarize peer-reviewed studies conducted throughout the African continent that examine the effects of ENSO, IOD, and other relevant climate modes on malaria transmission. We map these effects over different regions and denote the most important climate mode-associated environmental factors, such as minimum or maximum daily temperature, rainfall, humidity, and vegetation indices, that drive malaria transmission in each studied locale. Based on these findings and the timescales of predictability associated with each climate mode, we make inferences about which climate mode(s) may be best suited for use with early warning systems in each region. We conclude by suggesting high priority pathways for future research on climate mode-driven malaria forecasts, informed by a review of the processes that currently generate the largest uncertainties and complexities in the use of seasonal predictions.

0724

UNDERSTANDING MALARIA IN PREGNANCY SERVICE DELIVERY QUALITY IN SEVEN AFRICAN COUNTRIES

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Coverage of malaria in pregnancy (MiP) interventions, in particular, intermittent preventive treatment of malaria in pregnancy (IPTp), lags behind other key malaria indicators. In 2019, only 34% of eligible pregnant women received at least 3 doses of IPTp across sub-Saharan Africa although antenatal care (ANC) attendance in the region is relatively high. In order to better understand barriers to achieving global MiP targets, we reviewed MiP supportive supervision checklist data collected by the PMI Impact Malaria project and malaria control programs in 7 countries to explore the quality of care (QoC) for MiP prevention and treatment services as measured by a standard checklist as part of Outreach Training and Supportive Supervision Plus (OTSS+) activities. OTSS+ visits took place between October 2019 and March 2021 in Cameroon, Cote d'Ivoire, Ghana, Kenya, Mali, Niger and Sierra Leone. The data collected during these visits was analyzed and included provider training and behavior, elements of respectful care, facility readiness including MiP commodity stock status, and record keeping. Competency scores were calculated based on observations recorded in the supervision checklist. Average competency scores in MiP prevention range from 67% to 91% at baseline and 68-97% at follow up measurement, with five countries improving by an average of 6 percentage points. Average competency scores in MiP treatment range from 50% to 89% at baseline and 73% to 93% at follow up, with 5 countries improving by an average of 11 percentage points. Initial findings highlight the impact of known stock-outs on competency scores and inconsistent application of respectful maternity care principles on MiP QoC. The full analysis to be presented

will explore variations in and implications of individual elements of MiP service delivery on the overall QoC to identify notable successes and key challenges in implementing quality MiP service delivery. This review will generate targeted recommendations for improving MiP service QoC based on trends in MIP service delivery seen across countries, thereby contributing to potential improvements in MiP intervention coverage.

0725

SEX-SPECIFIC EFFECT OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA WITH ARTEMISININ COMBINATION THERAPIES AMONG SCHOOL CHILDREN IN MALI: A THREE-ARM OPEN LABEL RANDOMISED CONTROLLED TRIAL

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Single or multiple courses of intermittent preventive treatment in schoolchildren (IPTsc) has been shown to reduce clinic visits, asymptomatic parasitaemia, and anaemia. To our knowledge, the differential effects of IPTsc by sex have not been the subject of a longitudinal study in Africa. In this analysis, we investigate the overall efficacy of IPTsc and explore differences by sex. We conducted an open-label, rolling cohort randomized controlled trial among schoolchildren aged 6–13 years between September 2007 and February 2013 in Kollé, Mali. Students were randomized into three groups: sulfadoxine-pyrimethamine plus artesunate (SP+AS), amodiaquine plus artesunate (AQ+AS), and control (C). All students received two full treatment courses every two months while age-eligible and for the duration of the study period. We investigated overall effect and sex-specific effect of treatment on *Plasmodium falciparum* infection, haemoglobin and standardized grade-point average (SGPA). Over a six-year period, there were 4,564 observations from the 296 students who were randomised. Antimalarial treatment reduced the odds of *P. falciparum* infection in SP+AS and AQ+AS compared to C (OR 0.33, 95%CI 0.26-0.43, and OR 0.46, 95%CI 0.36-0.59). Strong evidence of increased haemoglobin level (g/dL) was observed in the SP+AS compared to C (difference +0.37, 95%CI 0.13-0.58), although AQ+AS produced similar protection to C (+0.15, 95%CI -0.08-0.37). There was evidence of improved SGPA in the AQ+AS compared to control (difference +0.36, 95%CI 0.02 to 0.69) and evidence of sex-specific difference in treatment effect on SGPA: in the SP+AS the difference in SGPA relative to control was -0.27, 95%CI -0.71-0.16 in boys versus +0.50, 95%CI -0.02-1.02 in girls, whereas in the AQ+AS the difference in SGPA was +0.33, 95%CI -0.12-0.78 in boys versus +0.40, 95%CI -0.10-0.90 in girls, relative to C. IPTsc with SP+AS reduced *P. falciparum* malaria and anaemia among school children and increased the SGPA among girls relative to boys. This evidence supports a more regular investigation of sex-specific effects of malaria interventions in children in sub-Saharan Africa.

0726

INSIGHTS ON FOREST GOER'S EXPERIENCE AND KNOWLEDGE OF MOSQUITO REPELLENT IN CAMBODIA USING RESPONDENT-DRIVEN SAMPLING

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Despite Cambodia's significant progress in reducing the prevalence of malaria over the past decade, some populations like forest goers are at higher risk of malaria infection due to their occupational profile. In August-September 2020, a representative survey of 654 forest goers was conducted using respondent-driven sampling to understand the experiences of repellent use among forest goers. One seed from each of 16 villages in Kampong Chhnang and Pursat provinces was recruited and referred up to three forest-goer contacts. Eligible participants reported spending at least one night in the forest in the past month. Recruitment continued until the required sample was reached, with up to 9 waves for some seeds. There is limited knowledge and awareness on repellents among all forest-goers. Only 25% of all forest-goers have previously heard about repellents before and only 17% of them reported using repellents recently or within the last three months. Among forest goers who have heard about repellents, less than half of them (48%) know where they can get them. Of those who have been exposed to or heard about repellents, 63% think it is very effective while 36% think it is only somewhat effective. Almost half of all forest goers (46%) expressed concerns about repellant use while the rest were not concerned or were unsure about repellents. Main concerns about repellent use revolve around worry about health effects (83%), skin reactions (30%) and possible side effects (18%). Although many forest goers express an intention to use repellents (85%), they think ITN is more suitable for forest goers (70%) over repellents (55%) for prevention purposes. Preventive behaviors are critical to achieve malaria elimination objectives. Repellents remain little known and used among forest goers in Cambodia; many believe they are effective and portable but many also have concerns about side effects. Repellents are not yet a WHO recommended intervention and further studies are required to explore effectiveness of repellents in this context, thus social and behavior change activities for improving prevention behaviors should remain focused on ITN use.

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FINDINGS FROM A RESPONDENT DRIVEN SAMPLING SURVEY EXPLORING CARE-SEEKING BEHAVIORS FOR FEBRILE ILLNESS AMONG FOREST GOERS IN CAMBODIA

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Malaria continues to disproportionately affect Cambodian forest workers and migrants. Due to forest goers' hard-to-reach and mobile nature, novel data collection methods are required to gain behavioral insights to inform appropriate malaria elimination interventions. In 2020, PSI conducted a survey of forest goers using respondent driven sampling (RDS) to understand determinants of care-seeking behaviors, with a total of 654 forest goers identified and included in the study. Only 57% of forest

goers reported seeking care for fever outside the home during their last febrile illness. Among those who sought care, 39% reported receiving care within 24 hours. Forest goers' care-seeking behavior was generally influenced by previous experience of febrile illness, provider preference, and social norms. Forest goers who suspected their last febrile illness was malaria were twice as likely to seek treatment within 24 hours (48% vs 31%). Forest goers who chose to go to a public sector provider were more likely to seek care within 24 hours than who chose a non-public provider (73% vs 34%). Additionally, forest goers who believe members of their community seek care within 24 hours were more likely to do so. Choice of provider is mainly influenced by perception of effective treatment (52.1%), proximity (49.4%), and trust in provider (43.6%). Main reasons for not seeking care were perceptions that the illness was not serious (50.9%) and being present in forest during symptom onset (38%). These findings show levels of care-seeking behavior significantly below targets required to achieve malaria elimination by 2025. Malaria interventions should capitalize on factors that promote prompt care seeking amongst forest goers, including building social norms, and enhancing treatment access for forest goers while in the forest. In particular, targeted social behavior change interventions that focus on improving timely care-seeking amongst forest goers is urgently required in order to meet malaria elimination goals in Cambodia.

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NET USE BEHAVIOURS AND HIGH PREVALENCE OF MALARIA PARASITEMIA AMONG CHILDREN UNDER FIVE IN ZAMFARA STATE, NIGERIA

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In Zamfara state, Nigeria's 2015 National Malaria Indicator Survey found ownership of at least one long-lasting insecticidal net (LLIN) of 88.6%, LLIN use of 56.3%, and malaria parasitemia among children under five (U5) of 69.9%. We assessed LLIN use, and perceived risk factors associated with this high level of parasitemia. A cross-sectional household (HH) survey was conducted in August 2018 in 60 HHs each in six local government areas (LGA) to assess LLIN ownership and use. Data on LLIN ownership and use for HHs were obtained through interviews with household heads. Each LGA conducted six focus group discussions (FGDs) of U5 caregivers (36 FGDs) and one FGD of facility healthcare workers (HCWs). Each FGD included 6-12 participants. Individual discussions were held with five state malaria program officers and six LGA malaria program officers. Semi-structured questionnaires guided the FGD discussions; these were audio-recorded, transcribed, and translated into English. Frequencies and proportions were calculated for HH data; FGD findings were analyzed for themes. A total of 358 HHs (99.4%) of those targeted were surveyed; 333 (93.0%) had at least one LLIN and 181 (50.6%) had at least one LLIN for every 2 persons. 2,134 (73.3%) persons slept under an LLIN the night before the survey. Caregivers attributed the high rate of parasitemia to rains, U5s staying outdoors late (watching films, attending night Arabic schools) without protective clothes, and improper use of LLIN (e.g., use of torn nets). HCWs reported staying outdoors late without wearing protective clothing, proximity of houses to irrigation projects, and inadequate supply of LLIN. LLIN access and use in Zamfara should be improved; behavioral change communication on use of protective clothing for children during evenings and public sensitization on the effectiveness and use of LLINs may be beneficial to reducing high levels of parasitemia in Zamfara.

0729

A NEW COLLABORATION PLATFORM TO SUPPORT THE DEVELOPMENT OF NEXT-GENERATION MEDICAL INTERVENTIONS FOR MALARIA PREVENTION

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A gap remains in providing personal protection for preventing infections in children and other vulnerable populations experiencing the highest burden of malaria. The development of next-generation medical interventions and immune therapies is guided by Target Product Profile documents that support decisions to prioritize candidates and ensure unmet health needs and targets are reached. Mathematical modelling can provide quantitative evidence to support identifying minimum necessary product requirements to reach a desired health goal. Potential impact is estimated by capturing the interaction of new interventions, malaria transmission dynamics, and health system properties in models of malaria transmission and control. Together with key stakeholders, the Bill & Melinda Gates Foundation and Swiss Tropical and Public Health Institute developed a collaboration platform to foster effective communication between stakeholder groups and align product requirements with health targets throughout the research and development (R&D) process. Within this ongoing collaboration platform, we aim to shape key performance characteristics guiding future R&D efforts. Quantitative evidence to support priority questions on use cases, intervention characteristics and implementation specifics is provided by combining malaria modelling and simulation experiments with machine learning approaches. This allows us to map a large parameter-space of candidate properties and investigate their potential public-health impact. Here, we present conclusions from a first stakeholder convening including a roadmap towards consensus decision making between stakeholder groups. Additionally, we report results from a modelling exercise in which we evaluated the product characteristics of a novel anti-infective intervention over varying age eligibility, seasonal patterns, deployments, and health system accessibilities. Subsequently, we carved out the trade-offs between further optimisation of intervention characteristics through further research and delay in implementation of new interventions.

0730

SMARTNET INITIATIVE: HARNESSING MOBILE TECHNOLOGY TO ANSWER KEY QUESTIONS ABOUT INSECTICIDE-TREATED NETS (ITN) USAGE AND MALARIA ILLNESSES IN MALAWI

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Malaria remains a leading cause of morbidity and mortality in Malawi, with over 5 million confirmed cases in 2019. As the country targets elimination of malaria by 2030, increasing the distribution and use of insecticide-treated mosquito nets (ITNs) could help to reduce malaria incidence. To obtain real-time data on ITN ownership, use, and malaria cases, the Malawi SmartNet Initiative used 2/3/4G mobile technology to reach ITN owners across Malawi. Of the 300,000 recipients of ITNs at antenatal clinics in March 2020, 54,682 subsequently voluntarily dialled a short code and of those 18,555 (34%) completed an Unstructured Supplementary Service Data (USSD) mobile phone survey; 14,947 (80%) respondents completing surveys also provided the unique ITN code. Of the respondents between March 2020 and March 2021, 12,671 (68%) were male and 38% were 15-24 years old. Respondents reported owning an average of 1.9 ITNs per household. The total number of household members was reported as 71,694 (average 4) of whom an average of 75% members reported as sleeping under a net the previous night. Nearly 2,561 (17%) respondents reported a household member had malaria in the past three months. Limitations include potential lack of representativity of this sentinel sampling approach; biases include increased response from those who are literate and have mobile phones. Although generalizability of these data remains to be determined, through future surveys, this sentinel engagement with 21,434 individuals is one of the largest conducted in African malaria control and provides opportunities to both disseminate and collect information in real time.

0731

ACCESS, ACCEPTABILITY AND UPTAKE OF AN EXTENDED MALARIA SERVICE PACKAGE FOR REACHING UNDERSERVED POPULATIONS OF FOREST GOERS

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Reported malaria cases in Myanmar declined 88% from 2012-2019. However, the declining trend has stabilized over the past 3 years, particularly among forest goers. Conventional malaria services are often not accessible among forest goers. A mixed method was applied to assess the access, acceptability and uptake of an extended malaria service package including topical mosquito repellent, a long-lasting insecticidal net, and health messaging. A voucher system was introduced to identified eligible forest goers through Village Malaria Workers (VMW) and Mobile Malaria Workers, allowing participants with vouchers to access the continuum of malaria service packages including replenishment of topical repellents, and malaria diagnosis and treatment. The study was conducted in 38 villages of Tanintharyi Region and Rakhine State where there were stable malaria transmission and presence of a VMW. Forest goers ≥5 years of age were screened, and 2,624 participants (69% male) were enrolled and provided vouchers for enhanced and ongoing malaria services during March to August 2020. Participant ages ranged from 5-9 years with a mean and standard deviation of 36±14 years. Most were of the Kayin (50%) and Bamar (43%) ethnics. A total of 2,624 tubes of repellents were distributed at enrollment while 2,601 tubes were replenished until February 2021. Of the participants returning from the forest, 54% reported consistent use of repellents while in the forest. Over half of the participants (51%) accessed malaria testing with 2,210 rapid diagnostic tests conducted resulting in 30 (1.4%) malaria positives. Key informants revealed that the vouchers created the social bond between VMWs and forest goers for providing and accepting continuum of malaria service packages over 1 year of field survey period. Forest goers accepted the vouchers as tokens for their right to receive free care services from VMWs. Vouchers were kept by VMWs who used them as a tool for tracking the malaria services provided to every voucher holder. This client-oriented voucher mechanism represents an innovative approach to deliver malaria services packages to reach underserved forest goers.

0732

EVALUATING INDIVIDUAL AND COMMUNITY-LEVEL FACTORS ASSOCIATED WITH MALARIA KNOWLEDGE, ATTITUDES, AND PRACTICES USING BASELINE DATA FROM A COMMUNITY-RANDOMIZED TRIAL ASSESSING THE EFFECTIVENESS OF TARGETED ACTIVE MALARIA CASE DETECTION AMONG HIGH-RISK POPULATIONS IN SOUTHERN LAO PDR

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Despite great global progress towards malaria elimination, it remains a substantial source of morbidity and mortality in many regions. The Health Belief Model suggests that an individual's knowledge and perception of disease risk can influence the likelihood of prevention behaviors. However, few studies have investigated the relationship between socioeconomic factors (SES) and knowledge, attitudes, and practices (KAP) regarding malaria in Asia. The literature regarding malaria-specific KAP in the Greater Mekong Subregion of Southeast Asia, including Laos, is particularly sparse. We evaluated associations using baseline cross-sectional data from the population-based AcME-Lao study, a randomized trial among 1313 households in Laos from 2017 - 2018. Heads of households completed a questionnaire including questions on SES and malaria-specific KAP. Socioeconomic variables include highest level of education, primary occupation, and owned assets as a proxy for income. Composite variables were derived separately for knowledge, attitudes, and prevention practices. Knowledge was evaluated with six questions regarding malaria signs, symptoms, and transmission. Attitudes were evaluated with six questions regarding the acceptability of malaria testing and treatment for themselves and their children. Prevention practices were assessed with a single question that asked about a variety of practices; outcomes were recorded as the total number of behaviors utilized. Non-composite variables for treatment-seeking behavior, including type of treatment sought and time to treatment, were evaluated for the overall study population and for those who reported illness in the previous two weeks using ordinal logistic regression models with adjustment for potential confounders. Analyses are ongoing with results anticipated in August 2021. This study is innovative in being the first to study the relationship between SES and malaria-specific KAP among a general population sample in the Lao People's Democratic Republic to support evidence-based programming to address residual malaria transmission.

0733

PROTECTING ADOLESCENT GIRLS AGAINST MALARIA: THE NEED FOR ADAPTED POLICIES AND HEALTH SYSTEMS TO BENEFIT AN UNDERSERVED NEGLECTED POPULATION

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Malaria is a largely unrecognized and under-investigated, but significant, cause of death in adolescent girls. However, a review of the literature reveals limited evidence on the specific impact of malaria on adolescent girls. Lack of recent age and sex-disaggregated data leaves policymakers without solid evidence to develop sound policies and programmes to reduce the burden of malaria in adolescent girls. This policy paper, developed by ISGlobal, MMV and UNICEF, presents the socio-cultural, socio-economic and biological factors that make adolescents girls more vulnerable to malaria. Regional mortality and morbidity estimates from the World Health Organization's data portal for adolescents living in sub-Saharan Africa and South-East Asia were assessed to understand whether adolescent girls are at higher risk of malaria in comparison to adolescent boys. Adolescent girls (aged 10-19) in sub-Saharan Africa have a higher risk (7%) of dying from malaria than adolescent boys. This is slightly more pronounced in younger adolescent girls (aged 10-14) who have a 12% higher mortality rate than younger adolescent boys and a 36% higher mortality rate than older adolescent girls (aged 15-19). Adolescent girls also have higher malaria morbidity rates (9% more DALYs) than adolescent boys. Younger adolescent girls have 11% more DALYs than younger adolescent boys and 40% more DALYs than older adolescent girls. In South-East Asia, adolescent girls have a 36% higher malaria mortality rate than adolescent boys. Younger adolescent girls have a 28% higher mortality than younger adolescent boys and a 46% higher mortality rate than older adolescent girls. Although some progress has been made, malaria mortality rates among adolescents, especially girls, have stagnated. The authors advocate for a revision of malaria policies and plans to include strategies tailored for adolescent girls to accelerate towards malaria elimination targets. They call for the generation of in-depth evidence on the impact of malaria on adolescent girls, increased political commitment, and community mobilization.

0734

ACTIVE SCREENING FOR MALARIA BY ENHANCED COMMUNITY CASE MANAGEMENT AND MONTHLY SCREENING AND TREATMENT SIGNIFICANTLY REDUCES THE INFECTIOUS RESERVOIR OF MALARIA IN BURKINA FASO

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Many malaria-infected individuals in endemic countries do not experience symptoms that prompt treatment-seeking and can remain infectious to mosquitoes for many months. These asymptomatic infections are a major obstacle in the path to malaria elimination, and reductions in malaria burden could be achieved by detecting and treating them early. In a cluster-randomized trial in Burkina Faso, in an area of intense, seasonal malaria, we assessed the impact of two interventions on the prevalence and transmissibility of malaria infections. The interventions included (i) enhanced community case management (CCM), comprising active weekly screening for fever, with treatment of rapid diagnostic test positive febrile individuals, or (ii) monthly screening and treatment (MSAT) using standard rapid diagnostic tests. 180 compounds with 906 subjects were randomized to: Arm 1 - control; Arm 2 - enhanced CCM; or Arm 3 - enhanced CCM combined with MSAT. Interventions were implemented over 16 months, with parasitemia and gametocytemia monitored in 4 start/end season cross-sectional surveys and a rolling survey. At the start of the study, parasite prevalence by qPCR was 64%, and 61% of these infections carried gametocytes. Most infections in cross-sectional surveys were asymptomatic (98.6% [1336/1355]), and asymptomatic infections were as transmissible to mosquitoes in membrane feeding assays (4.5% [35/771]) as symptomatic infections (5.7% [7/123]). A total of 1159 clinical malaria episodes were passively detected at local health facilities; 108 infections detected by CCM; and 629 infections detected by MSAT. In each cross-sectional survey there was a significant impact of CCM plus MSAT. Notably, at the end of the dry season, qPCR parasite prevalence was reduced to 7.6% in Arm 3 compared to 36.6% in Arm 1, and transmissible gametocyte prevalence (>1000p/mL) was reduced to 1.1% in Arm 3 compared to 17.2% in Arm 1. This study could pave the way for a simple yet effective method to control malaria burden in areas of high transmission by abrogating infections before clinical presentation and the development of transmissible gametocytemia.

0735

FROM CONTROL TO ELIMINATION: A SPATIAL-TEMPORAL ANALYSIS OF MALARIA ALONG THE CHINA-MYANMAR BORDER

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Malaria cases have declined significantly along the China-Myanmar border in the past 10 years and China has achieved elimination. The aim of this study is to investigate the epidemiology of malaria along the border, will identify challenges in the progress from control to elimination. National reported malaria cases from China and Myanmar, along with the data of 18 Chinese border counties and 23 townships in Myanmar were obtained from a web-based diseases information reporting system in China and the national malaria control program of Myanmar, respectively. Epidemiological data was analyzed, including the number of reported cases, annual parasite index and proportion of vivax infection. Spatial mapping of the annual parasite index at county or township level in 2014 and 2018 was performed by ArcGIS. The relationship of malaria endemicity on both sides of the border was evaluated by regression analysis. The number of reported malaria cases and API declined in the border counties or townships. In 2014, 392 malaria cases were reported from 18 Chinese border counties, including 8.4% indigenous cases and 91.6% imported cases, while the highest API (0.11) was occurred in Yingjiang County. There have been no indigenous cases reported since 2017, but 164 imported cases were reported in 2018 and 97.6% were imported from Myanmar. The average API in 2014 in 23 Myanmar townships was significantly greater than that of 18 Chinese counties ($P < 0.01$). However, the API decreased significantly in Myanmar side from 2014 to 2018 ($P < 0.01$). The number of townships with an API between 0-1 increased to 15 in 2018, compared to only five in 2014, while still four townships had API > 10. *Plasmodium vivax* was the predominant species along the border. The number of reported malaria cases and the proportion of vivax infection in the 18 Chinese counties were strongly correlated with those of the 23 Myanmar townships ($P < 0.05$). In order

to sustain the elimination along the China-Myanmar border and prevent the malaria re-establishment, continued political, financial and scientific commitment is required.

0736

UNDERSTANDING MALARIA KNOWLEDGE, PREVENTIVE PRACTICES, AND CARE-SEEKING BEHAVIOR AMONG COMMUNITIES IN THE SHARED BORDER AREAS OF ANGOLA, NAMIBIA, AND ZAMBIA, AND IDENTIFYING OPPORTUNITIES FOR IMPROVEMENT

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A cross-sectional survey of 10,143 households assessed malaria knowledge, insecticide treated net (ITN) use and access, indoor residual spraying (IRS) acceptance, and care-seeking behavior among communities in six Provinces/Regions of the Isdell:Flowers Cross Border Malaria Initiative program areas along the shared borders of Angola, Namibia, and Zambia—countries collaborating through the Elimination Eight Initiative to eliminate malaria in the region by 2030. The survey, conducted from June - September 2020 using a structured questionnaire on the KoboCollect platform, established baseline indicators that will be tracked annually for five years and indicated areas for further attention. To reduce malaria incidence, there is need for increased ITN access: though reported population ITN use (average of each household's percentage) was low: 36.5% (Angola), 56.4% (Namibia), and 59.6% (Zambia), the ITN use:access ratio was high: 0.85 (Angola), 0.74 (Namibia), and 1.04 (Zambia, indicating that mean users per net exceed 2), showing the gap in ITN use can be largely attributed to lack of access. IRS coverage must increase in areas where it is indicated and be well announced (the primary reason for not receiving IRS was that it was not offered, and the secondary was not being home at time of spraying), and in some regions, demand generation should increase (IRS refusals were low [<1.0%] in Angola and Namibia, but considerably higher [15.0%] in Zambia). In addition, to reduce malaria deaths, treatment seeking must be more prompt: among respondents whose child <5 years had a fever in the past two weeks, 91.6% sought care from a health facility or community health worker, though only 57.1% did so within 24 hours of fever onset. To eliminate malaria, there is need for increased knowledge about low-density infections; though knowledge of the cause and symptoms of malaria was high in most areas (≥80.0%), only 63.0% in Namibia, an area nearing elimination, knew of the possibility of infections without symptoms. These findings can inform strategic planning and behavior change messaging that is targeted and tailored to this key cross-border area.

0737

IMPACT OF SPATIAL VARIATION IN ACCESS TO COMMUNITY HEALTH WORKERS ON MALARIA OUTCOMES AMONG CHILDREN IN A HIGH TRANSMISSION REGION IN ZAMBIA

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Integrated community case management (iCCM) was implemented in Nchelenge District in northern Zambia where malaria parasite prevalence is greater than 50 percent and malaria cases make up 35 percent of all pediatric hospital admissions. The iCCM program in Nchelenge District deployed 386 community health workers (CHWs) trained to diagnose and treat malaria in the community and refer patients who require hospital level care, with the aim to facilitate earlier treatment of malaria and prevent malaria deaths. Geolocations were available for 332 of the 386 CHWs. We assessed the impact of iCCM-expanded access to care in a 1:2 time-matched case-control study (n=159 cases). Access to care was approximated by the density of CHWs within 1 km of a patient's village, and the association between CHW density and in-hospital mortality was examined using adjusted conditional logistic regression models. Secondary analyses were conducted to assess the potential impact of CHW access on mortality in subgroups of patients. In sensitivity analyses, we examined larger (2 km) and smaller (0.5 km) radii and limited the analysis to an 8-month period during which CHWs were verified to be active. Access to CHWs was not associated with in-hospital mortality and this was seen across all subgroups and sensitivity analyses. The negative finding may be explained by saturation—almost 100 percent of patients came from villages with 1 or more CHWs within a 1 km radius—or the presence of other barriers to access that have not been surmounted by the current CHW deployment, such as the low availability of emergency transport systems and pre-referral therapies.

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CUREMA: AN OPERATIONAL RESEARCH PROJECT AIMING MALARIA ELIMINATION AMONG HARD-TO-REACH MOBILE POPULATION IN THE GUIANA SHIELD.

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Malaria elimination faces the challenge of taking into account a specifically vulnerable category: isolated and mobile (often cross-border) populations, usually linked to clandestine activities. We previously developed an innovative approach for improved management of malaria attacks in these situations: the Malakit project. Implemented among illegal gold miners, reached at their staging areas at French Guiana's borders, its strategy consists in distributing kits for self-testing and self-treatment following specific training. The last challenge to meet is the radical cure of latent *Plasmodium vivax* infections, which otherwise lead to spontaneous recurrences and fuel vector-borne transmission. CUREMA will is a mixed-methods interventional multicentric study based on a pragmatic design. Our purpose is to test an intervention model that addresses malaria elimination in a mobile and isolated population, producing results that can be transferred to many contexts facing the same challenges around the world. Our target is the hard-to-reach, mobile population working

in informal gold mining in the Amazon forest of the Guiana Shield. Our innovative approach is to combine several new tools for malaria case management and offer them at strategic cross-border staging areas. Our intervention will have an effect on asymptomatic carriage of *Plasmodium vivax* hypnozoites (radical cure of individuals at risk, Module A) and on malaria episodes occurring in extremely remote areas (distribution of Malakits, Module B). With this study we will: (1) assess the effectiveness of the overall intervention on malaria transmission among the target population; (2) identify the best medication (primaquine or tafenoquine) for Module A in terms of acceptability and compliance; (3) assess levers and obstacles to carrying out this intervention in a pre-elimination setting. The intervention evaluation will be conducted by remote longitudinal follow-up of participants and by quantitative (pre/post) and qualitative surveys.

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SUB-NATIONAL MALARIA ELIMINATION - GENERATING EVIDENCE OF DISTRICT LEVEL INTERRUPTION OF MALARIA TRANSMISSION IN THE SOUTHERN AFRICAN DEVELOPMENT COMMUNITY MALARIA ELIMINATION E8 SUBREGION

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Spatial and temporal malaria distribution and the progress towards malaria elimination is known to be heterogenous in countries that have transitioned their malaria strategies from control to elimination. A malaria stratification exercise can categorise the levels of transmission intensities in districts and their potential to reach and maintain zero malaria status. The Southern African Development Community Malaria Elimination 8 (E8) sought to use the WHO subnational verification criteria to identify districts that reach and maintain zero malaria for a period of three years. Border districts are of particular interest since border malaria often impedes progress towards elimination in low burden countries. The E8 subregion collects monthly malaria data from border districts from its member countries to track progress towards elimination at district level. A criterion for subnational elimination, modelled from the WHO malaria elimination certification toolkit was used. Malaria receptive border districts which reported zero local malaria for three consecutive years were identified and evidence of receptivity and the interruption of transmission verified by a sub-national verification committee. Subnational elimination of malaria can provide an incentive to intensify efforts, particularly at local level, and pave the way towards national elimination and WHO malaria free certification in a country. Districts that reduce malaria and ultimately reach zero will require sustained resources to prevent the reestablishment of malaria in a context where country priorities may shift to medium or high transmission districts.

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ANOPHELES MODEL: A PLATFORM TO QUANTIFY THE EFFECT OF VECTOR BIONOMICS ON THE IMPACT OF VECTOR CONTROL INTERVENTIONS AGAINST MALARIA TRANSMITTED BY ANOPHELES MOSQUITOES

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Good quality, comprehensive bionomic data capturing the ecology and behavior of different malaria vectors is challenging to collect. Nevertheless, considering evidence about the ecology of mosquitoes is crucial for predicting impact of vector control interventions. Mathematical models can be used to infer the likely impact of malaria interventions from entomological field data. With most vector control studies carried out in sub-Saharan Africa, most impact predictions are specific to African vectors, and these may not apply for settings with other vector species, leading to suboptimal policy guidelines for other geographical locations. Currently, there is no available tool which systematically incorporates field data and allows estimating location-specific intervention impact accordingly. We developed AnophelesModel, an online, open-access platform that quantifies the impact of vector control interventions according to mosquito species-specific bionomics and human behavior. AnophelesModel is based on a comprehensive, curated database of field entomological data from over 50 Anopheles species assembled from the Malaria Atlas Project and additional literature. Furthermore, the database contains patterns of human activity collected at 77 sites in 26 countries from Africa, Asia and Central America as well as vector control effectiveness estimated from experimental hut trial data. The considered interventions include indoor residual spraying, long-lasting insecticide-treated nets and house screening. Within AnophelesModel, the data are used to parameterize a discrete-time, state transition model of the mosquito feeding cycle and infer the location-specific impact of vector control on the vectorial capacity. Using AnophelesModel, we demonstrate how key implications for intervention impact change for various vectors across locations in Africa, Asia and Central America. Our platform can thus be used to make predictions about the key factors determining intervention impact according to human activity and vector-specific bionomics, guiding thinking towards more robust and efficient policy recommendations.

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DOES LARVAL HABITAT MODIFICATION AND MANIPULATION CONTROL MALARIA? FINDINGS FROM A SYSTEMATIC REVIEW

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Malaria continues to be a major public health problem. Although long-lasting insecticidal nets and indoor residual spraying of households with insecticide have proven highly effective, due to emerging and expanding insecticide resistance, there has been renewed interest in other vector control approaches, including larval source management (LSM). This systematic review summarises the effectiveness of larval habitat modification and manipulation on epidemiological and entomological outcomes. Intervention studies were identified from searches of six databases and other grey literature sources. Two authors independently screened studies, performed data extraction, and assessed the risk of bias. A narrative synthesis approach was used. GRADE was used to assess the certainty of the evidence for each intervention. A total of 13 controlled intervention studies were included, of which only four assessed epidemiological outcomes. There was very low evidence that using spillways across streams or floodgates on a dam reduce malaria parasite

prevalence and/or incidence. Also, there was low evidence that repairing and clearing of drains, cutting grasses and minor repairs reduces malaria parasite prevalence and/or incidence. Stronger certainty of evidence was seen for entomological outcomes, particularly relating to interventions based on frequent/intermediate clearing of grass and replenishing water in larval habitats, or the use of Napier grass for shading. Without epidemiological evidence or sufficient studies demonstrating that a significant reduction in vector densities translates to reduced disease, it is currently difficult to provide firm recommendations for the use of habitat manipulation/modification to control malaria.

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RESTING BEHAVIOR OF MALARIA VECTORS IN GHANA AND ITS IMPLICATION ON VECTOR CONTROL

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There is widespread use of long-lasting insecticidal nets (LLINs) and Indoor residual spraying (IRS) to control the density of malaria transmitting mosquitoes in Ghana. An understanding of the interactions between increased insecticide resistance and resting behaviour patterns of mosquitoes is important for an effective vector control programme. Mosquitoes were sampled during the dry and rainy seasons of 2019 from 3 ecological landscapes in Ghana, namely, Sahel savannah (Kplasogou, Pagaza, Libga), Coastal savannah (Ada Foah) and Forest (Konongo) zones. PCR based molecular techniques was used to determine the species, knockdown resistance mutation (L1014F), G119S *Ace*-1 mutation, host blood meal and sporozoite infection in field collected mosquitoes. The study found 90% of vectors were *Anopheles gambiae* s. l. and the dominant sibling species was *An. coluzzii* (63%), followed by *An. gambiae* s. s. (27%), *An. arabiensis* (9%) and *An. melas* (1%). The frequency of *kdr* west (L1014F) mutation for mosquitoes resting indoors was 0.90 and 0.84 for outdoors collection in the Sahel savannah zone. However, in the Forest zone, the frequency of *kdr* mutation was 0.9 in outdoor resting and 0.79 indoor resting mosquitoes. The frequency of *Ace*-1 mutation was higher in mosquitoes resting outdoors (0.50) than indoors (0.38) in the coastal zone. However, similar *Ace*-1 mutation was detected in outdoor and indoor resting mosquitoes from the Sahel savannah (0.39 vs. 0.36). Outdoor resting mosquitoes had a higher (67.5%) human blood index (HBI) than indoor (67.0%) resting mosquitoes in the Sahel savannah. However, in the Coastal zone, the HBI for in indoor resting mosquitoes was 66.7% and 50% for outdoor resting mosquitoes. Overall, the sporozoite rates was high in indoor resting *An. coluzzii* from the Sahel savannah (5.0%) and Forest (2.5%) zones. These results underline the difficulties of controlling malaria vectors resting and biting outdoors using the current interventions. Continuous monitoring of resting behavior of mosquitoes and the implementation of new vector control interventions that will target outdoor resting vectors are urgently needed in Ghana.

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CHARACTERIZATION OF ANOPHELES SPECIES TEMPORAL COMPOSITIONS AND BIONOMIC CHARACTERISTICS IN BANDARBAN, BANGLADESH

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With over 10% of the population of Bangladesh at risk of contracting malaria, it isimperative that we have a better understanding of mosquito drivers of the disease withmalaria elimination on the agenda. This study sought to characterize temporal andspatial species compositions and behaviors throughout the rainy season towardsunderstanding present gaps in transmission that result in residual malaria transmission. *Anopheles* mosquitoes were sampled from four villages in malaria-endemic Bandarbandistrict, Bangladesh, from May through October, 2018. Sampling methods usedincluded CDC-Light traps (CDC-LT), human landing catches (HLC), and pyrethrumspray catches (PSC). Species identification of female specimens was conducted bothmorphologically as well as molecularly - using ITS2 and *Co1* sequences. A total of 5376mosquitoes were collected belonging to 17 species - *Anopheles vagus* was thedominant species (39.7%) followed by *An. nivipes* (12.5%). Other species identifiedincluded *An. baimaii, An. campestris, An. dissidens, An. jamesii, An. jeyporensis, An.karwari , An. kochi, An. maculatus, An. peditaeniatus, An. philippinensis, An.sawadwongporni, An. splendidus, An. subpictus* (Forms A and B) and *An. varuna*. Themonths following peak rainfall in June had the highest Anopheles biomass. Outdoorcatches were usually higher than indoor catches at all four sites. A large variation inspecies-specific biting behaviors demonstrates a complex vector ecology with *An.vagus* demonstrated the highest endophily (95% of indoor resting mosquitoes) as wellas the highest anthropophagy. This study points to the primary vector at these sitesbeing *An. vagus* with contributions to disease transmission from other species. Gaps inprotection with indoor-based interventions include vectors and humans overlapping inthe early evening and outdoors. Targeting *An. vagus* indoor resting behavior with non-pyrethroid-based indoor residual spraying (IRS) may be effective against this vector.Other interventions targeting transmission that occurs outside the scope of LLINs andIRS are required with malaria elimination being the goal.

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PHYSICAL DURABILITY MONITORING AND USE ASSESSMENT OF INSECTICIDE TREATED NETSIN MALI OVER A THREE-YEAR PERIOD

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In Mali, insecticide treated nets (ITNs) are distributed through regional mass campaigns and through routine health services to pregnant women and infants. In 2018, the ITN national coverage was 95% (one ITN for every two persons) in 2018. Both net durability and the "average useful life" of a net are critical factors for determining the frequency at which nets need to be replaced and the types of nets to be procured. The objectives of this study were to: 1) assess the physical durability of deltamethrin based ITNs (Yorkool and PermaNet 2.0) in two sites, 2) compare their durability and 3) identify major determinants of net performance.The study was conducted in Kenieba and Kita districts which have similar malaria prevalence profiles. Cohorts of 461 ITNs (Yorkool) in Kenieba and 515 ITNs (PermaNet 2.0) in Kita were identified for monitoring with evaluations carried out at 6, 12, 24 and 36-months after a mass net distribution carried out in the area in December 2017. Physical durability monitoring was performed by taking into account the rate of net loss (attrition), the physical condition of surviving nets which was measured by the WHO standardized proportionate hole index (pHI) and the proportion of nets in good condition (pHI<64) after three years. At the 36 months post-distribution evaluation, 245 ITNs in Kenieba and 193 ITNs in Kita were active. Attrition due to wear and tear was higher in Kenieba (44.8%) than in Kita (22.3%) (p=0.0187), with tears being the main type of damage at both sites: 70% in Kenieba and 59% in Kita. The proportion of nets in good condition was lower in Kenieba (33%) than in Kita (63%) (p=0.0125). The proportion of nets surviving in good condition was lower

in Kenieba (25%) than in Kita (58%) (p=0.002). The median survival was 2.1 years [1.82-2.61] in Kenieba (Yorkool) and 3.4 years [2.67-5.04] in Kita (Permanet 2.0).The reasons for the significantly lower performance of Yorkool nets in Kenieba could be associated with product specifications, as PermaNet 2.0 has a thicker fabric of 100 denier polyester compared to 75 denier for Yorkool. Socio-cultural differences between the districts could have an impact and remain to be assessed.

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DEFINING A VECTOR CONTROL PRODUCT FOR THE BUILT ENVIRONMENT

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The risk of malaria is greatly influenced by the built environment; for example, house modifications such as metal roofs, ceilings, closed eaves, well-fitted doors, and screened windows can reduce the risk of malaria in households. Combining these features with targeted insecticide treatment may produce an even greater protective effect, by killing mosquitoes as they attempt to enter a house. Because these modifications cover a broad set of interventions, it can be difficult to define targets for product development and routes to market for new products. One tool to help guide the process is a target product profile (TPP). TPPs define the desired characteristics of a public health product, such as a new vaccine or insecticide-treated bed net, which helps address product development issues and prevent late-stage development failures. Understanding need and demand is essential for developing a TPP. To better understand demand for a screening product for the built environment, we conducted interviews with malaria control experts in six countries: Cameroon, Democratic Republic of the Congo, Ghana, Guinea, Nigeria, and Zambia. The survey sought to explore questions such as: 1. What is the level of interest for an insecticidal screen product (e.g. window screen)? 2. What drives interest in such a product? 3. Who would use such a product? 4. How important is a WHO recommendation for product adoption 5. What are the expected technical and operational challenges? Respondents were generally interested in a screen product, particularly window screening. They felt that screening could be effective for vector-borne disease control and serve as a passive tool to deploy in areas with poor bed net usage. However, they also voiced concerns around cost and durability, and how households could acquire and install such a product. Whilst preliminary, the survey does demonstrate many recurrent themes in ongoing discussions around vector control and the built environment. We intend it as a launching point for further discussion with key stakeholders, and further development of a TPP for a screen product.

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RAPID AGEING AND SPECIES IDENTIFICATION OF NATURAL MOSQUITOES FOR MALARIA SURVEILLANCE

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Vector surveillance is essential to monitor and optimize adult mosquito control to prevent vector-borne diseases. Specifically, accurate and high-resolution estimation of both the mosquito abundance of morphological identical species and their longevity are essential for the assessment of the impact of vector control measures. Up to date no simple and precise method allows measuring these mosquito traits. The current tools in use are expensive and time-consuming. So, our new approach underway development is based on the amount of light absorbed by the different molecules of the mosquito cuticle through mid-infrared spectroscopy (MIRS), which changes between different mosquitoes species and during ageing. By applying convolutional neural networks to MIRS spectra, we have been able to predict with an unprecedented accuracy of up to 95% the age and species of lab and semi-field mosquitoes *Anopheles gambiae, An. coluzzii* and *An. arabiensis* from Bobo-Dioulasso, Burkina Faso and Ifakara, Tanzania. To account for the genetic and ecological variation, mosquitoes were collected from the field and then reared under lab standard conditions and in semi-field facilities. We then validated our model on wild mosquito species. First, we collected wild mosquitoes from different villages and their gonotrophic cycles assessed upon dissection and microscopic analysis of the ovaries, then measured by MIRS. Here we will present the age and species prediction model of wild mosquitoes based on convolutional neural networks. This new approach is easy-to-use, cost-effective, robust and high-throughput and presents several advantages by predicting simultaneously the age and species which could be of great value in vector surveillance programmes.

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A PROSPECTIVE MICRO-COSTING LARVAL SOURCE MANAGEMENT TOOL TO INFORM DECISION MAKING IN TANZANIA

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In the face of stagnating malaria budgets, only the most cost-effective interventions should ideally be implemented. However, cost-effectiveness estimates rest on accurate costing, a challenging activity when interventions have not yet been deployed in a given setting. Here, we aim to prospectively cost microbial larval source management for prospective deployment in Tanzania. We developed a micro-costing model to evaluate economic and financial costs associated with a microbial larval source management intervention in low and very low transmission areas. We used budgeting data from an infield intervention together with global guidance and the literature to inform economic and financial costing models. Costs were stratified based on uni-modal and bi-modal rainfall patterns. Resource use and costs were derived for the following sub-activities: micro-planning, quantification and procurement of larvicide, training and capacity building, implementation and monitoring and evaluation. Sensitivity analysis was used to explore how uncertainty in key parameters, such as the cost of larvicide, number of spray cycles, area and number of breeding sites, affected costs. Larvicide procurement accounted for 50% of the total costs, human resource including training and supervision accounted for 21%. Estimated financial unit costs of microbial larval-source management were US$2.45 per person protected per year. Results were highly sensitive to the unit cost of the larvicide and staff payments. Standardized prospective costing tools offer a methodological framework that can be used to evaluate the financial and economic viability of malaria interventions at different scales. This costing tool demonstrates that larval source management is a low-cost vector control method which can supplement malaria control efforts. Costing frameworks like this help to better plan for implementation in specific settings, such as Tanzania, and can be adapted to different epidemiological and health system contexts that may consider implementing larval source management.

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HABITAT CHARACTERIZATION AND LARVAL ABUNDANCE OF ANOPHELES FUNESTUS IN A HIGHLAND AND LOWLAND SITE IN WESTERN KENYA

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Anopheles funestus is a major Afrotropical vector of human malaria. In the last few years, there have been reports of the resurgence of this vector in western Kenya after the introduction of insecticide-treated nets. This might be influencing malaria transmission in western Kenya. However, there is limited study on the larval ecology of this efficient primary vector. A larval survey was carried out in Kombewa in lowland and Bungoma in the highland of western Kenya from November 2019 to December 2020. All potential mosquito breeding habitats were identified, characterized, georeferenced and carefully examined for the presence of mosquito larvae and predators using the standard dippers technique and 10L bucket. The larvae were morphologically identified and confirmed using PCR. In all, 151 mosquito aquatic habitats were assessed. Of this, 58/71(82%, 95%, CI, 0.73-0.91) were found in Kombewa and 62/80 (78%, 95%, CI, 0.68-0.87,) in Bungoma were positive for mosquito larvae. A total of 3,193 mosquito larvae were sampled and *An. funestus* larvae constitute 38% (1224/3,193). Bungoma recorded a higher number of *An. funestus* larvae (85%, 95%, CI, 8.722- 17.15) than Kombewa (15%, 95%, CI, 1.33- 3.91). Fifty-nine percent (59%), 35% and 5% of *An. funestus* larvae co-exist with *An. gambiae s.l*, *Culex spp* and *An. coustani* in the same habitats. *An. funestus* breeds in a wide range of habitats with man-made ponds having the highest abundance of larvae. Typically, *An. funestus* breeds in a permanent and semi-permanent aquatic habitat with or without aquatic vegetation, slow-moving/disturbed or stagnant water that is either clear, opaque, cloudy or brownish near human habitations. Multiple regression analysis and principal component analysis identified the distance to the nearby house as the key environmental factors associated with the abundance of *An. funestus* larvae. This study serves as a guide to the control of aquatic stages of *An. funestus* and other mosquito species using larval source management or larviciding to complement the existing vector control tools.

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THE INTERPRETATION OF EXPERIMENTAL TRIAL DATA FOR EVALUATION OF INDOOR VECTOR CONTROL INTERVENTIONS AGAINST MALARIA

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Experimental hut trials are the most important entomological assay for assessing the efficacy of anti-mosquito interventions used in the home. These assays are being increasingly used to evaluate new products as resistance to pyrethroid insecticide spreads and new insecticide treated nets (ITNs), indoor residual sprays, spatial repellents and attractive toxic sugar baits are developed. As several sources of variation are present in the assay and complexity is introduced due to the use of wild mosquitoes, the interpretation of hut trial data can be somewhat unclear and there is the potential for considerable measurement error. Product development or prioritisation of interventions often relies on relatively modest differences in mosquito mortality or blood-feeding inhibition. Though these differences appear statistically significant when the data is aggregated, as large numbers of mosquitoes are collected, they may not necessarily mean that different interventions have an underlying different efficacy, even if the products were compared side by side in an experimental hut trial. The talk draws on a meta-analysis of 135 experimental hut trials to show how results from different sites should be interpreted. Using data from previous hut trials to understand the level of variation typically present in the assay, we utilise simulation-based methods to guide our work. For example, with a commonly used trial design, analyses indicate that the advantage that pyrethroid-piperonyl butoxide ITNs over standard pyrethroid-only ITNs can vary by up to 29% mortality in the same trial. The work highlights the minimum set of results that need to be presented to allow different results to be accurately compared. Implications for comparison of products, policy and the prioritisation of different vector control tools are discussed.

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MOSQUITOCIDAL CONCENTRATION OF IVERMECTIN OVER SIX MONTHS THROUGH A LONG-LASTING FORMULATION: TOWARD A SUSTAINABLE CONTROL OF MALARIA VECTORS IN A ONE-HEALTH APPROACH WITH THE HELP OF PERIDOMESTIC ANIMALS?

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In malaria endemic areas, because humans are protected by bednets, peridomestic animals represent an alternative blood source for *Plasmodium* vectors, allowing residual transmission of the parasite. Previous studies show that ivermectin (IVM) treatments of *Anopheles* hosts provoke systemic insecticidal effects. This is therefore seen as an alternative to complement existing malaria control tools, albeit with the strong limitation of the poor remanence of the product, which represents a clear limit toward a mass implementation.The ANIVERMATE project was designed to evaluate the killing effect of a new formulation of IVM, using the Bepo® technology, on wild *A. coluzzii* females.Twenty-one calves were used as mosquito hosts for this 3-arms design study (control, classical formulation Ivomec-D® (monthly injections of 0,4 mg/kg) and Bepo®-IVM (one injection of 2,4 mg/kg)). The Bepo®-IVM has been designed to target at least the LC50 plasma concentration of IVM over 6 months. 15 000 *A. coluzzii* females were allowed to feed directly on the calves' skin at different time points after the hosts treatments. Mosquito survival was assessed over 30 days post feeding. As expected, IVM concentrations in the hosts treated with the Bepo®-IVM were lower and steadier than when using Ivomec-D®, with a significant mosquitocidal activity measured for up to 6 months. The efficacy using the Ivomec-D® was variable, depending on the time elapsed from the treatment, and reached concentrations below the LC50 on the last week of each month. By comparison, the Bepo®-IVM released IVM concentrations remained above the LC50 during the whole study.The entomological data will feed a model expected to show the benefit of Bepo®-IVM formulation for significantly reducing Malaria transmission. In addition, the potential use of a long lasting IVM treatments in peridomestic animals has been studied in the fields, where we examined IVM behaviour in the dung and in local soils as well as deleterious effects of the molecule on non-targeted coprophilic fauna, that are essential to ecosystem's health and productivity (see companion abstract by Heinrich *et al*.)

0751

MALARIA VECTOR CONTROL IN THE AMERICAS: MEASURING THE IMPACT OF INSECTICIDE TREATED NETS AND INDOOR RESIDUAL SPRAYING

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Malaria vector control in the Americas depends on insecticide-treated nets (ITNs) and indoor residual spraying (IRS) of insecticide. However, there is limited evidence regarding the effectiveness of these tools against neotropical malaria vectors that exhibit host-seeking behaviors distinct from those observed in Africa. While the Americas have achieved reductions in malaria transmission, important foci remain despite the widespread use of ITNs and IRS. We are conducting a two-armed cluster-randomized trial to determine the entomological impact of ITNs and IRS in Colombia, where *Anopheles albimanus* is the primary vector. The trial began in February 2021 in Cauca department, on the Pacific coast of Colombia, in the municipalities of Guapi and Timbiquí. The study was designed to detect a 40% reduction in indoor host-seeking *Anopheles* spp. with 80% power, comparing the IRS and ITN arms. As such, the study required 20 clusters per arm, sampling four houses per cluster for the primary indicator (indoor and outdoor human landing catches, HLCs), for a total of 160 houses measured monthly. Clusters were randomly allocated to receive either ITNs (alpha-cypermethrin) or IRS (deltamethrin), implemented by the Colombian Ministry of Health. In addition to HLCs, mosquito resting density, parasite inoculation rates, and insecticide susceptibility are also being monitored. Finally, qualitative household KAP surveys, combined with human blood sampling to measure antibodies to mosquito salivary antigens are being conducted to better understand the impacts of the interventions. At baseline, 18,256 *Anopheles* spp. were collected throughout the night in both indoor and outdoor HLCs, with a peak of activity between 18:00 and 22:00 hours. When tested for insecticide resistance, the *Anopheles* spp. were susceptible to both deltamethrin and alpha-cypermethrin using CDC bottle bioassays. Entomological data collection will be ongoing for 18-months post-intervention. Results from this study will provide evidence for the entomological impact of ITNs and IRS in a malaria transmission setting in the Americas and will highlight gaps in use of the current tools.

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NITISINONE AS A POTENTIAL ALTERNATIVE ECTOPARASITIC DRUG FOR A COMPLEMENTARY MALARIA VECTOR CONTROL STRATEGY

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The residual transmission of malaria is driven by outdoor biting and insecticide resistant vectors. In the search for novel tools that target the current challenges facing malaria elimination, repurposing ivermectin as an endectocide and complementary tool for vector control has been prominent. The tyrosinemia drug, nitisinone, is selectively toxic to blood-feeding arthropods, inhibiting the essential tyrosine metabolism that follows bloodmeal ingestion. Nitisinone is a promising candidate for an ectoparasitic drug, owing to its favourable pharmacokinetic/pharmacodynamic (PK/PD) properties. Drug-spiked blood meals were fed to *Anopheles gambiae* in standard membrane feeding assays in a direct comparison between the drugs. A Cox regression survival analysis assessed mortality. Proportional hazard ratios were fitted to a non-linear squares model and PK/PD modelling simulated mosquitocidal activity over time. Monte Carlo population simulations were applied to dosing regimens to attain clinically meaningful comparisons. Nitisinone demonstrated a longer lasting mosquitocidal effect than ivermectin at relevant dosing regimens (3x300µg/kg IVM, 3x1mg/kg NTBC). At a modest dose, nitisinone maintained a superior mosquitocidal effect for one week longer than a high dose of ivermectin (19 days, 12 days). Nitisinone was superior to ivermectin at both low and single dosing regimens, which is important from a clinical safety and logistical perspective, respectively. Although the lethal concentration (LC50) for ivermectin was comparatively lower than nitisinone (15.66ng/mL, 136ng/mL), the PK profile of nitisinone means that higher blood concentrations can be attained and remain above the LC50 for longer. Our work presents initial results of a potential ectoparasitic drug that is comparative to ivermectin as a complementary vector control tool. Even when dosed at a tenth of the recommended regimen, a mosquitocidal effect was predicted for a week. It is important to preserve the existing efficacy of ivermectin. In exploring other compounds, we can alleviate a reliance on a sole eligible compound for this strategy of vector control.

0753

EFFICACY OF BROFLANILIDE (VECTRON™ T500), A NOVEL META-DIAMIDE INSECTICIDE, AGAINST PYRETHROID-RESISTANT ANOPHELINE VECTORS IN NORTHERN TANZANIA

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Insecticides with a different mode of action are urgently needed to counter resistance in mosquitoes. A new meta-diamide insecticide for vector control, broflanilide (TENEBENAL™), was evaluated in northern Tanzania. We present the potential of this insecticide as an addition to the arsenal of IRS products needed for rotational use to maintain both control of malaria and resistance management of malaria-transmitting mosquitoes. Bottle bioassays indicated that broflanilide was effective against both susceptible and mosquito strains with multiple resistance mechanisms, demonstrating an absence of cross resistance between broflanilide and pyrethroids. Two consecutive experimental hut trials were conducted; the first evaluating the efficacy of three concentrations using a prototype formulation of broflanilide, and the second trial evaluating an improved formulation; VECTRON™ T500. The experimental hut trial showed a dosage-mortality response of broflanilide and 3-8 months of residual activity, with longer activity on concrete than mud. The second trial with VECTRON™ T500 showed prolonged residual efficacy to 5-6 months on mud, and high mosquito mortality for the full duration of the trial on concrete. Results with free-flying, wild *Anopheles arabiensis* echoed the mortality trend shown in cone assays, with the highest dose inducing the highest mortality and the improved formulation showing increased mortality rates. No blood-feeding inhibition or insecticide-induced exiting effects were observed. Following on from these promising results in the experimental hut studies, a community study in northern Tanzania with broflanilide was initiated in February 2021 with approximately 2000 households.

0754

REDUCTION IN MALARIA CASES AND ANNUAL PARASITE INCIDENCE AFTER REINTRODUCTION OF INDOOR RESIDUAL SPRAYING IN ESCUINTLA, GUATEMALA

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The department of Escuintla, Guatemala, is an important malaria endemic region, accounting for an average of 60% (50-70%) of the country's cases from 2013-2019. However, just 29% of Guatemala's cases were reported from the department last year coinciding with Escuintla's fourth straight year of case declines. In 2017, the National Vector-Borne Disease Program reintroduced indoor residual spraying (IRS) in high burden localities of Escuintla as a strategy to accelerate toward malaria elimination. This was done in conjunction with ongoing surveillance and case management strengthening activities and continued distribution of long-lasting insecticidal nets in endemic localities. IRS spray rounds were conducted at the beginning of the malaria high transmission season (defined as epidemiological weeks 40 to 18) in 1 (2017), 10 (2018), and 5 (2019) highly endemic localities. IRS coverage averaged >80% each year. We examined the impact of IRS on the reduction of malaria cases during the high transmission season and on annual parasite incidence (API) in the areas receiving IRS. We calculated the percent reduction from baseline (2016-2017 season) for each subsequent season after IRS was introduced. The total number of cases during the high transmission season in 2016-2017 (before IRS reintroduction) was 737 cases, compared to 646 (2017-2018 season), 346 (2018-2019 season), and 82 (2019-2020 season), reflecting a 12.3%, 53.1%, and 88.9% reduction in total cases in IRS localities when compared to the baseline, respectively. Because not all localities received three spray rounds, separate grouped analyses were conducted dependent on the total number of rounds received. Results for a subset of four localities receiving two spray rounds during consecutive high transmission seasons demonstrated an average API of 176.2 in 2018 (before IRS reintroduction), 106.2 in 2019, and 14.8 for 2020. These results suggest that the introduction of IRS in targeted, high-risk areas as part of an integrated and strengthened malaria elimination strategy contributed to Escuintla's 88% decline in total cases since 2017.

0755

WHICH TRAP IS BEST? ALTERNATIVES TO HUMAN LANDING CATCHES FOR MALARIA VECTOR SURVEILLANCE: A META-ANALYSIS

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Human landing catches (HLCs) are an entomological collection technique in which humans are used as attractants to capture medically relevant host-seeking mosquitoes. This method is considered the gold standard in malaria vector surveillance as it provides metrics such as human biting rate, and entomological inoculation rate (EIR). Despite the high-resolution data obtained from HLCs, the use of this method has been a topic of debate for decades mainly due to safety concerns for the collectors. Many alternatives to HLCs have been proposed; however, to date, no quantitative review of studies comparing HLCs to alternative trapping methods has been conducted. Here, we present a meta-analysis of published literature on HLCs and alternative trapping methods for outdoor *Anopheles* spp. collections. We identified 17 studies comprising 59 comparisons representing 16 countries. Using data from these studies, we conducted a meta-analysis comparing treatment effects for each study (Hedges' G). Three moderators were used: mosquito (*An. gambiae* s.l., *An. funestus* s.l., and *Anopheles* spp.), trap type, and sub-trap classification (biological, chemical, physical, etc.). Our analysis showed that outdoor HLCs did not perform statistically better than any of the alternative trap types, although there were differences among the sub-traps including specifically between physical/chemical traps and biological, chemical, and physical traps alone. Traps with humans as attractants were the only sub-trap type to capture significantly more mosquitoes than HLCs (g = -0.3940). Alternative trapping methods captured significantly more *An. gambiae* s.l., primary vectors, when compared to HLCs (g = -1.8516). These results suggest that there is no significant difference between alternative trapping methods and outdoor HLCs for *Anopheles* collections and that these methods can be considered comparable. However, human-baited traps may be superior for the collection of *An. gambiae* s.l. Regional decisions about the use of HLCs for outdoor mosquito collection methods should consider factors such as target vector species and access to alternative trapping methods.

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IMPROVING INDOOR RESIDUAL SPRAYING DEPLOYMENT THROUGH A SPATIAL DECISION SUPPORT SYSTEM

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Indoor residual spraying (IRS) has been implemented yearly on Bioko Island since 2004. IRS is expensive due to the costs of insecticide and the workforce needed to deliver the activity in a limited time frame. Robust deployment and monitoring strategies are needed to ensure optimal productivity and homogenous coverage across deployment units. The Bioko Island Malaria Elimination Project (BIMEP) developed a robust spatial decision support system (SDSS) that comprises a household mapping system, a server-based data collection platform, and real-time data access through live dashboards. In 2021, IRS was aimed at ~46,000 households across Bioko Island. The SDSS was used to cluster 100 x 100m map sectors into three equal deployment units. Map sectors within these clusters were grouped into equal size workloads taking into consideration accessibility and distance to cover in a given day by fieldworkers. One workload was given to a team of three spray operators and one community mobilizer at a given time, defining a deployment. To avoid stalling of productivity and over or under-spraying, feedback was provided daily to instruct field teams to prioritize map sectors with large denominators and/or high numbers of households remaining to spray to achieve optimal coverage. Secondary spraying visits were oriented towards map sectors with low IRS refusal rates. Once the target coverage was achieved in a map sector, the latter was eliminated from the workload, and the teams were prompted to exit and move on to other map sectors. After two months of spraying, 30% of the target coverage has been achieved with a team of 102 spray operators, spraying on average 4.7 households per sprayer per day, above the anticipated 3.7 and higher than the historical average for all previous spray rounds. Spray coverage was homogeneous across map sectors. Using a spatial, data-driven approach to optimize IRS will ensure optimal coverage and productivity as well as distributive equity, substantially improving the protection of the population and saving critical resources.

0757

OBSERVATIONAL IMPACT OF INSECTICIDE TREATED NETS ON MALARIA INCIDENCE IN 16 DISTRICTS ACROSS BURKINA FASO, MOZAMBIQUE, NIGERIA, AND RWANDA

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Dual active ingredient insecticide-treated bed nets (ITNs) that contain more than one active ingredient (AI) and are effective against pyrethroid-resistant mosquitoes were recently distributed as part of mass distribution campaigns in Burkina Faso, Mozambique, Nigeria, and Rwanda. Interceptor® G2 (IG2) (BASF) ITNs were distributed in all four countries, and Royal Guard® (RG) (Disease Control Technologies) ITNs were distributed in Mozambique and Nigeria. Each campaign also included standard pyrethroid-only ITNs and ITNs containing the insecticide synergist piperonyl butoxide (PBO). As part of the New Nets Project, observational studies are underway in 16 districts across the 4 countries to determine the effectiveness and cost effectiveness of IG2, Royal Guard, and PBO ITNs compared to pyrethroid-only ITNs. Surveillance data from each country's health management information system were used to monitor trends in malaria case incidence across all age groups. Cases represented patients who sought care at public health facilities or from community health workers and tested positive for malaria by rapid diagnostic test and/or microscopy. Monthly malaria incidence was calculated by dividing the total number of positive malaria tests in each district by its corresponding population. Baseline malaria incidence rates using monthly incidence from the 12 months prior to each country's mass distribution campaign varied across countries and districts. Incidence rates up to one year post-distribution will be presented for each study district. Interim results from these routine data analyses can help inform country- and global-level decision makers on the effectiveness of different ITNs across a wide range of transmission settings and are critical in establishing calculations of cases averted for cost-effectiveness analyses. These preliminary epidemiological outcomes, showing the diversity in study sites, represent only one year of net life; more complete analyses with two years post-distribution data using a difference in difference approach are forthcoming.

0758

RESCUE THERAPY FOR ALVEOLAR ECHINOCOCCOSIS PATIENTS WITH BENZIMIDAZOLE TREATMENT FAILURE OR INTOLERANCE WITH LIPOSOMAL AMPHOTERICIN B

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Although curative resection is the treatment of choice for alveolar echinococcosis (AE), in most cases complete resection is impracticable due to extended involvement of critical anatomic structures. Benzimidazole treatment (BMZT) is the only available medical treatment approved in such cases, but may not be tolerated or insufficient. Thus, new drugs are urgently needed. Amphotericin B treatment (AMBT) has been used with success in such desperate situations. We report long-term follow-up data from 8 patients with AMBT at our centre who were diagnosed with unresectable AE and BMZT intolerance or treatment failure. Initial AMBT loading phase of 2 weeks was followed by 1-3 applications per week, depending on response. Close monitoring was conducted for the first month and afterwards every 3 months. Responses were confirmed by clinical and serological response, as well as PET-CT scan. All patients had complex, disseminated disease manifestation with pulmonary, cardiac, vertebral, peritoneal and cerebral manifestations. Patients showed intolerance (n=4) and progression (n=4) under BMZT. Age at diagnosis ranged from 29-58 years and AMBT was initiated 1-25 years after diagnosis. With AMBT, 4 patients achieved stable disease (SD), of which 3 are still on therapy after 0.5, 1.5 and 13 years, respectively. One patient received curatively intended surgery under AMBT and treatment response has yet to be confirmed. The other two patients died after having had achieved SD for 4 months and 3 years, but AMBT had to be discontinued due to ileus or renal toxicity, respectively. One patient had shown only a partial remission for 17 months and achieved SD with alternative treatment. Treatment response of the last patient has yet to be evaluated. Side effects under AMBT were mostly manageable, however deteriorating renal function prompted the halt of AMBT in one patient. Salvage AMBT might be a valuable treatment option in cases of BMZT failure and can induce persistent disease control, although with potentially significant side effects, high treatment costs and the need for repeated intravenous therapy.

0759

A NOVEL MULTIPLEX REAL-TIME PCR ASSAY FOR THE MOLECULAR DIAGNOSIS OF METACESTODE INFECTIONS IN HUMANS

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For the clinical outcome of larval cestodiases in humans such as neurocysticercosis (NCC), cystic echinococcosis (CE) and alveolar echinococcosis (AE), fast therapeutic treatment based on early diagnosis at the initial stage of infection is key. Despite remarkable development of imaging technologies and serology for the diagnostic identification of invasive cestodiasis, a reliable high-throughput molecular method on biopsy or cytology specimens would ensure a more rapid species identification. This holds specifically true for diagnosing NCC, CE and AE in patients with unusual imaging data and/or negative serology due to immunosuppression, or diagnosing rare taeniid species. A broad variety of PCR protocols for the detection and differentiation of taeniid species have been published. However, most approaches are based on conventional PCR techniques and either require gel electrophoresis, an additional sequencing step or are limited to certain *Echinococcus* or *Taenia* species, taxa or genotypes. In the present study a quadruplex real-time PCR has been established which allows the differentiation of *Echinoccus granulosus sensu lato (s.l)*, *E. multilocularis* and *Taenia spp.* but also the detection of an internal control in a single processing step. Subsequent Sanger sequencing of *E. granulosus s. l.* and *Taenia spp*. amplicons further allows the differentiation of all *Echinococcus* and *Taenia* species including recently reported human infecting species such as *T. crassiceps*, *T. serialis*, *T. martis* and *Versteria sp*. This simple, fast and reliable multiplex real-time PCR has been successfully assessed for the specific detection of *E. granulosus sensu lato (s.l)*, *E. multilocularis* and a broad spectrum of *Taenia spp.* cyst fluids and fine-needle biopsies of clinical and veterinarian cases. All reference samples were from clinical cases well documented upon imaging, serological and parasitological/morphological means. To our knowledge, this is the first report of a quadruplex real-time PCR, which can be routinely used in a clinical microbiology lab on biopsy or cytology specimens for the detection of *E. granulosus s.l*, *E. multilocularis* and *Taenia spp.*

0760

CORRECTION OF THE SELECTION BIAS IN THE ESTIMATION OF CYSTIC ECHINOCOCCOSIS PREVALENCE IN COMMUNITY SURVEYS

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Cystic echinococcosis (CE) is a zoonotic disease caused by the larval stage of the dog's parasite *echinococcus granulosus*. Epidemiological surveys performed in endemic areas of Peru reported prevalence values between 5% and 10%; however, the majority of them have a potential selection bias produced by the location of the homes in the city and their proximity to the place where the ultrasound evaluation is performed resulting in an underestimation of the real prevalence. One approach that have been used by ecology to correct this bias is the use of the spatial distribution of each individual at the moment they had selected to the sample. We performed a study to determine the prevalence of CE in Corpacancha, a highly endemic village located in Peruvian central highlands. Based on the function of sample intensity is equal to the population function intensity multiplied by a constant plus a function that depends on space, we determine that each observation doesn't have the same probability to be chosen at the sample. Therefore, in order to obtain an unbiased value of the prevalence, we fit a weighted model considering the spatial effect and other covariates that have been considered as a risk factor; in this model the weights are based on the sampling effect. Using the traditional method to estimate the prevalence (based on binomial distribution likelihood), the prevalence of CE in Corpacancha was 24.1% (95% CI: 18.9% - 29.3%); however, after adjusting this by the spatial distribution we observed a prevalence of 26.9% (95% CI: 19.6% - 34.2%). The main advantage of this methodology is that allows to correct the underestimated prevalence without increasing the sampling coverage. Additionally, it also allows the correction of risk at a spatial level through a correction factor that is mathematically equivalent to the value of the Odd Ratio of the disease between sample and population. Finally, this model makes it possible to extrapolate the results obtained in a biased sample to the entire population.

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ASSOCIATION OF HYDATID CYST VOLUME AND THE DEVELOPMENT OF PREOPERATIVE COMPLICATIONS IN PEOPLE WITH HYDATIDOSIS TREATED IN TWO PERUVIAN CITIES DURING 2000 - 2011

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Human cystic echinococcosis (CE) is a chronic zoonotic disease caused by the larval stage of *Echinococcus granulosus* that is characterized by the development of a cystic lesion in the organ involved such as liver or lungs. Infected subjects remain asymptomatic during several years until the cyst is enough large or develop a complication to produce symptoms. Several studies reported clinical characteristics of the disease as well as cyst characteristics; however there is not clear if the organ involved, morphological characteristics such as size, or vicinity with other cystic lesions are risk factors to develop cyst's complications. We performed a cross-sectional study in a cohort of subjects surgically treated between 2000 to 2011; using a bivariate and multiple analysis with a generalized linear model with Poisson family of robust variances and log linkage links. From a total of 714 subjects, more than half of them were female (53.8%), the average age was 27.5 years (±18.6). Three hundred forty one presented liver involvement (47.8%) and the majority of them were CE1 stage (30.8%).Regarding lung cysts, it was more common to be in the right lung (56.3%), the total volume of cyst(s) ranged from 4.2 to 24268.4 mm3. It was observed that the average total volume of cysts in the liver was 1392.3mm3 (4.2 - 24268.5), in the right lung it was 486.3mm3 (6.7 - 3783.7) and in the left lung it was 557.4mm3(5.0 - 5575.3). There were 380 people (53.6%) with complicated cyst, where 227 were due to infection (59.7%) and 153 due to rupture (40.3%). In the multiple analysis it was observed that the left lung (PR: 1.29, CI95% 1.06-1.56) and right lung (PR: 1.30, CI95% 1.08-1.58) presented a higher prevalence of complication compared to the liver, both associations were statistically significant. Based on this findings, we conclude that the total volume of cysts has different characteristics according to the organ affected and also the presence of complication, suggesting the importance of performing lung evaluation during the epidemiological surveys in order to determine lung cystic lesion before being complicated .

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CAROTID PORCINE MODEL OF NEUROCYSTICERCOSIS: ASSESSING THE LINK BETWEEN INJECTION DOSE WITH CNS INFECTION RATES AND SERUM ANTIGEN DYNAMICS

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Animal models in neurocysticercosis (NCC) allow a better understanding of the immunopathogenesis, host-parasite interactions, and disease progression in the central nervous system (CNS), and are also useful for assessing novel therapies. The pig is the ideal model for NCC as is the natural intermediate host of *Taenia solium* and closely resembles human pathology. Oral infection models require a high dose of eggs to produce NCC resulting in low CNS infection rates and poor reproducibility. Carotid injection of activated *T. solium* oncospheres in pigs overcomes these problems and provides a more feasible and consistent NCC model. We assessed the effects of oncosphere dose on infection outcomes and antigen dynamics in the carotid pig model. Twenty-nine piglets were infected via carotid injection of activated oncospheres (2500, 5000, and 10000). Pig sera were collected at days 0, 7, 14, 30, 50, 70, 100, 130 and 150 (necropsy). At necropsy, viable and degenerated cysts were recorded in the CNS and pig muscles. Antigen detection (Ag-ratios) was assessed using a sandwich Ag-ELISA assay. A panel of sera from 8 pigs orally infected with a *T. solium* gravid proglottid was used as a comparison group. NCC was achieved in 21/29 pigs (72%), with higher infection rates at higher doses (4/9 [44%], 9/11 [82%] and 8/9 [89%] for 2500, 5000, and 10000 oncospheres, p=0.038). All CNS cysts were viable, whereas viable and degenerated cysts were found in muscles. Degenerated cysts were more frequent in pigs in the lower dose group (mean 57.7%, 6% and 9.1% for the 2,500, 5,000 and 10,000 oncosphere groups, p=0.020). At day 14 Ag-ratios increased substantially in all groups, more in pigs that received 5000 and 10000 oncospheres. Ag-ratios in NCC pigs remained higher and not different between 5000 and 10000 oncosphere groups, reaching plateau levels at day 30 until necropsy (mean Ag-ratio: 59.2 and mean Ag-ratio: 52.7, p=0.812). Ag-ratios over time in the high oncosphere groups were similar to those observed in orally infected pigs. Intracarotid doses of at least 5000 oncospheres provide high NCC rates and antigen dynamics similar to the oral model, providing an appropiate model for NCC.

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EXPLORING SOURCES OF FALSE-POSITIVE URINE CYSTAG SCREENING RESULTS IN A REGION ENDEMIC TO TAENIA SOLIUM

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Cysticercosis is an infection by the metacestode stage of the *Taenia solium* tapeworm. When infection involves the central nervous system, the disease is called neurocysticercosis (NCC), and when cysts or cyst membranes invade the subarachnoid spaces, it is called subarachnoid neurocysticercosis (SANCC), an aggressive form of the disease with poor prognosis. We are evaluating early detection and medical intervention for SANCC using a population screening approach by testing for high levels of cysticercosis antigen (cystAg) excreted in urine, using an ELISA with Peruvian monoclonal antibodies (TsW8/ TsW5). A positive urine screen result, however, requires magnetic resonance imaging (MRI) of the brain for definitive diagnosis of NCC, including SANCC. Approximately 75% of individuals with high levels of urinary cystAg do not have cysts detectable by MRI. The objective of this study is to evaluate potential sources of high cystAg levels in the absence of NCC. In this cross-sectional study, we are enrolling consecutively 30 individuals with high urine cystAg (optical density ratio ≥ 3) and no evidence of NCC on brain MRI, to investigate other potential sources of urine cystAg. Procedures include computed tomography (CT) without contrast of the head, chest, abdomen, and proximal limbs to evaluate for the presence of *T. solium* cysticercosis of the skeletal muscles, as well as other larval cestode infection (e.g. *Echinococcus* sp.) of the lungs, liver, and abdominal cavity. We also examine the skin for subcutaneous cysts. We use Western Blot to evaluate for the presence of antibodies against other larval *Taenia* cestodes known to infect humans including *Echinococcus* sp. and *T. multiceps*. We will report frequency and proportion of findings for all exams. Characterizing the reasons for false positive results is important for improving the performance of the assay and interpretation of its results.

0764

PROCESS MAPPING TO UNDERSTAND THE STEPS REQUIRED TO IMPLEMENT A CONTROL PROGRAM FOR CYSTICERCOSIS IN RURAL PERU

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Ring treatment is a strategy to control cysticercosis that is efficacious when delivered by research teams. We are using implementation science tools to transfer ring treatment to the public health system in Northern Peru. Process mapping is a system engineering and quality improvement tool used in health care settings to break down complex processes into sequenced steps, identify efficiencies, and streamline work. Our objective was to develop process maps to facilitate early stages of ring treatment implementation. The Systems Analysis and Improvement Approach guided our process mapping activities. We collected examples of existing protocols and procedures documents that have been applied in other infectious diseases in Peru and designed a prototype for community control of cysticercosis. We are conducting interviews and walk-through exercises with health system staff to detail each step of ring treatment, from community case identification and verification of T.solium infection to the presumptive treatment of humans/people/community members with niclosamide in 100-meter 'rings' ,and follow-up. We use Visio software to display our final process maps. We will present the steps to develop a process map in collaboration with stakeholders from the health system and provide recommendations for training staff and conducting fieldwork to build the process map flow chart. We will include examples of completed process maps in the poster. It will serve as a guide for applying process mapping when the processes are not well standardized but need to be established, in low-resource settings. Quality improvement tools, such as process mapping, can be applied in health care and community health settings with low resources to implement and scale-up evidence-based interventions, conserve resources, and establish sustainable control strategies/processes.

0765

DESCRIBING VARIABILITY OF URINE CYSTICERCOSIS ANTIGEN EXCRETION IN RURAL PERU, A REGION ENDEMIC TO TAENIA SOLIUM

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Neurocysticercosis (NCC) is a central nervous system infection by the cestode *Taenia solium* that accounts for 30% of seizure disorders in endemic regions. We are currently validating a population screening approach to detect the most severe form of infection, subarachnoid NCC, to allow medical management in early disease stages. We collect urine samples in the community setting and test these for the presence of cysticercosis antigens (cystAg) using ELISA and a monoclonal antibody (mAb) set developed in Peru (TsW8/TsW5). Understanding the natural variability in cystAg levels over time is critical to establish optimal cutoff levels for a positive result, which will help limit false positive results leading to unnecessary neuroimaging for diagnostic confirmation. Our objective is to describe the short-term variability of urine cystAg excretion among individuals living in a region endemic to *T. solium*. We are conducting a prospective cohort including 200 participants across 3 strata of baseline cystAg levels (Strong positive: Optimal density ratio (ODR) ≥ 3, n= 50; Weak positive: ODR ≥1 and <3, n=50; Negative: ODR <1, n=100) in Tumbes, Peru. We collect urine samples from each participant once every 6 hours for 24 hours, once daily for 7 days, and once weekly for 4 weeks. Samples are processed by ELISA using TsW8/TsW5 mAbs. We will report the results of multilevel mixed-effects linear regression to compare mean ODR and standard deviation across time intervals (day, week, month), overall and according to ODR group, while evaluate effects of age and sex. Understanding the variability of urine cystAg will inform optimal ODR screening thresholds and reduce the likelihood of false positives and unnecessary neuroimaging.

0766

INCIDENCE OF CYSTICERCOSIS ANTIGEN EXCRETION IN THE URINE IN A POPULATION ENDEMIC TO TAENIA SOLIUM IN NORTHERN PERU

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Taenia solium is a zoonotic cestode with a transmission cycle involving pigs as an intermediate host and humans as a definitive host. Infection of the human brain, neurocysticercosis (NCC), is a leading cause of epilepsy and other neurologic disease across many regions of Latin America, Africa, and Asia. NCC occurs when people ingest *T. solium* eggs passed in the feces of a human with an intestinal tapeworm. Although there have been many cross-sectional studies describing the prevalence of exposure and infection in human and pigs, few studies have described the incidence of exposure to *T. solium* eggs among humans in an endemic community setting. The objective of this study is to evaluate the cumulative incidence of cysticercosis antigen (cystAg) excretion in urine, a marker for prior exposure to *T. solium* eggs, and one indicator of the overall level of transmission in the community. As part of a large cross-sectional study to validate population urine screening for NCC, we collected 4017 urine samples in several rural villages in Piura, Peru, in March 2020. Days later, Peru entered national lockdown due to COVID-19 restrictions, forcing us to return 9 months later to recollect these samples for their original screening purposes. Both sample sets were stored frozen at -20C, including paired sets from 3759 participants sampled at both time points. We are currently processing these paired urine sets by ELISA, using Peruvian monoclonal antibodies (TsW8/TsW5), to evaluate for the presence of cystAg. Results are characterized as strong positive (Optical density ratio (ODR) ≥3), weak positive (ODR ≥ 1 & <3), and negative (ODR < 1). We will report the cumulative incidence of conversion of results between categories, as well as risk factors for both conversion and persistence of positive results. We will discuss the implications of our results for public health control and prevention.

0767

ASSESSING THE EFFECT OF COMBINED THERAPY WITH ALBENDAZOLE PLUS PRAZIQUANTEL VERSUS ALBENDAZOLE MONOTHERAPY IN TWO DOSES ON LIVER TRANSAMINASES IN PATIENTS WITH VIABLE PARENCHYMAL NEUROCYSTICERCOSIS

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Combined albendazole (ABZ) plus praziquantel (PZQ) is more effective for viable parenchymal neurocysticercosis (NCC) in comparison with ABZ monotherapy. ABZ therapy may induce elevation of liver transaminases, and is unclear whether this elevation is even greater when combining ABZ+PZQ, which may lead to temporary discontinuation of treatment. This study assessed whether elevation of liver transaminases (alanine aminotransferase [ALT] and aspartate aminotransferase [AST], U/L) from baseline to days 4, 7, 11 and 30 was associated with different therapy schemes in parenchymal NCC using data from two trials in patients who received 10 days of standard ABZ (15mg/kg/d), increased ABZ (22.5mg/kg/d) or standard ABZ plus PZQ (50mg/kg/d). We compared the proportions of patients whose transaminases increased from baseline to follow-up between groups. Maximum differences (Δmax) of liver transaminases were calculated by subtracting the highest measurement at follow-up with the measurement at baseline. Regression coeficients of Δmax values between therapy schemes were estimated in linear regression models adjusted by type of anti-epileptic drug, age and sex. The proportion of patients with elevated ALT levels from baseline to follow-up was statistically lower in the combined ABZ+PZQ group versus increased ABZ (35/47 [74.5%] and 27/29 [93%], p=0.041), but not different with standard ABZ (41/47 [87%, p=0.116). The regression model showed significantly lower Δmax ALT values in the combined ABZ+PZQ group versus standard ABZ (B=-30.9 U/L, p<0.001) and increased ABZ (B=-58.2, p=0.020). The proportion of patients with increased AST levels from baseline to follow-up was also lower in the combined ABZ+PZQ group (28/47 [60%]) versus standard ABZ (37/47 [70%, p=0.044) and increased ABZ (23/29 [79%], p=0.075), and the regression also showed lower Δmax AST values in the combined ABZ+PZQ group versus standard ABZ (B=-14.7 U/L, p<0.001) and increased ABZ (B=-27 U/L, p=0.010). The likelihood and magnitude of liver transaminases elevation increases following antiparasitic treatment in NCC and are lower when combining ABZ+PZQ instead of ABZ monotherapy

0768

COMPARISON OF TWO MONOCLONAL ANTIBODY BASED AGELISA ASSAYS FOR ASSESSING THE DYNAMICS OF CIRCULATING ANTIGENS IN PIGS EXPERIMENTALLY INFECTED WITH TAENIA SOLIUM CYSTICERCOSIS

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Antigen dynamics in *Taenia solium* cysticercosis reflect the processes during infection as well as host-parasite interactions and can be properly assessed in the pig model. The reference assay for antigen detection is the B158/B60 Ag ELISA assay detecting excretory/secretory products of *Taenia saginata* cysts, but also detecting *T. solium* cysticercosis antigens due to cross-reaction. Our group has previously developed 22 moAbs against *T. solium*, of which two (TsW8/TsW5) can detect antigen in serum or urine samples. This study compares the performance of our novel anti-*T. solium* (TsW8/TsW5) moAbs in a sandwich Ag-ELISA format versus B158/B60 Ag-ELISA assay for assessing antigen dynamics in experimental porcine cysticercosis using a panel of sera collected at days 0, 7, 14, 21, 28, 42, 56, 70 and 90 in pigs infected with T. solium eggs by oral route. Mean optical density (OD) readings at different follow-up time-points were compared between Ag-ELISA assays, and correlations between cyst burden and antigen OD readings for each assay were calculated using the Spearman rank correlation coefficient. Antigen dynamics showed similar performance between Ag-ELISA assays with no decline in OD readings in pigs positive at necropsy. At baseline and at day 7, mean antigen OD readings were low (<1) and not different between assays. Mean antigen OD readings increased at day 14, being slightly higher when using TsW8/TsW5 moAbs compared to B158/B60 moAbs (mean OD:1.07, and mean OD: 0.90, p=0.014), and reached a plateau at day 28 onwards with no statistical differences between assays (mean OD:1.80 and mean OD:1.87 at day 90, p=0.382). Viable cyst burden had a stronger correlation with antigen OD readings measured with the TsW8/TsW5 moAbs (r=0.87, p<0.001) compared to antigen OD readings measured with the B158/B60 moAbs (r=0.75, p=0.003). Our findings showed a similar performance between both Ag-ELISA assays for assessing antigen dynamics in

experimentally infected pigs, with an apparent higher avidity of our moAbs TsW8/TsW5 to detect living cysts, providing a promising tool for studying host-parasite interactions.

0769

VALIDATED NON-LOCAL PARAMETRIZATION OF AN AGENT-BASED MODEL TO REPRESENT LOCAL-SCALE TAENIA SOLIUM TRANSMISSION IN RURAL VILLAGES OF AN ENDEMIC AREA IN NORTHWEST PERU

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Taenia solium is an important cause of epilepsy and economic loss in many rural areas of the world. Although there has been some progress in developing and testing interventions to control and eliminate transmission, optimal strategies are yet to be defined. Validated models that simulate transmission and interventions within regions may guide design and evaluation of the most effective strategies to control or eliminate *T. solium*. Our objective was to develop a new version of CystiAgents, an Agent Based Model of local-scale *T. solium* transmission, that does not rely on local calibration of every modeled village. Based in MASON with in-house Java coding, the model covers relevant aspects of *T. solium* transmission, including processes of pig and human infection, spatial distribution of human and pig populations in several endemic villages in northwest Peru, pork production within villages for human consumption, and movement of humans and pigs in an out of simulated villages. We applied a new approach to model calibration, based on approximate Bayesian computation, in which model outputs are fit simultaneously to observed prevalences of human taeniasis and pig cysticercosis for several endemic villages. Despite large underlying variance associated with empirical measurement of *T. solium* epidemiological data, the calibrated model reproduces observed prevalences with acceptable precision, not only for empirical data of villages used to calibrate the model, but also for empirical data from villages not included in the calibration process. The calibrated model can be successfully transferred to accurately simulate transmission in other villages within the same region. We plan to use CystiAgents with non-local calibrated model parametrization, as a universal tool to conduct *in silico* experiments to guide design and optimization of *T. solium* control and elimination interventions for northwest Peru.

0770

SUBARACHNOID NEUROCYSTICERCOSIS

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Subarachnoid neurocysticercosis (SANCC) is the most serious form of neurocysticercosis; its clinical manifestations vary depending on the location, size and host response. Cysticercus antigen detection is useful for diagnosis and assessment of activity. Calcification has been reported after treatment but its frequency and clinical implications have not yet been elucidated. We report the baseline characteristics of patients with SANCC, frequency of calcification at presentation and after treatment, and correlation with cysticercosis antigen in a non-endemic region. Methods: We reviewed the symptoms, imaging and serology of patients with SANCC who presented to Jacobi Medical Center, New York City. Cysticercosis antigen by ELISA was measured in serum and cerebrospinal fluid (CSF) at CDC. 91 patients with SANCC were included; 34 patients had exclusive SANCC, 44 patients had SANCC and parenchymal disease, 4 patients had SANCC and intraventricular disease, and 9 patients had involvement of all 3 spaces. The most common location was sylvian fissure alone (37.4%), followed by basilar alone (28.6%), sylvian and basilar (25.3%), cisterna magna (1.1%), interhemispheric (1.1%) and spinal (6.6%). Thirty-four patients (37.4%) had calcified SANCC at presentation. Seventy patients (76.9%) were assessed to have symptoms due to subarachnoid disease; headache was the most common symptom (81.4%). Of them, 32 patients had intracranial hypertension (45.7%). Less common presentations included seizures (15.7%), spinal symptoms (15.7%), aseptic meningitis (10%), and vascular complications (7.1%). Delay in diagnosis was noticed in 41 patients (58.6%). Among asymptomatic patients, 22.8% had concomitant spinal disease. The cysticercus antigen in serum and CSF was positive in 49 patients (59.8%) and 21 patients (38.2%). The most common presentations of SANCC are headache and intracranial hypertension. Concomitant spinal involvement is common. A third of patients had calcifications at presentation. Calcifications were associated with enhancement; further studies are needed to assess its significance.

0771

EVALUATING THE ROLE OF CORRALS AND INSECTS IN THE TRANSMISSION OF PORCINE CYSTICERCOSIS: A COHORT STUDY

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The widespread dispersion across endemic villages of pigs infected with cysticercosis, the low average cyst burden among infected pigs, and the low prevalence of taenisias, all suggest that direct pig ingestion of human feces is not the only mode of transmission for *Taenia solium*. Alternate mechanisms of egg dispersal away human fecal depositions are likely to play an important role in transmission. Insects are known to transmit many cestode parasites, including those of the Taenidae family. Our objective was to evaluate the risk of porcine cysticercosis associated with exposure to human feces and to insects in an endemic community setting. We used a cluster-randomized cohort design to compare the risk of developing antibodies and infection among 120 seronegative piglets raised in either free-roaming (FR; exposed to human feces and insects), standard corral (SC; protected from human feces, exposed to insects), or netted corral environments (NC; protected from human feces and flying insects). We processed monthly blood samples by LLGP-EITB to detect antibodies and necropsied all pigs after 10 months to identify cysts. 64 piglets developed antibodies for a seroincidence (95% CI) of 0.07 (0.04 - 0.11), 0.08 (0.05 - 0.13) and 0.15 (0.11 - 0.22) cases per pig-month in NC, SC, and FR groups, respectively. The relative risk of seropositivity in FR vs. corralled pigs was similar in the first 2 months, then increased to 5.3x (3-6 months) and 8.7x (6+ months). Of 108 pigs necropsied, 15 had *T. solium* cysts (range 1-2387 cysts), all belonging to the FR group. Corrals were protective against infection but less so against seropositivity. Netted corrals, which did not completely exclude insects, did not provide added protection against seropositivity compared with standard corrals. Results suggests that flying insects do not play an important role in infection. Alternate routes, such as egg persistence in soil, water, or feed should be explored.

0772

RACINE SCALE EVALUATION FOR THE ANALYSIS OF EPILEPSY IN A RAT MODEL OF NEUROCYSTICERCOSIS

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Neurocysticercosis (NCC) is caused by larval stage of *Taenia solium* or cysticerci infecting the central nervous system and is the leading cause of acquired epilepsy and convulsive conditions worldwide. The Racine scale is a method used in experimental epilepsy animal models that characterizes seizures at different levels according to their severity. The optimal evaluation of seizures in rats with NCC, would help in the prevention and treatment of epileptogenesis. The behavior of 41 Holtzman rats with NCC (8 months post-infection) was recorded using cameras for 5 days. Subsequently, the presence of seizures was evaluated according to the Racine scale. For 41 rats with NCC, 95% developed some level of seizures. During the evaluation, 37 rats had multiple levels of seizures. It was found that level 2 (85%, nodding the head) is the predominant, followed by level 3 (83%; clonus of forelimb), level 1 (73%; movement of the mouth and face), level 4 (36%; breeding) and level 5 (13%; breeding, and fall). However, some behaviors, which were not included in the scale, were observed. Such as sudden jumps, fleeting runs, myoclonic spasms, and fusion of several levels at the same time. In conclusion, the Racine scale must be complemented and adapted for the evaluation of epilepsy in a rat model of neurocysticercosis, which will allow the evaluation of antiepileptic drugs in this NCC model.

0773

ADDRESSING ACUTE FEBRILE ILLNESS AMONG NON-SEVERE PATIENTS IN RIO DE JANEIRO, BRAZIL: A PROSPECTIVE STUDY

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Identifying etiologies of acute febrile illness (AFI) is challenging in settings with limited laboratory capacity. We aimed to describe the causes of AFI among non-severe patients presenting at the primary level in Rio de Janeiro when a Chikungunya (CHIKV) outbreak was taken place. We conducted a 10-month prospective AFI study in outpatient participants aged 2-65 years. Patients with fever (axillary temperature of 37.5° C or higher) for seven or fewer days were offered enrollment, and clinical, and laboratory data were gathered for consecutive participants. Blood cultures and rapid diagnostics tests (RDTs) were performed regardless of presentation. Others tests were performed only if specific signs or symptoms were present. Flavivirus infection was defined when concurrent Dengue (DENV) and Zika (ZIKV) ELISA antibodies were present, while DENV infection was when reactivity against DENV without ZIKV occurred. Logistic regression model determined predictors of laboratory-positive CHIKV (defined as CHIKV RT-PCR positive). Follow-up visits were conducted 14-28 days after the index visit. A total of 500 participants (median age 26 [15-41] years, 50.4% females) yielded 708 diagnoses, 49.8% of whom were multiple diagnoses. Systemic infection was the most frequent febrile syndrome (380/500, 76%), followed by acute respiratory infection (150/500, 30%) and urinary infection (18/500, 3.6%). The diagnosis most prevalent was CHIKV (284/500, 56.8%), followed by Flaviviruses (214/500, 42.8%), DENV (42/500, 8.4%), and viral upper respiratory infection (40/500, 8%). Etiology remained undetermined in 124 (25%). According to arboviruses RDT, 80% were exposed to an arbovirus, and the most prevalent was DENV (25.8%). Predictors of laboratory-positive CHIKV were the absence of cough, arthralgia, rash, temperature, and leucopenia. At follow-up, 40% reported persistence of any symptoms. CHIKV-infected patients were more likely to experience persistent arthritis than CHIKV negative. Evidence of a viral process was found in almost 80%, and the practice of empirical antibiotic prescription in non-severe febrile patients should be reconsidered.

0774

ETIOLOGY OF FEBRILE NEUROLOGICAL SYNDROMES IN LOW- AND MIDDLE-INCOME COUNTRIES: RESULTS FROM THE FIEBRE STUDY

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Fever is one of the most common symptoms leading to health care seeking and hospital admission worldwide. Neurological symptoms associated with fever are a major concern given the difficulty and expense in making a diagnosis, and the high mortality of infection related neurological syndromes. In low- and middle-income countries (LMICs), there is little preexisting knowledge regarding the underlying etiology and microbiology of febrile neurological syndromes to adequately guide their management beyond empirical broad-spectrum antimicrobial regimens. This analysis aims to provide a description of patients with fever and neurological symptoms in the FIEBRE (Febrile Illness Evaluation in a Broad Range of Endemicities) observational study. We present a subset of patients enrolled in FIEBRE with neurological signs and symptoms on presentation, in order to describe their clinical characterization, treatment, outcome, and final etiology. Child and adult in- and outpatients were recruited and systematically investigated in Mozambique, Malawi, Lao PDR and Zimbabwe from 2018 to 2021. Clinical description on presentation, treatment and outcome were recorded for each participant. Point-of-care tests (including blood culture, urine dipstick and culture, and HIV and malaria tests) were performed, and further samples were shipped to reference laboratories for gold standard testing for a broad range of infectious agents. Across all sites 457/7145 patients (13% of all inpatients and 1.7% outpatients) presented with at least one neurological sign or symptom. Of those, 71% had seizures, 22% impaired consciousness, and 13% neck stiffness. The majority (78%) received antibiotics. Central nervous system infection was suspected by clinicians at arrival in 55/457. We will present all laboratory diagnostic results stratified by age, site and clinical presentation, and an analysis of sub-group outcomes. Neurological symptoms on presentation are frequent among febrile inpatients in LMICs and antibiotics are widely prescribed. Detailed etiological diagnoses are crucial to better target the scarce therapeutic interventions available.

0775

RECONCILING IMPERATIVES. CLINICAL GUIDELINES AND THE ENACTMENT OF GOOD CARE IN LOWER-LEVEL HEALTH FACILITIES IN TORORO, UGANDA

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In the face of rising threats of antimicrobial resistance, health workers are urged to reduce the unnecessary prescription of antimicrobials. Clinical guidelines emerge through evaluations of best practice – bundling clinical, technological and economic dimensions – and they create metrics through which to assess practice. On the global health stage, guidelines become a device through which to address complexities of care across multiple contexts; by altering the algorithm of a guideline in one setting, the number of antibiotic prescriptions could be reduced in another. Prescribing practice can then be benchmarked against the latest guidelines, a key tenet of antimicrobial stewardship programmes. Drawing on ethnographic data gathered in lower-level health care facilities in rural Eastern Uganda for 10 months between January and October 2020, extending previous work carried out over the past decade, we describe how clinical guidelines are translated and implemented in settings where resources are limited. In a context of scarcity where 'care' is characterized by delivery of medicines, and is constituted beyond these algorithmic outputs, we observed that clinical practice in lower-level health facilities in Nagongera, Tororo was shaped by patterns of resources, professional and patient expectations as much as by the written algorithms. For stewardship to care for patients as well as for medicines, a better understanding is required of clinical practice and expectations of care, in relation to and beyond the framework of the guidelines.

0776

PREVENTION, DIAGNOSIS AND MANAGEMENT OF PODOCONIOSIS; INTEGRATION INTO PRIMARY HEALTH CARE IN SOUTHERN NATIONS, NATIONALITIES, AND PEOPLE'S REGION (SNNPR), ETHIOPIA

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Podoconiosis is a non-infectious, neglected tropical disease (NTD) whose global burden is estimated to be about 4 million people, concentrated in the highland areas of tropical Africa, Central America and north-west India. Ethiopia bears the highest burden of podoconiosis globally, with more than 1.5 million people affected, causing detrimental economic, physical and social impact. The absence of diagnostic point-of-care tests, coupled with inadequate knowledge among healthcare providers, contributes to the under-reporting of podoconiosis cases in a context where funding is limited. However, the World Health Organization (WHO)'s 2021-2030 roadmap calls for greater integration and mainstreaming of NTD approaches into national health systems. Additionally, Ethiopia's 2016-2020 NTD Master Plan listed podoconiosis as one of eight priority NTDs for integration into its primary healthcare (PHC) system. This study aimed to develop and pilot an evidence-based, multi-component intervention to strengthen and integrate podoconiosis detection, management and reporting into PHC in Ethiopia. In the formative research phase of the project, mapping and review of existing national guidelines was conducted to identify current gaps in the PHC system. Key informant interviews were held to elicit community perceptions of podoconiosis and barriers to care-seeking. A health system capacity assessment was conducted in a primary hospital, health centre and five health posts to assess gaps and identify requirements for integration. Stakeholders included community members with and without podoconiosis, health extension workers, health workers and programme managers from national to district level. Results informed the design and validation of intervention materials and processes which were piloted for six months with end users across all levels of PHC system. Formative research and the evaluation of intervention feasibility, acceptability and implementation cost will be presented.

0777

BACTERIAL ILLNESS IN LIBERIAN CHILDREN UNDER FIVE YEARS

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Fever without apparent source (FWAS) is one of the most common reasons why children seek urgent medical treatment across the world (1). When diagnostic microbiology became available in Monrovia in 2019, we undertook a study of children under 5 who were admitted to the hospital with acute febrile illness to determine possible etiologies of their fever. We conducted a prospective study of febrile illness in children admitted to a Liberian tertiary care center from May 2019-June 2020. Parental consent was obtained for children aged 6 mo to 5yr who presented with fever within 24 hours. Demographic and clinical information, CBC, blood culture, and malaria smears were obtained for every participant. Tests such as CXR, urine, throat and CSF cultures were conducted when clinically indicated. Study staff were trained on sterile technique using alcohol and povidone prior to venipuncture. Of the 220 children who were included in the study,31 had positive blood cultures. Of these samples, 21 had positive blood cultures associated with skin contaminants: 19 coagulase negative *Staph spp. (*CoNS*),* 2 Corynebacterium, and *Enterococcus* spp. (1). Thus, the overall contamination rate seen in our study was 9.5% (21/220 patients). In performing a study on acute febrile illness in Liberian children, we noted a high rate of contaminated blood cultures. This rate of contamination greatly exceeds the 2-3% benchmark rate for incidental culture contamination that has been cited previously. This is similar to findings within pediatric populations in other African tertiary care centers such as in Nigeria (11%), and Kenya (8.8%). CoNS is the primary cause of BC contamination worldwide and contamination is typically higher in pediatric populations than adults. Blood culture contamination leads to inappropriate antibiotic use and increased cost. Patient care may suffer from misdiagnosis. Several studies have noted improved rates of blood culture contamination with education directed at scrupulous sterile technique. Such strategies will be important to pursue in countries where diagnostic microbiology is newly available, such as Liberia.

0778

THE HEART OF THE MATTER: CARDIAC MANIFESTATIONS OF CHAGAS DISEASE AT A TROPICAL MEDICINE CLINIC IN BOSTON

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There are limited data about Chagas disease (CD) screening and cardiac manifestations of CD for people living in the United States. This study aimed to describe disease severity by American Heart Association (AHA) stage and Rassi mortality risk prediction score and cardiac medication management for patients with CD. This retrospective cohort analysis included patients with CD (diagnosed with two positive tests) identified through a community-based screening program of immigrants at a

local community health center and seen at a Boston tropical medicine clinic between January 2016-February 2021. We analyzed cardiac testing (electrocardiogram [EKG], echocardiogram, cardiac monitor, stress tests, and cardiac MRI) and medication management. Of the 63 patients, 34 (54%) were female, and the mean age was 45 years (range 22-73). Among the 4 patients who underwent cardiac MRI, 3 (75%) had late gadolinium enhancement findings suggestive of Chagas cardiomyopathy (CCM). Overall, 34 (54%) patients were AHA stage A (no EKG/echocardiographic changes), 22 (35%) were stage B1 (EKG or echocardiographic changes, normal ventricular function), 4 (6%) were stage B2 (ventricular dysfunction), and 2 (3%) were stage C (ventricular dysfunction and symptomatic heart failure). No patient was stage D (heart failure refractory to medical therapy) and 2 were unstageable due to limited data. The majority of patients (n=55, 87%) were in the low Rassi mortality risk category, 6 (10%) were intermediate risk, none were high risk, and 2 (3%) were unable to be risk stratified. After their cardiac workup, 4 patients were started on goal-directed therapy for heart failure, and 4 patients were started on anticoagulation (1=warfarin, 3=aspirin). Almost half of patients in our cohort had evidence of CCM and 10% had elevated 10-year mortality risk. We demonstrate that screening individuals from endemic countries in a community-based primary care setting identifies those at high risk and in need of medical therapy for cardiomyopathy.

0779

PREVALENCE AND FACTORS ASSOCIATED WITH HEART FAILURE IN HAITI: FINDINGS FROM A POPULATION-REPRESENTATIVE COHORT STUDY

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Cardiovascular disease is the leading cause of mortality in Haiti and other low- and middle-income countries (LMICs). However, there is a scarcity of data on the epidemiology, etiologies and risk factors of heart failure (HF) in these areas, hampering our ability to devise preventive interventions and treatment guidelines. We conducted a population-representative cohort study of Haitian adults ≥18 years of age to estimate the prevalence of HF in Port-au-Prince, Haiti. Sociodemographic and clinical data, including echocardiography, electrocardiography, and blood pressure measurements were collected from March 2019 - April 2020. HF was defined as the simultaneous presence of at least 2 major Framingham HF criteria or 1 major criterion in conjunction with at least 2 minor criteria. Associated factors (age, sex, education, income, smoking, alcohol, hypertension, and BMI) were assessed using univariate logistic regression models. Of 1,420 participants, the prevalence of HF was 3.2% (N = 45). Of those with HF, 11 (24.4%) had a reduced ejection fraction of less than 50% and 34 (75.6%) had a preserved ejection fraction greater than 50%. 68.9% of participants with HF had concurrent hypertension. Importantly, 301 (21.9%) participants without HF had diastolic dysfunction on echocardiography. In participants ≥50 years of age (N = 289), the prevalence of hypertension, diastolic dysfunction, and HF was 75.3%, 58.6%, and 7.7%, respectively. Female sex, increased age, lower education levels, hypertension, and increased BMI were all significantly associated with increased rates of HF (p-value < 0.05). HF has been shown to be the most common cause of hospital admission in Haiti, and we report that the prevalence of HF in a population-representative sample was 3.2%, which doubles in those 50 years of age and older. The high prevalence of hypertension, diastolic dysfunction, and HF with preserved ejection fraction demonstrate that early interventions like hypertension management may greatly improve cardiovascular health and reduce long-term morbidity and mortality in Haiti and other LMICs.

0780

BUILDING THE FULL CONTINUUM OF CLEFT CARE IN EL SALVADOR

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Six percent of all surgical procedures are performed in the poorest countries that are home to 1/3 of the world's population. Disparate rates of cleft palate malformation repairs are one example of an inequity that disproportionately affects low income nations. The goals of comprehensive cleft care include repair of the birth defect, achievement of normal speech, adequate breathing, and dental health, and facilitation of optimal psychosocial and developmental outcomes. These goals are accomplished in America through coordinated, multidisciplinary care, which is challenging to provide in a global context with limited resources and surgical training. Investing in craniofacial care in these nations will save lives, improve quality of life, and promote economic growth. We describe a model for providing such care developed in 2009 by an American Craniofacial Surgeon in El Salvador at Bloom Children's Hospital wherein the focus was to both provide medical care and improve surgical capability and expansion within the country. While efforts started with the traditional focus of performing cleft palate surgeries, the program was developed into a multidisciplinary effort to provide craniofacial surgical care year-round. This progress largely resulted from establishing partnerships with local stakeholders and providing culturally-sensitive care that garnered trust from the local community. This trust enabled the group to train local orthodontists and oral/plastic surgeons in cleft care, allowing for longitudinal and multidisciplinary care for patients to span the continuum of care. Over three years, services expanded to include speech therapy, 12 cases of Le Fort I surgeries, 12 cases of alveolar bone grafting, 4 cases of distraction osteogenesis, and 3 cases of bilateral sagittal split osteotomies. Through offering educational opportunities, such as craniofacial fellowship training in America, as well as providing equipment advancements for the hospital, El Salvador is on its way to having an independent and sustainable foundation with which to provide comprehensive, multidisciplinary craniofacial care administered by local providers.

0781

THE MAIN PATHOGENS CAUSING FEBRILE ILLNESS AND IMPLICATIONS FOR FEVER MANAGEMENT IN LAOS; RESULTS FROM THE FIEBRE STUDY

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Management of febrile illness in Laos typically relies on clinical assessment and empiric treatment, as laboratory confirmation is often not available, except malaria and dengue rapid tests. The standard empiric treatment of inpatients with sepsis or febrile illness in Laos is parenteral ceftriaxone. Vientiane Provincial Hospital in northern Laos was one site of the multicentre FIEBRE (Febrile Illness in a Broad Range of Endemicities) study which performed a comprehensive evaluation of the causes of febrile illness in inpatients and outpatients of all ages. We aimed to describe the leading pathogens diagnosed from FIEBRE patients recruited in Laos. Between October 2018 and October 2020, 1972 patients were enrolled. Laboratory testing included blood culture, malaria microscopy, molecular

or serological testing for histoplasma-antigen, PCR and serology for dengue, Zika, chikungungya, and JEV, PCR for respiratory pathogens, plus *Leptospira* and rickettsial serology (results awaited). Among 1972 patients, 135 (6.8%) had positive blood cultures. Of these, 17 (12.6%) grew *Burkholderia pseudomallei*, 8 (5.9%) *Escherichia coli* (4 ESBL positive), 7 (5.2%) *Staphylococcus aureus* (2 MRSA), 3 (2.2%) *Klebsiella pneumoniae*, 2 (1.5%) *Talaromyces marneffei*, and 2 (1.5%) *Streptococcus pneumoniae*. None of our patients tested positive for malaria. From the first batch of testing, 5/382 (1.3%) samples were positive for Histoplasma-Ag. From 605 samples, 24 (3.9%) were positive for dengue. Of 669 pharyngeal samples, 218 (32.6%) tested positive for respiratory viruses. 1207 (61.4%) patients received antibiotics, of which 799 (66.2%) were cephalosporins. Our results reveal the leading infectious causes of febrile illness in rural Vientiane. Results to date show viruses accounted for most diagnoses, particularly respiratory viruses and dengue. Despite this, antimicrobial prescribing rates were high. While the diagnostic yield from blood cultures was low. We demonstrated melioidosis and ESBL-producing Enterobacterales are prevalent in this part of Laos, with important implications for empiric prescribing in severely ill patients with sepsis.

0782

ACUTE FEBRILE ILLNESSES IN CHILDREN PRESENTING TO A TERTIARY HOSPITAL IN SOUTHERN ETHIOPIA: MANAGEMENT AND OUTCOMES

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The management of febrile illnesses is challenging in settings where diagnostic laboratory facilities are limited. We conducted a longitudinal study in febrile children to assess the appropriateness and outcomes of their management. We consecutively enrolled febrile children aged 2 months and under 13 years presenting to Hawassa University Comprehensive Specialized Hospital from May 2018 through February 2019. Information was obtained from history-taking, physical examination, and laboratory investigations. Culture results available within one week of enrolment provided further guidance on diagnoses and management. Participants were managed based on available findings on day of enrolment. Outcome data were collected on days 7 (±1) and 14 (±1). The appropriateness of management was retrospectively evaluated in accordance with guidelines. Of 433 enrolled children, pneumonia and acute diarrhoea were diagnosed at presentation in 177 (40.9%) and 82 (18.9%), respectively. Urinary tract infection (UTI) was detected in 74 (18.4%) of 402 children. Antibacterial agents were prescribed to 360 (84.7%) of 425 children, but 36 (34%) of 106 children without an initial indication received inappropriate antibacterials. Antimalarial drugs were prescribed to 47 (11.1%) of 425 children, but 30 (7.3%) of 411 children with negative malaria microscopy received inappropriate antimalarials. Fever had resolved in 357 (89.7%) of 398 children at day 7 (±1), and in-hospital death within 7 days occurred in 9 (5.9%) of 153 admitted patients. Among children with pneumonia, being infants or underweight, and absence of vomiting were predictors of death or persisting fever at 7 days. Inappropriate use of antimalarials was associated with older age, presence of anaemia, absence of cough, and higher fevers, while inappropriate antibacterial prescriptions were more likely among infants and those without tachypnea. Our study underscores the need for improving the diagnostic support to properly guide management decision and enhance adherence by clinicians to treatment guidelines.

0783

PODOCONIOSIS INSTRUCTION IN NURSING CURRICULA IN UGANDA, RWANDA, AND KENYA

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Podoconiosis is a chronic, disfiguring, and non-infectious type of elephantiasis that predominantly affects communities marginalized by poverty. Nurses play an important role in early detection and response in rural Africa. However, it is unclear whether they receive adequate training to effectively diagnose and manage this disease. This study aimed to characterize podoconiosis curricula at all post-secondary institutions offering nursing education in three endemic countries. Between July and September 2020, we conducted a cross-sectional quantitative survey of certificate (84), diploma (148) and degree (56) programs across Kenya, Rwanda, and Uganda. Measures focused on podoconiosis knowledge, quality/quantity of podoconiosis instruction, and barriers to podoconiosis education. In total, 109 individuals participated, providing information on 145 of 228 invited nursing schools (63.6%). Knowledge of podoconiosis was poor; 68.6% knew that podoconiosis was caused by contact with soil but 17.6% believed it was caused by mosquitoes. Podoconiosis-specific content was highest in diploma (54.2%), followed by degree (28.6%) and certificate (23.1%) programs. Perceptions towards quality and quantity of instruction varied, ranging from high satisfaction across certificate programs (87.5%) in Kenya to low satisfaction (33.3%) across degree programs in Rwanda. The importance of providing podoconiosis training was ranked high by 41.7% of participants. Exclusion from government curricula, rarity of disease and low faculty knowledge were cited as barriers to sufficient podoconiosis inclusion. This study identified widespread gaps in podoconiosis knowledge among nursing faculty in three East African countries as well as low inclusion in nursing curricula. Well-trained nurses can play a critical role in prevention by promoting simple and low cost measures such as WASH and footwear. Interventions to improve nurses' knowledge could include the development and free distribution of podoconiosis teaching materials, designed for integration into pre-existing courses.

0784

FEASIBILITY OF COMMUNITY BASED EPILEPSY TREATMENT USING TRAINED COMMUNITY HEALTH WORKERS IN MALI: DATA OF THREE MONTHS FOLLOW UP

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In Mali, a prevalence study to identify persons with epilepsy (PWE) by an active research method using community health workers (CHWs) was conducted in six Health Districts (HD) in Kolokani, Kayes, Kéniéba, Sikasso, Kadiolo and Tominian of two ecoclimatic zones of Mali. Following this study, a cohort of PWE treated with phenobarbital has been implemented to evaluate the feasibility of community-based epilepsy with CHWs. A closed cohort study started in September 2020 and running until August 2022 was implemented. PWE from previous prevalence study were selected and checked for their eligibility for phenobarbital treatment. We took in each HD the health area that had the highest number of confirmed cases of epilepsy during the prevalence study. All confirmed cases of epilepsy aged two years and above, not pregnant, not having absence seizures and not taking other antiepileptic drugs were included in the

study. In each health area, the CHWs were trained in the correct use of the follow-up booklet and how to administer the drugOf a total of 181 patients selected, 90.61% (164/181) were eligible for the community-based epilepsy treatment. In the six HD, 36 CHWs were used, including 9 women. The majority of CHWs (47.22% (17/36) had between 11 and 15 years of experience. Most of the CHWs had a basic education level with 69.44% (25/36) for primary education level or Medersa (Arabic school) with 42.75% (56/131). During the inclusion phase, 18.29% (30/167) of the patients were seizure-free compared to 59.21% (90/152) at the three months follow up. Drowsiness was the main side effect reported with 44.44% (8/18). The mean duration between two seizures was significantly reduced from 74.39% (122/164) at inclusion to 25.66% (39/152) at the three months follow up. Dosage errors were the most frequent error caused by the patients with 61.90% (13/22). Among the difficulties reported by the CHWs, those related to a long distance were the most frequent with 89.29% (25/152). Our preliminary data showed that CHWs seem to be a good alternative for the therapeutic follow up of patients with epilepsy in community-based epilepsy treatment in resource limited settings.

0785

A PROSPECTIVE COHORT STUDY OF CLINICAL CHARACTERISTICS, MANAGEMENT, AND OUTCOMES FOR ADOLESCENTS AND ADULTS WITH SEPSIS IN NORTHERN TANZANIA

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Despite a high burden of sepsis in sub-Saharan Africa (sSA), clinical data for sepsis in adolescents and adults are limited. We sought to describe sepsis clinical characteristics, management, severity scores, and their associations with outcomes in a prospective cohort of adolescents and adults at Kilimanjaro Christian Medical Centre, Moshi, Tanzania from September 2019-March 2020. We enrolled participants using a modified Sepsis-2 definition, which excluded the white blood cell criterion due to lack of routine availability, and we collected data on demographics, clinical characteristics, severity indicators, and management, with an emphasis on hours 0-6 after arrival to the Emergency Department. Poisson regression was performed to estimate incidence rate ratios (IRR) and 95% confidence intervals (95%CI) between demographics, clinical factors, and severity scores, and our primary outcome of in-hospital death. There were 86 participants included in our analysis, of whom median (IQR) age was 46.5 (32-60) years, 27 (31.4%) were female, 13 (15.3%) self-reported HIV infection, and 25 (29.1%) died in-hospital. Clinical factors associated with an increased rate of inpatient death included: inability to drink unassisted, IRR 3.75 (95%CI 1.66-8.49), requirement for oxygen supplementation, 2.39 (1.07-5.32), and altered mentation, defined as status other than alert on the Alert-Verbal-Pain-Unresponsive scale, 3.63 (1.38-7.07). Rate of in-hospital death was increased for participants with a quick Sequential Organ Failure Assessment (qSOFA) score ≥2, IRR 3.12 (95%CI 1.38-7.07), and a Universal Vital Assessment (UVA) score >4, 6.68 (2.15-20.71). Within 6 hours after arrival, 29 (33.7%) participants received antimicrobials and 49 (57.0%) received intravenous fluids. Neither intervention was associated with in-hospital outcome. Sepsis in northern Tanzania was associated with high in-hospital mortality. Further data on clinical factors, particularly management practices, and relationships to outcomes are needed to establish the highest-yield interventions suited to the unique characteristics of sepsis in sSA.

0786

FIGHTING ADULT MORTALITY THROUGH ETIOLOGY OF FEVER STUDIES: DESCRIPTION OF HIGH MORTALITY IN AN ADULT INPATIENT POPULATION IN MOZAMBIQUE

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Fever is a common symptom leading to health care seeking and hospital admission in Africa. Mortality rates in febrile adult inpatients in Mozambique are high, and are associated with high underlying prevalence of co-morbidities like HIV and cardiovascular diseases, and delays in care-seeking. Better characterization of febrile teenage and adult patients in terms of clinical presentation, diagnostic laboratory findings, and outcomes may be important for a more evidence-based evaluation of current clinical management algorithms, with the aim of decreasing preventable mortality. The observational study "FIEBRE: Febrile illness evaluation in a broad range of endemicities" recruited febrile patients in Mozambique, Zimbabwe, Malawi and Lao PDR to identify infectious causes of fever and antimicrobial susceptibility of bacterial pathogens. Here we present mortality data from Mozambican adults enrolled from Jan 2019 to Feb 2021. Demographic, clinical and outcome data at enrolment and ≥26 days later were collected, and laboratory tests (blood culture, mycobacterial blood culture, urine dipstick and culture, malaria and HIV testing, serum cryptococcal antigen (CrAg) and urine lipoarabinomannan (uLAM)) were conducted on site. Samples were sent for centralized reference laboratory testing for other specific infectious pathogens. We enrolled 469 outpatient and 300 inpatient adults, with median age 33 years and majority female 529 (68.8%). Among inpatients 53 (17.7%) died within 28 days of enrolment, of which 36 (68%) were HIV-infected. Among HIV-infected inpatients 10% tested positive for uLAM and 7% were positive for CrAg. We will present the diagnostic profile of these patients, in comparison to survivors, with detailed characterization of co-morbidities and socio-demographic factors associated with deaths. Mortality among adult Mozambican inpatients with febrile illness is high, due in part to preventable and treatable opportunistic infections in the context of underlying HIV infection. Comprehensive strategies to address HIV infection at all stages (prevention, diagnosis and treatment) are needed to decrease mortality.

0787

IMPROVING SURVEILLANCE AND MANAGEMENT OF MALARIA, DIARRHEA AND PNEUMONIA IN PRIVATE FACILITIES IN UGANDA

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In Uganda, more than 60% of the population seeks health care in the private sector as their first point of contact. The sector is characterized by poor quality and availability of malaria commodities, challenges with adherence to test results for malaria treatment, and lack of reporting. In 2018, there was a 6-month pilot of mTrac (a USSD based case self-reporting system previously used only in the public sector) for private sector malaria surveillance in 108 facilities in 3 districts which led to an increase in HMIS weekly reporting rates. In 2020, the MoH requested CHAI to assess the impact of scaling up mTrac while strengthening case

management in the private sector in another 4 districts. In September 2020, after a baseline assessment in 136 private clinics and drug shops to understand knowledge, practices, and reporting for malaria, pneumonia, and diarrhea, providers were trained on integrated management of childhood diseases as well as mTrac reporting. Data review meetings were conducted to identify testing, appropriate treatment, and reporting gaps reported through mTrac and followed by routine supportive supervision by district health teams. Testing and reporting rates reported through mTrac were monitored weekly to assess the impact of supervision visits implemented between October 2020-March 2021. Reporting rates showed trends closely linked with patterns of engagement and supportive supervision, i.e dropping to 40% in between supervision, climbing up to above 88% (compared to baseline of 6%) following review meetings, on site mentorship and supportive supervision. Testing rates for malaria, as well as adherence to test results, increased from 76% to 90% between October 2020 and March 2021. Continuous follow up and refresher trainings through onsite mentorships and targeted supervision to underperforming private clinics and drug shops resulted in improvement in reporting rates as well as case & stock management practices. A key driver of the sustainability of this intervention will be the maintenance of continuous targeted support supervision visits as well as the quarterly data review meetings as per the study protocol.

0788

PUBLIC HEALTH INTERVENTION USING THE HEALTH BELIEF MODEL IMPROVES COVERAGE AND ACCEPTANCE TO IVERMECTIN MASS ADMINISTRATION

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Poor community awareness and social mobilization serve as a major barrier by increasing absenteeism and downplaying the relevance of the ivermectin mass distribution by community members. Inadequate awareness also creates confusion among community members especially when one intervention is mistaken for the other. We designed a targeted SBCC intervention with clearly defined and tailored messages of ivermectin MDA program targeting onchocerciasis in endemic communities. At baseline, 63.9% respondents did not receive Ivermectin during the previous year (2019) MDA programme and more than half of them (53.3%) were not aware of the drug distribution. The communities that received the intervention at endline revealed a significantly higher increase in coverage (SATT=0.123, 95% CI=0. 0.073, 0.173, p<0.001). At baseline, uptake rate of 91.0% was recorded. Post the intervention, there was an increase in the proportion of respondents who ingested the MDA drugs (ivermectin) from 91.0% to 95.45%. Previous uptake of MDA drugs (AOR=10.67; 95%CI: 5.59-20.38, p<0.001), Perceived benefit of MDA drug (AOR=4.13; 95%CI: 1.69-10.15, p<0.001) and being aware of the MDA programme (AOR=2.28; 95%CI: 1.00-5.02, p=0.049) was associated with improved receipt of Ivermectin. The findings of this study reveal that SBCC intervention improves ivermectin coverage and uptake rate in mass drug administration. Further research with technological innovations which can enhance SBCC is recommended taking hind sight of the limitations of the study due to the COVID-19 pandemic.

0789

IMPLEMENTATION OF THE NATIONAL INTEGRATED HELMINTH CONTROL PROGRAM IN A PROVINCE IN WESTERN VISAYAS, THE PHILIPPINES: IMPLICATIONS IN THE COVID-19 PANDEMIC

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We performed qualitative semi-structured interviews of 14 individuals actively involved in the soil-transmitted helminth (STH) infection control program in Negros Occidental from different administrative divisions to determine how COVID-19 affected the program at various levels of implementation. Interviews were conducted from July to August 2020, audio-recorded, transcribed verbatim, and coded in an open inductive manner. Thematic analysis reveals that public health activities in the province were focused on detecting and controlling COVID-19. School-based deworming was suspended and the responsibility has been assumed by local government units through house-to-house or health center delivery; however, human resources are lacking as opposed to the education sector. There has been a large shift toward information and communications technology for continued operations. Advocacy continues online through websites and social media with a large emphasis on water, sanitation, and hygiene (WASH). The WASH component of the program has seen improvements as activities are in line with both COVID-19 and STH control and prevention. Communication has been challenging due to barriers such as lack of access to internet, cellular coverage or hardware, and the fear of exposure to the virus. Logistic support for preventive community activities like toilet construction continues to be underprovided, while financial and pharmaceutical resources for deworming are sufficient despite delays. Key informants agree that multi-sectoral collaboration, although lacking at the time, and integration with the activities of other programs are essential, especially when COVID-19-related. The STH control program implementation in Negros Occidental has been disrupted almost completely as public health efforts focus on COVID-19 and infection precautions such as social distancing and community quarantines are observed. As the country continues to face challenges in controlling the virus, there is a need to provide alternative and catch up strategies for NTD service delivery and build a new roadmap to accommodate the gaps identified by this study.

0790

MISSED TREATMENT ROUNDS AMONGST MOBILE POPULATIONS IN MALI: A POTENTIAL RISK TO NEGLECTED TROPICAL DISEASES (NTDS ELIMINATION

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Neglected tropical diseases (NTDs) are a group of diseases targeted for control, elimination and eradication by the World Health Organization. Five NTDs use a strategy of mass drug administration (MDA) using preventive chemotherapy for all eligible people living in endemic areas. Certain populations, including nomadic and migrant mobile populations, may not be reached during drug distribution. Stopping infection transmission through MDA will not be possible as long as significant groups of untreated people remain. This study aims to understand the characteristics of nomadic and migrant populations and how to improve MDA drug coverage. We carried out a mixed methods study in two health districts of Mali. Research sites included internally displaced persons as a result of terrorist and ethnic violence. Data were collected through individual in-depth interviews (IDI), focus group discussions (FGD) and a questionnaire using a micro-narrative survey to capture the experiences and practices of nomads and migrants in relation to their participation in previous MDA rounds. A total of 1,067 people were included in the survey and 19 IDI and 10 FGD were done. Survey participants included 22.7% transhumants and 25.1% farmers. More than 50% said they had not participated in the last MDA campaign. Some participants said they missed the MDA in their home village because of their mobility. Others reported that they did not feel connected to the host districts: *We are not very convinced that these medicines are of any use to us. Even if we go there we would not be considered because we are not planned here.* The movements of these populations do not align with the MDA campaign in their district of origin. These populations do not feel connected to the communities they migrate to, further reducing their opportunities to take MDA treatment. This study suggests that nomadic and migrant populations may be missing multiple rounds of MDA, introducing an important barrier to eliminating some NTDs in Mali. Improved coverage to these populations could be achieved by understanding their movements and strengthening collaboration among stakeholders.

0791

IMPROVING NEGLECTED TROPICAL DISEASES (NTDS) SERVICES AND INTEGRATING INTO PRIMARY HEALTH CARE IN SOUTHERN NATIONS, NATIONALITIES, AND PEOPLE'S REGION (SNNPR), ETHIOPIA

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Neglected tropical diseases (NTDs) are a group of parasitic and bacterial infectious diseases that affect more than 1.5 billion of the world's population. Over 40% of the global NTD burden is concentrated in sub-Saharan Africa, and Ethiopia bears one of the highest burdens. Several NTDs are endemic in the country, including trachoma, schistosomiasis and lymphatic filariasis. Previous NTD control has focused on developing and distributing safe and effective drugs to affected populations, mainly through mass drug administration. However, the World Health Organization (WHO)'s 2021–2030 roadmap calls for greater integration and mainstreaming of NTD approaches into national health systems. Additionally, Ethiopia's 2016–2020 NTD Master Plan lists the integration of NTDs into its primary healthcare (PHC) system as a strategic objective. In 2017, Malaria Consortium conducted a small-scale pilot and based on the results, is now running a larger scale pilot. This study aimed to develop and pilot an intervention to strengthen and integrate NTD detection, management and reporting into PHC in Ethiopia. The project consisted of formative and intervention stages; in the formative stage, key informant interviews with different stakeholders aimed to understand current case management of NTDs, the impact of COVID-19 on NTD services and elicit perceptions of future system requirements. Twenty interviews were conducted with community members, health extension workers, health workers and programme- and national-level stakeholders. A health system capacity assessment was conducted in a primary hospital, health centre and five health posts to assess health infrastructure gaps and identify requirements for NTD integration. Quantitative and qualitative results from the formative research informed the development of intervention materials and processes which were tested for 6 months across all levels of the health system. Results from both the formative research and intervention evaluation regarding feasibility, acceptability and implementation cost will be presented.

0792

PILOTING COVID-19 PREVENTION MEASURES DURING TRACHOMA SURVEYS IN THE DEMOCRATIC REPUBLIC OF CONGO

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Eye examination during trachoma surveys requires close person to person contact thus appropriate measures are needed to mitigate potential risk of COVID-19 spread. We report on methods and results piloting COVID-19 preventive measures during trachoma surveys in DRC. Before surveys, COVID-19 risk assessment of community spread was done and deemed to be low. Standard operating procedures (SOPs) were developed on COVID-19 prevention, including: PCR-based testing of surveyors for SARS-Cov2; daily COVID-19 symptoms check for surveyors; COVID-19 symptoms screen for survey participants; physical distancing during travel, training and fieldwork; face masks for surveyors and survey participants; integrated loupe-face shields (in addition to face masks) for graders; and gloves and alcohol hand sanitizing gel for graders. In a sub-sample of households, supervisors assessed compliance of preventive measures using a checklist of observations and interviewed household heads on acceptability of COVID-19 preventive measures. A focus group discussion (FGD) was done with surveyors to obtain feedback on COVID-19 preventive measures. A total of 79 households participated in the supervisor observations and household feedback. Compliance of COVID-19 preventive measures was high across 11 observations (range 87% to 100% of households). Majority of households agreed that: COVID-19 precautions were appropriate (100%); they felt safe, during survey (99%); and they would participate in survey again with same precautions (100%). Few households (5%) had concerns with potential risk of COVID-19. FGD found that integrated loupe/face shields: were easy to use and comfortable to wear; did not interfere with examination for trachoma signs; there were no difficulties disinfecting face shields between households; and the loupe/face shield assembly held firmly together. This pilot showed that the SOPs on COVID-19 prevention measures were practical and provided additional protection for both survey teams and communities. Based on this successful pilot, surveys were expanded to cover other areas of DRC where COVID-19 risk is deemed to be low.

0793

PRE-INTERVENTION PHASE IDENTIFICATION OF CHALLENGES AFFECTING UPTAKE OF ONCHOCERCIASIS AND LYMPHATIC FILARIASIS PREVENTIVE CHEMOTHERAPY AMONG PEOPLE WITH DISABILITIES IN EJIGBO AND ORIADE LOCAL GOVERNMENT AREA, OSUN STATE, NIGERIA

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Preventive chemotherapy using ivermectin and albendazole is the main thrust of controlling and eliminating onchocerciasis and lymphatic filariasis in Nigeria. This study evaluated factors affecting accessibility and uptake of these medicines among people with disabilities(PWDs) and selected community members in eight hard-to-reach areas of Ejigbo and Oriade Local Government Areas of Osun State, Nigeria. The study was conducted at the pre-intervention stage of the annual treatment. A total of two hundred and twenty (220) PWD's, community stakeholders, and key informants participated in Focus Group Discussions to determine their community needs. Problems identified at the pre-intervention stage are presented as follows; need to compile and update household registers by the CDDs to include a column for PWDs. Emphasis on training for community Health Educators and CDDs to increase sensitization and mobilization using appropriate IEC materials 67.3% (x^2 12.082, df 3, p 0.003), need to improve sensitization and advocacy visits to leaders of the PWDs groups to ensure inclusion 87%(x^2 02.041, df 6, p 0.042). Also, a subset 31% (x^2 23.115, df 3, p 0.000) of the persistent refusers of the medicines among the PWDs believed that the medicines are only suited for women and are of no use to them, while the remaining advocated for a special treatment day to cater for their peculiarities. In general, there is the need to increase the number of CDDs who will be engaged to follow up PWDs who may have missed treatment during the treatment rounds. There is a need for increased and concerted efforts targeted at treatment to suit the peculiarity of this marginalized group. This will ensure equitable access to health services for all without leaving anyone behind.

0794

GAPS OF NEGLECTED TROPICAL DISEASES KNOWLEDGE AFTER MASS DRUG ADMINISTRATION INTERRUPTION IN BURKINA FASO

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The burden of Neglected Tropical Diseases (NTDs) remains high in developing countries with over one billion people being at risk.Despite the joint efforts for NTDs control through high coverages mass drug administration campaigns and case management, several Sub-Saharan countries are failing to reach the target of NTDs elimination.Possible causes for the persistence of these diseases include socio-cultural factors,namely knowledge and attitudes.Innovative and collaborative approaches have been initiated to accelerate the elimination of selected prioritized NTDs in Burkina Faso, Mali and Niger through the Malaria/Neglected Tropical Diseases control project.We assessed the gaps of knowledge relative to NTDs among the children aged 9-14 years in Burkina Faso.An electronic lot quality assurance sampling survey (LQAS) was conducted in 2020 in 15 eligible districts for mass drug administration against at least one prioritized NTDs in Burkina Faso.Overall,266 participants children aged from 9 to 14 were interviewed in each district using standardized LQAS survey questionnaire.Soil-transmitted helminthiasis, trachoma, onchocerciasis schistosomiasis and lymphatic filariasis were known by 59.77%, 25.56%, 17.29%, 35.90% and 45.49% of the participants respectively.School and parents were the main sources of information about NTDs.The main symptomss of schistosomiasis and soil-transmitted helminthiasis was known by 87.97% and 84.96% of participants respectively.NTDs routes of transmission were known by 14.42%, 53.96%, 29.6%, 28.10%, and 13.04% of participants for schistosomiasis, soil-transmitted helminthiasis, trachoma, lymphatic filariasis and onchocerciasis, respectively.School-aged children had insufficient knowledge about NTDs particularly for the NTDs for which MDA was interrupted.There is a need to strengthen sensitization and improve schools' managers' involvement in NTDs control.The inclusion of NTDs in schools' curricula might be a winning approach.

0795

OVERCOMING COVID-19 THREAT TO CONDUCT LYMPHATIC FILARIASIS TRANSMISSION ASSESSMENT SURVEYS IN 36 HEALTH DISTRICTS IN CAMEROON

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Cameroon declared its first COVID-19 case on March 6, 2020. The national government then instated COVID-19 control measures, including mass gathering bans and school closures. Following the issuance of updated World Health Organization guidance on safe resumption of field-based NTD activities in July 2020, the National NTD Program (NTDP) undertook a risk assessment of NTD activities. Based on this assessment, the NTDP resumed NTD field activities. Here we describe the restart and implementation of the lymphatic filariasis transmission assessment survey 2 (TAS2) in 36 health districts (HDs), grouped into 13 evaluation units (EUs), in the context of the COVID-19 pandemic. The NTDP worked with partners to conduct a risk assessment and then developed standard operating procedures (SOP) for MDA and DSAs in the COVID-19 context, and also received support from partners for COVID-19 preventive measures, such as handwashing stations, bleach, water containers, soaps and hand sanitizers, alcohol, masks, and for production of social mobilization materials. Training sites selection considered larger space for physical distancing of 2 meters between participants. All supervisors, laboratory technicians and drivers were screened by COVID-19 test prior to traveling to the field for data collection. Three community mobilizers per cluster, instead of one, enforced social distancing and managed crowd control. Teams and participants were required to comply with systematic masks-wearing, handwashing before entering survey testing areas, use of a new pair of gloves for each child, survey-site access strictly reserved for enrolled children, and bleach to disinfect the survey testing area before and after teams' work. To mitigate rumors about testing or COVID-19, administrative, traditional, and religious authorities were involved in community sensitization. All 13 EUs achieved the minimum sample size required and all selected clusters were visited, indicating that the TAS2 was implemented successfully and that mitigation measures were accepted by the surveyors and the community.

0796

MASS DRUG ADMINISTRATION FOR SCHISTOSOMIASIS IN SIERRA LEONE IN THE CONTEXT OF COVID-19

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In 2008, baseline mapping for schistosomiasis (SCH) was conducted in 16 districts of Sierra Leone, where prevalence was found to be high (>50%) in five and moderate (between 10% and 50%) in four districts. Mass drug administration (MDA) with praziquantel (PZQ) started in 2009 and impact assessments in 2016 showed a reduction in overall prevalence from 42.2% to 20.4%, no district had high prevalence, and only 2.0% of infections were moderate or high intensity. MDA schedules were adjusted per World Health Organization (WHO) guidelines. In March 2020, WHO declared the COVID-19 pandemic and recommended postponing field-based NTD activities, like MDA. Preparations were already complete for the SCH MDA in nine districts, but the government postponed the campaign following the WHO announcement. The government limited public gatherings and closed all schools. The SCH MDA was deferred to October 2020, following WHO recommendations, and schools had re-opened. The MDA was modified to include a risk assessment, contingency plans and standard operational procedures. Trainings were held in smaller, socially distanced sessions, and included handwashing and mask wearing. Enhanced community engagement included the identification of negative influences on social media on MDA compliance and targeted 'push-backs' to build trust in the health sector. PICO videos were translated in five local languages to increase health worker and public awareness of the SCH campaign and COVID-19 safety. A supervisory checklist was adapted to capture compliance with COVID-19 prevention measures during the MDA. MDA data showed that 84.5% (678,929/803,437) of school-aged children received PZQ (range 76.1-97.5%). Impacts of COVID-19 on the MDA included initial delay, misinformation through social media even to remote communities and NTD staff reassignment to COVID-19 surveillance. Some parents were reluctant to send children to school or allow them in the MDA. Careful messaging emphasized safety measures and informed health workers and communities on adverse events so they were not mistaken for COVID-19 symptoms, which enabled effective coverage in all nine districts.

0797

CONTEXTUAL FACTORS ASSOCIATED WITH EPIDEMIOLOGICAL COVERAGE DURING MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS ACROSS TEN WEST AFRICAN COUNTRIES

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Effective mass drug administration (MDA) is the cornerstone of the elimination of lymphatic filariasis (LF) and a critical component in combatting all neglected tropical diseases (NTD) for which preventative chemotherapy is recommended. Although MDA coverage is the essential metric of any MDA's success, trends in coverage are rarely investigated systematically and informal anecdotes often blend assumptions with evidence. Coverage evaluation surveys can provide some explanation of coverage trends but have limited external validity and rely on questions the lay public can answer (e.g., a respondent's own behavior and demographics) leaving more macro-level trends unexplored. As a result, national NTD programs are only partially equipped to explain past MDA performance, be it high or low, and make informed programmatic decisions for the future. Here we present a regression analysis of reported epidemiological coverage (proportion of those at risk who are treated) from 3,875 district-level LF MDA campaigns across ten West African countries from January 2009-June 2021 (analysis ongoing). Coverage to date averaged 72.7% (24.5 – 121.0, SD 16.1) amongst 647 health districts, each having conducted between one and twelve (mean 5.9) rounds of LF MDA. Using a hierarchical linear model (HLM) to account for variation between districts and countries, we examined the relationship between each MDA's coverage rate and contextual factors relevant to its date and/or location including rainfall, temperature, road access, Ramadan, population density, reported social unrest, COVID-19, and the number of previous rounds of MDA. Key findings include an estimate of COVID-19's relationship to coverage as well as that of rainfall and temperature, both with implications for climate change.

0798

COMPLIANCE WITH COVID-19 PREVENTIVE AND BARRIER MEASURES DURING RECENT MDA AND DSA IN SIX ACT | WEST COUNTRIES

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In March 2020, the World Health Organization (WHO) declared COVID-19 a pandemic, prompting governments to implement measures, such as closing borders, mandating masks and limiting public gatherings. In July 2020, WHO issued new guidance recommending countries conduct a risk assessment and develop a risk mitigation plan prior to resuming neglected tropical disease (NTD) activities. Burkina Faso, Cameroon, Guinea, Mali, Niger and Sierra Leone restarted NTD activities with support from USAID's Act to End NTDs | West Program, following risk mitigation steps and donor approval. To monitor COVID-19 risk mitigation plan in the field, Act | West developed COVID-19 Supervisors Checklists for mass drug administration (MDA) and disease-specific assessments (DSA). Participating supervisors completed one MDA checklist per community drug distributer (CDD) team, observing their performance during a single interaction with MDA participants. For DSAs, supervisors completed one checklist per site. A total of 329 MDA checklists were completed. MDA strategies included door-to-door (282), fixed point (27) or school-based (18) distribution. CDDs wore masks (91.7%) more than MDA participants (48.1%). Handwashing or hand sanitizer was used by 71.4% of participants, and 94.6% of CDDs did not touch the participants when giving the drug. Physical distancing (two meters) between participants and CDDs was maintained in 84.1% of households. Willingness to participate in MDA was high among both participants (95.7%) and CDDs (97.3%). A total of 100 DSA checklists were completed. Masks were worn by 57.0% of participants and 99.0% of surveyors. All DSA sites had hand sanitizers and 98.0% had a handwashing facility with soap. Physical distancing was maintained in 92.9% of sites, and crowd control measures were implemented at 99.0% of sites. Results from both MDA and DSA checklists indicate compliance with COVID-19 preventive and barrier measures was high, except mask-wearing among participants. The willingness of the countries to adopt these measures indicates the commitment of the national governments and partners to safely continue the fight against NTDs.

0799

INVESTIGATION PRELIMINARY REPORT: RIFT VALLEY FEVER OUTBREAK IN FATICK, SENEGAL, 2020

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Located in central-western Senegal, Fatick Health District is a pastoralist area and covers 40 Facilities including *Ndiaye Ndiaye* from where an RVF case was confirmed when an outbreak is noted in a border country and an epizootic in the north of Senegal. Indeed we were alerted on 10-08-2020, during the 41st epidemiological week (EW). Thus we had to investigate immediately this RVF outbreak. A cross-sectional study, respecting ethical considerations and one health approach, was conducted and followed by a riposte from 10-29-2020 in Fatick. In fact, the data management needed: observation, literature reviews, interviews, rolling and picking logistics, multidisciplinary team, other investigation tools as Biogents Sentinel, laptops, etc. And this, from data collection to results presentation. The investigation found, according to Senegalese adapted WHO's case definitions, RVF outbreak in Fatick with already two human cases in 40th and 42nd EW, and one animal case in 44th EW. Among human cases, 19.8 percent consulted at the 42nd EW, 89 percent complained of headaches versus 34 percent of asthenia. The age group most affected was between 25 and 50 years old with women predominance, 38 percent were students, 32 percent housewives, 8 percent shopkeepers. Dakar and Touba for 31 percent were the most visited cities through travel in the last 15days and the most facilities were *Ndiaye Ndiaye* for 35 percent then *Peulga* for 20 percent versus *Emetteur* for 2 percent which was less visited. Regarding animal cases spread over 5 herds, 40 were sampled of which 35 percent were less than a year old, 67.5 percent females, 95 percent ovines, and 32.5 percent local breed. Finally, the entomological and environmental surveys showed 33 percent of vector species identified were competent, including *Culex quinquefasciatus*, 139 ticks collected from livestock, and an ecological environment favorable to RVF. The risk assessment placed Fatick in the orange zone. Results prove the importance of community-based surveillance and one health approach response to public health emergencies.

0800

EGYPTIAN FRUIT BATS ROUSETTUS AEGYPTIACUS ARE RESERVOIRS OF CCAMPYLOBACTER COLI EXPRESSING ANTIMICROBIAL RESISTANCE GENES

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Many infectious diseases in human including campylobacteriosis are directly attributed to wildlife sources at the human-animal interface and these poses increasing threat to public health. Interactions between humans and animals have also led to acquisition of antimicrobial resistance (AMR) genes in pathogens that are transmitted from animals to humans in shared environments. Emerging AMR from wildlife reservoirs especially bats is underestimated yet contributes to the pattern of antimicrobial resistance in nature. In this study, a total of 51 freshly voided bat guano were randomly collected in a zoological garden and analysed. The process was humane and without the need for invasive procedure after obtaining necessary permit from the park authority. Following microbial culture and isolation, 13 (25.5%) *Campylobacter* isolates were obtained. Molecular identification using specie specific PCR technique revealed all the strains obtained by isolation to be *Campylobacter coli*. The predominant extended spectrum beta-lactamase (ESBL) genes were bla_{CTXM} (31%) and bla_{SHV} (31%). Beta-lactam are commonly used antibiotics and resistance to these can hamper their effectiveness in chemotherapy of infectious diseases. This investigation determined the presence of *Campylobacter coli* and elucidate AMR genes in a colony of *Rousettus aegyptiacus* at a zoological park in Jos Nigeria. We showed that bats are carriers of zoonotic bacteria pathogens with antimicrobial resistant genes. The potential for human transmission including zoo workers and tourists through contact with feces, food contamination and aerosols in the environment is of public health concern. It is therefore important to implement interventions including enlightenment, hygiene and biosecurity at the human-wildlife interface especially in places where contact with bats is inevitable.

0801

USING A ONE HEALTH SYSTEMS ASSESSMENT TOOL TO STRENGTHEN ZOONOTIC DISEASE OUTBREAK RESPONSE IN LIBYA

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Communication and coordination among national disease surveillance and response networks are vital in ensuring the timely response to emerging infectious diseases. Libya, however, faces a number of challenges due to recurrent political and social conflicts that have weakened public and animal health systems infrastructure and disrupted rapid detection, surveillance and response efforts throughout the country. Our study aim is to determine whether resources to develop vertical programs can be leveraged to build sustainable One Health laboratory and surveillance networks for priority zoonoses. In collaboration with the Libyan National Centre for Disease Control (NCDC) and the National Centre for Animal Health (NCAH), we deployed our One Health Systems Assessment for Priority Zoonoses (OH-SAPZ) tool to map the existing laboratory and surveillance networks for detecting and reporting priority zoonotic diseases. The tool helps identify nodes of communication, coordination and decision-making where health and veterinary sectors intersect, as well as priorities and gaps that limit information-sharing for action. Working with officials at the national and subnational level, we selected five zoonoses as case studies to map Libya's current response capacity and determine overlaps and gaps in communication and coordination across the surveillance and laboratory sectors. Our major findings indicate a strong desire for and commitment to multi-sectoral coordination for zoonoses detection and response across public health and veterinary sectors. Nevertheless, rapid disease surveillance and detection in Libya is heavily impacted by the ongoing national security crises and the diminished workforce and resources available. Therefore, assessing The current coordination and communication between implicated health sectors can assist in determining areas for improvement and target actions. We anticipate this project will result in the formal creation of One Health rapid response teams composed of personnel from both NCDC and NCAH, responsible for coordinated management of zoonotic disease outbreaks in the country.

0802

ENVIRONMENTAL CONTAMINATION WITH FASCIOLA EGGS PASSED IN LIVESTOCK STOOL IS ASSOCIATED WITH HUMAN INFECTIONS IN THE ANTA PROVINCE OF CUSCO, PERU

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Fascioliasis is an emergent infectious disease but little is known about the dynamics transmission. We hypothesized that environmental

contamination with *Fasciola* eggs passed in livestock stool is associated with human fascioliasis. We analyzed data from a cross-sectional study among children aged 3-16 years old in 3 districts of the Anta Province in the Cusco region of Peru. Three stool specimens were collected from each child and livestock samples around the children's residence were collected and analyzed using microscopy for *Fasciola* eggs. Each sample was geographically tagged. A total of 2070 children were enrolled, 49.5% were female, the average age was 9.1 (± 3.5) years, and the *Fasciola* prevalence was 7.2%. The most common district of residence was Anta (50.8%). Stool from 2627 livestock was collected. *Fasciola* prevalence in livestock stool was 31.3% with the highest prevalence in the Ancahuasi (45.1%) and Anta (44.4%) districts. Donkey stool had the highest prevalence of *Fasciola* (60.4%), followed by sheep (40.31%) and cattle (32.2%). The logistic regression analysis showed no association between infected children in a residence and finding livestock stool with *Fasciola* eggs in a 50 m, 100 m, and 200 m radius. A significant association was found between having infected children in a residence and finding cattle stool infected with *Fasciola* in a 50 m (OR 1.46, 95%CI 1.12-1.9, p= 0.005), 100 m (OR 1.23, 95%CI 1.04-1.46, p= 0.018) and 200 m (OR 1.1, 95%CI 1.00-1.22, p= 0.041) radius around the residence. The median distance to a positive animal stool from a residence with infected children was 40.7 m (IQR=86.7) compared to 237.9 m (IQR=448.7) from a non-infected residence (p< 0.0001). The need for large-scale analysis to understand the dynamics of infection is imperative, as strategies for infection control are urgently needed.

0803

DETECTION OF THE SIMULTANEOUS PRESENCE OF SPECIFIC ANTIBODIES TO CAUSATIVE AGENTS OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME, CIRCOVIRUS INFECTION, TESCHEN DISEASE, AND AUJESZKY'S DISEASE IN WILD BOARS

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Wild pigs play an important role in the maintenance and circulation of viruses that affect domestic pigs. The distribution of viruses in wild pigs is thus an important One Health issue because the causative agent of Aujeszky's disease can infect humans (animal care staff). Using Elisa, we screened wild boar blood sera (6,840 samples) and detected the presence of antibodies to: Aujeszky's disease virus - 15.04%, Teschen disease virus - 19.94%, porcine circovirus infection (PCV type 2) - 31.51%, porcine reproductive and respiratory syndrome (PRRS) viruses - 2.38%. In 455 (6.68 %) blood sera, the simultaneous presence of antibodies to these diseases was detected in the following distribution: to two pathogens (Aujeszky's disease and Teschen disease - 119 (1.75%), Aujeszky's disease and PCV type 2 - 145 (2.13%), Aujeszky's disease and PRRS - 14 (0.21%), Teschen disease and PCV type 2 - 133 (1.95%), PCV type 2 and PRRS - 28 (0.41 %); simultaneously to three pathogens (Aujeszky's disease, Teschen disease and PCV type 2 - 7 (0.1%), Aujeszky's disease, PCV type 2, and PRRS - 2 (0.03 %). The obtained new serological data confirm the spread of these viral diseases in the wild boar population and do not exclude their associative course and may pose a threat to the pig industry and to the health of people.

0804

A ONE HEALTH PERSPECTIVE ON THE PREVALENCE OF PARALYTIC SHELLFISH TOXINS AND ITS IMPACT ON ALASKA NATIVE SUBSISTENCE

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Subsistence practices are imperative to many Alaska Natives, providing traditional and cultural meaning while serving societal and dietary needs. Potential threats to the security of subsistence foods such as shellfish includes the increased occurrences of harmful algal blooms (HABs) in southeast Alaska. The dinoflagellate genus *Alexandrium* produces saxitoxins which can bioaccumulate in shellfish, making the shellfish toxic to humans after consumption, a condition referred to as paralytic shellfish poisoning (PSP). A One Health perspective on this issue acknowledges the interconnectedness of Alaska Native health, shellfish, HABs, and climate change. The purpose of this study was to analyze biotoxin data collected from 2017-2019 by six tribal partners of the Southeast Alaska Tribal Ocean Research (SEATOR) group to establish paralytic shellfish toxin profiles for community locations. For the six locations included in the study, descriptive statistics were calculated and graphs showing trends in shellfish toxin levels over time were created. Receptor Binding Assay was used to detect paralytic shellfish toxins in samples from select locations. It was hypothesized that shellfish toxin levels would exceed the regulatory limit for safe consumption at 80µg/100 g in non-summer months in addition to the expected spikes in toxin levels in summer months. It was also hypothesized that toxin levels would vary across the six locations samples were collected from. A total of 786 shellfish samples from these sites were analyzed and it was found that 51.0% of samples above the regulatory limit were in non-summer months. The data also suggest the prevalence of biotoxins in shellfish differs across the six locations, with Petersburg having no samples above the limit while Juneau presented the highest overall toxin level with 40.9% of all samples above the limit. Identifying the prevalence of toxins in areas of shellfish harvest can help inform communities on the safety of this subsistence resource, identify potential prevention strategies, and protect Alaska Native health.

0805

A ONE HEALTH APPROACH TO DESCRIBE THE PREVALENCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS COLONIZING LIVESTOCK FARMERS, LIVESTOCK AND LIVESTOCK PRODUCTS IN SOUTHERN SRI LANKA

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pervasive multidrug-resistant pathogen which causes life-threatening infections in humans and animals. Based on genotypic and antibiotic susceptibility patterns, MRSA is classified as community-associated (CA), healthcare-associated (HA) and livestock-associated (LA). Being a zoonotic pathogen, LA-MRSA strains can be transmitted to humans via direct contact with animals, environmental contamination, and eating or handling contaminated livestock products. This study describes the *S. aureus* and MRSA colonization prevalence in livestock farmers, livestock and livestock products in the Southern Province of Sri Lanka using the One Health approach. From November 2020 to March 2021, nasal swabs were collected from farm personnel who had direct contact with livestock, as well as from randomly selected livestock (cattle, buffalo, pig, goat and poultry) in the respective farms. At least one livestock product (milk, meat or egg) was also collected from all farms. Standard microbiological testing and antibiotic susceptibility testing using Kirby Bauer disc diffusion were used to isolate *S. aureus* and identify MRSA. Nasal swabs were collected from 71 farmers, who were mostly male (65, 85%) and had a median age of 39 (21-63) years. A total of 22 (30%) farmers were colonized with *S. aureus.* Of them, 7 (9.8%) were colonized with MRSA. Nasal swabs were collected from 70 livestock animals (cattle-40%, pig-37%, goat-14% and paultry-9%). Of those, 19 (27.2%) were colonized with *S. aureus* (cattle-42%, goat-31% and pig-26%). None of the *S. aureus* isolates was identified as being MRSA. Among 35 livestock products tested (milk-60%, egg-25% and meat-14%), 10 (29%) showed the presence of *S. aureus* (milk-60% and meat-40%). MRSA was not isolated. MRSA was isolated from farmers, but not from the livestock and livestock products. However, the presence of *S. aureus* at a considerable level in livestock and livestock

products suggests the potential for transmission to humans. Continued surveillance of farmers and livestock is needed to detect whether LA-MRSA exists and to describe zoonotic transmission.

0806

HUMAN-ANIMAL INTERACTIONS TO ASSESS RISK OF EMERGING INFECTIOUS DISEASES IN KENEMA DISTRICT SIERRA LEONE: FINDINGS FROM A 2010 HOUSEHOLD SURVEY

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Most emerging infectious diseases have zoonotic origins. Understanding the relationships between human behavior and animal exposure in a given community is essential to understanding spillover events and taking steps to prevent them. In 2010 we conducted a pilot survey of 260 persons in Kenema District, Sierra Leone to characterize human-animal interactions. The population surveyed was a convenience sample of: Kenema town residents (n=100); three rural villages within close proximity to Kenema town (n=112); and purposively-selected hunters residing in surrounding areas (n=48). A total of 89% of participants reported someone in the household with a febrile illness in the past year. Participants reported a wide variety of animal interactions, including physical contact with rodents (93%), bats (23%), wild birds (75%), and monkeys (48%). Contact occurred both intentionally through farming and hunting and unintentionally through contact or bites from household pests. A total of 83% participants raised animals for food or sale. Behaviors and interactions varied by location, with animal exposures generally higher in rural villages than in Kenema town. For example, 80% of households in the rural villages reported the presence of bats in the household in the past year, compared to only 36% in Kenema town. Preparing or eating certain animals such as mongooses and wild birds was also more common in the villages (>95%) than in Kenema town (<50%). Hunting or opportunistic catching was reported by 43% of participants in the villages. Of particular interest was the high frequency of exposure to rodents, given Kenema District is endemic for the rodent-borne disease Lassa fever, and to bats, the putative reservoir of Ebola virus, considering that Kenema is within a few 100 kilometers of the sites where Bombali ebolavirus was first reported in 2018 and Ebola virus epidemics began in neighboring Guinea in 2013 and 2021. Pilot surveys such as this are relatively rapidly and inexpensively conducted and could help assess specific human-animal interactions to develop localized surveillance and prevention efforts.

0807

SEROPREVALENCE AGAINST VIRUS AND ZOONOTIC PARASITIC DISEASES IN SLOTHS OF WESTERN PANAMA

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Panama has played an important role in the discovery and control of emerging zoonotic diseases with impact on public health. These etiological agents involve different reservoirs with potential to cause outbreaks, such as sloths *Choloepus spp.* and *Bradypus spp.* involved in the transmission of parasitic agents *Trypanosoma cruzi, Trypanosoma rangeli, Leishmania panamensis*. Despite the isolation of *T. cruzi, T. rangeli* and arboviruses as *Oropouche, Punta Toro group virus (PTV), Utive virus* in the 1980s, the current status of these mammals and their role as a reservoir in Panama is unknown. This descriptive study aims to determine the current seroprevalence against arboviruses and parasites with epizootic potential in 50 sloths captured in rural areas of the province of West Panama endemic for Chagas, Leishmaniasis and Dengue. To detect neutralizing antibodies against arboviruses: *PTV, Madariaga (MADV), Mayaro (MAYV), Venezuelan Equine Encephalitis (VEEV), Una (UNAV BT 1495), Chikungunya (CHIKV), Yellow Fever (YFV), Dengue serotype 2 (DENV-2)* and *Pan Sloth 149* and *D50* viruses of genus *Orbivirus,* plaque neutralization assay as used. To determine the presence of antibodies against T.*cruzi, L. panamensis, T. rangeli*, Western Blot was used. If present, antibodies in sloth serum detect proteins of the parasites, and then were detected by a mouse secondary antibody anti sloth IgM and IgG, which in turn, binds to a commercial anti mouse antibody conjugated with peroxidase. As anti sloth antibody is not available commercially, we proceeded to immunize CFW mice using full immunoglobulin protein, the heavy chain and the light chain. Preliminary results indicate that 8% of the sloth sera have neutralizing antibodies for *VEEV,* while all were negative for *UNAV, MAYV, CHIKV*. The development of polyclonal mouse anti IgM and IgG antibodies from sloths was standardized, observing 100% recognition against both light and heavy chains. We aim to provide data on the presence of zoonotic viruses and parasites with emerging potential in sloths in the province of West Panama that is under increasing deforestation and urbanization, a risk factor for emergence.

0808

CAUSE OF ACUTE RESPIRATORY INFECTION IN NORTHERN LAOS

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With the advent of highly sensitive real-time PCR, a diversity of pathogens are identified from the nasopharyngeal swabs of patients with acute respiratory infections (ARIs). However, the detection of micro-organisms in the upper respiratory tract does not necessarily mean that they were the cause. We conducted a matched case-control study, nested in a large fever aetiology project, to facilitate the determination of the aetiology of ARI in hospitalised patients in northern Laos. Consenting febrile patients of any age admitted to Xiengkhuang Provincial Hospital were included in the case-control study if they had an ARI presentation (≥ one of: cough, rhinorrhoea, nasal congestion, sore throat, difficulty breathing or abnormal chest auscultation). One control for each included case, matched by sex, age and village of residence, was recruited within 2 weeks of patient enrolment if they did not have fever and ARI presentation. Nasopharyngeal swabs were collected from participants and tested for 33 pathogens by probe-based multiplex real-time (RT-)PCR (FastTrack Diagnostics Respiratory pathogen 33 kit). Attributable fraction of illness for a given microorganism was calculated by comparing results between patients and controls (=100 *[OR-1]/OR). Between 24th June 2019 and 24th June 2020, 205 ARI patients and 205 matching controls were recruited. After excluding 8 pairs due to age mismatch, 197 pairs remained in the analysis. Males were predominant with sex ratio 109:88 and children <5 years old accounted for 56% of participants. At least one potential pathogen was detected in 172 (87%) patients and 175(89%) controls. 17.8% of all ARI cases were attributable to influenza B, 17.2% to influenza A, 7.5% to human metapneumovirus, and 6.5% to respiratory syncytial virus (RSV). Determining aetiology of ARI remains challenging. Among hospitalised Lao ARI patients presenting at a provincial hospital in northern Laos, most were attributed to a viral cause, particularly influenza A, influenza B, human metapneumovirus, or

0809

RESPIRATORY SYNCYTIAL VIRUS-ASSOCIATED MORTALITY AMONG YOUNG INFANTS IN KARACHI, PAKISTAN: A PROSPECTIVE POST-MORTEM SURVEILLANCE STUDY

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Respiratory syncytial virus (RSV) is a significant cause of infant morbidity and mortality globally, and a potential target for maternal immunization strategies. However, data on the role of RSV in young infant deaths in developing countries is limited. We conducted a community-based mortality surveillance from August 2018 to March 2020 for infants ≤6 months old in Karachi, Pakistan. Nasopharyngeal swabs from deceased infants were tested (RT-PCR) for presence of RSV. We collected 490 nasopharyngeal specimens from 1,280 eligible infant deaths. Verbal autopsy was conducted to identify respiratory cases and controls. We calculated odds of RSV-associated mortality with 95% confidence intervals (CI) and used multivariable logistic regression to evaluate associations. There were 377/490 live births, of which 14 (3.7%) were RSV positive. Most deaths occurred at age 0-3 months (88.1%) or 0-28 days (67.4%), in the community (50.1%), and 64.3% (9/14) of RSV positive cases were captured during RSV high season. RSV infection was more likely to be detected among deceased infants with respiratory illness versus no respiratory illness (odds ratio (OR): 5.2; CI: 1.2 -23.7) and during high RSV season versus low RSV season (OR: 4.4, CI: 1.4-13.3). In the multivariable logistic regression analysis, having respiratory symptoms during fatal illness (OR: 6.6; CI: 1.3-32.5), RSV seasonality (OR: 6.1; CI: 1.8-20.4), and age (neonatal versus post-neonates) (OR: 9.2; CI: 2.6-33.1) were significant predictors of RSV-associated mortality. RSV has a significant mortality burden in early infancy, in Karachi, Pakistan. Age, RSV seasonality, and respiratory symptoms/illness during fatal disease were significant predictors of RSV-associated mortality in young infants.

0810

DETECTION OF RESPIRATORY PATHOGENS USING MULTIPLEX TAQMAN ARRAY CARD AMONG STILLBIRTHS AND UNDER-FIVE DEATHS IN EASTERN ETHIOPIA

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Respiratory infections are an important cause of morbidity and mortality in lower income countries like Ethiopia. The Child Health and Mortality Prevention Surveillance (CHAMPS) network has adapted a custom multiplex real-time PCR 384-well microfluidic array, TaqMan Array Cards (TACs), to identify multiple pathogenic microorganisms. The aim of this study is to identify potential respiratory pathogens in post-mortem samples among stillbirths and under-five deaths. Among children aged <5 years in Harar/Kersa, Eastern Ethiopia, we examined postmortem specimens of lung tissue (all deaths) and nasopharyngeal/oropharyngeal (NP/OP) swabs (deaths in liveborn children only). We used the TAC panel for detection of 46 respiratory pathogens. Total nucleic acid (DNA and RNA) were extracted using Qiagen EZ1 DSP Virus Kit and the TAC runs were performed using reverse transcriptase real-time PCR on the QuantStudio 7 Flex real-time PCR system. A total of 137 deaths were investigated; 73 (53%) were stillbirths. Among 64 deaths in live-born children, 50 (78%) lung tissue samples and 59 (92%) NP/OP swabs contained nucleic acid of one or more organisms; a total of 358 pathogen-specific target detections were identified from lung tissue (36%) and NP/OP swab (64%). These consisted of bacteria (81.6%), viruses (17%) and fungi (1.4%). Bacteria detected included *K. pneumoniae* (24%), *A. baumanii* (12%), *S. aureus* (9.2%), *M. catarrhalis* (7.8%) and *H. influenzae* (7.5%). Among viral detections, Rhinovirus (6.4%) was most commonly detected, followed by Human Cytomegalovirus (3.1%), and Measles virus (1.7%). Among stillbirths, 20% of lung tissue samples processed were positive for a pathogen; only 18 pathogen-specific targets were detected. Two-thirds (61%) of those detected were *A. baumanii,* Human Cytomegalovirus and *P. aeruginosa*. Bacteria were commonly detected as potential respiratory pathogens among post-mortem respiratory specimens from recently deceased children aged < 5 years. The TAC multi-pathogen detection platform provided an efficient laboratory method to detect a variety of infectious organisms.

0811

RESPIRATORY VIRUSES DETECTED AMONG SEVERE ACUTE RESPIRATORY INFECTION IN-HOSPITAL DEATH CASES DURING FIRST YEAR OF COVID-19 PANDEMIC IN BANGLADESH

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Severe acute respiratory infection (SARI) is one of the leading causes of morbidity and mortality globally with an extreme peak during this COVID-19 pandemic. We aim to describe the respiratory viral pathogens detected from SARI patients who died in-hospital. During March 2020 - February 2021, we collected surveillance data of SARI associated in-hospital death cases utilizing the national hospital-based influenza surveillance (HBIS) platform at nine tertiary level hospitals in Bangladesh. Surveillance physicians prospectively identified inpatients meeting WHO-SARI case definition. Oro- and nasopharyngeal swabs were collected from these patients and tested for a panel of respiratory viruses including influenza virus, SARS-CoV-2, respiratory syncytial virus (RSV), human metapneumovirus (HMPV), human parainfluenza viruses (HPIV 1,2,3) and adenoviruses by rRT-PCR. We conducted descriptive analyses to describe the proportions of different respiratory viral pathogens among these SARI deaths. There were 122 (5%) in-hospital deaths among 2,408 SARI inpatients. The median age of the SARI death cases was 55 years (IQR:1.5 - 65 years) and 71% of the death cases were male. Of them, 49 (40%) had respiratory viral etiology. The highest proportion of virus detection was for SARS-CoV-2 (25%, 31) followed by HPIV (2%, 3), influenza (2%, 2), RSV (2%, 2) and HMPV (2%, 2). Viral co-infections were detected in 9 (7%) death cases. Four death cases were co-infected with SARS-CoV-2 and adenovirus, 3 with HPIV and adenovirus, 1 with RSV and adenovirus and 1 with SARS-CoV-2, adenovirus and RSV. Fifty-one percent of the death cases (25/49) having a viral etiology had at least one co-morbid condition. Death cases aged ≥40 years were most commonly infected with SARS-CoV-2 (39%, 29/75). Respiratory viruses were detected in one-third of SARI inpatients who died in-hospital during first year of COVID-19 pandemic in Bangladesh. Highest rate of virus detection was due to SARS-CoV-2. Vaccination against SARS-CoV-2 and non-pharmaceutical interventions such as social distancing, hand hygiene and wearing face masks might contribute to reduction of SARI mortality.

0812

EASY ACCESS TO MODS CULTURE: EVALUATION OF DEHYDRATED MEDIA

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Tuberculosis (TB) is still one of the leading causes of death from infectious diseases in low- and middle-income countries (LMIC). Efficient diagnosis is crucial for TB control. Molecular tests show important advantages (i.e. short response time, and high sensitivity/specificity). However, they are not nationwide applied in LMIC. Culture-based methods remain a low-cost

option but with longer response times (≥24 days). The MODS culture is a low-cost assay with a mean response time of 10 days and a sensitivity/specificity of more than 90%. However, its implementation is limited in LMIC due to the low accessibility to culture supplies required for the enriched medium. Here, two dehydrated 7H9-OAD based media were evaluated: a powder-component blended media (BM) (not sterilized), and a sterile lyophilized liquid media (LM). Catalase, PANTA mixture, and gamma irradiation were evaluated for BM and LM variants. Culture performance was compared with the MODS standard medium (MM) using *M. tuberculosis* (MTB) isolates and 26 positive acid-fast smear sputum samples. Bacterial growth was reported as CFU/mL and growth-well percentage (GW%). Analysis with MTB isolates showed that catalase promotes bacterial growth regardless of the type of media. PANTA and gamma irradiation were evaluated to prevent culture contamination; however, both combined reduce bacterial growth significantly ($P < 0.05$) in all media variants. In contrast, gamma irradiation does not reduce bacterial growth. Overall, no important difference was observed in CFU/mL between the evaluated media and MM. Three variant media were selected for sputum samples screening (irradiated LM, non-irradiated BM, and irradiated BM). A median positivity day of 6 ± 5 days was observed, regardless of the type of media. The non-irradiated BM showed high contamination (30% samples). No significant difference ($P = 0.47$) was observed when GW% was compared between irradiated BM (100%), and LM (100%) with MM. In conclusion, the preliminary results show that these two variant media have a similar performance to the standard MODS medium, aiming their incorporation in a high-accessible MTB diagnostic kit.

0813

GLYCOSYLATED HEMOGLOBIN DYNAMICS PRE AND POST TUBERCULOSIS TREATMENT DIFFERENTIALLY IDENTIFY DIABETES RANGE GLYCEMIA IN DHAKA, BANGLADESH

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In recent non-pandemic periods, tuberculosis (TB) has been the leading killer worldwide from a single infectious disease. Patients with DM are three times more likely to develop active TB, have accelerated progression of TB disease and poor treatment outcomes. However, data are limited regarding glycemic dynamics from TB diagnosis through treatment and single glycemic measurements at treatment initiation may inaccurately diagnose DM or mischaracterize DM severity. We aimed to conduct a prospective study of glycemia dynamics in response to TB treatment measured point-of-care glycosylated hemoglobin (HbA1c) in patients presenting to TB screening centers in Dhaka, Bangladesh to determine the prevalence and risk factors of new DM prior to and at the end of TB treatment. Total of 429 adults with active TB infection were enrolled from October '18 to July '19 and divided into three groups based on initial HbA1c range: normoglycemic, prediabetes and DM group. DM was diagnosed in 158 (37%), of which 14% were newly diagnosed DM by HbA1c despite normal random blood sugar testing. At end of treatment, we found 14 (6%) additional patients from the normoglycemia and prediabetes groups had HbA1c level in the diabetic range &gt 6.5%, thus increasing the prevalence of DM to 39%. The number needed to screen to diagnose one new case of DM prior to TB treatment was 5.7 and was 16 when repeating at the end of treatment in the subgroup without DM at treatment initiation. Weight gain &gt % at end of treatment significantly increased the risk of diabetic range glycemia in patients who had normoglycemia or prediabetes at TB treatment initiation (OR 5.66, 95% CI 1.23-26.04, p&lt0.05). In conclusion, point-of-care HbA1c testing for undiagnosed DM prior to and at the end of TB treatment found a high prevalence of prediabetes and DM, including a proportion that was found only at the end of treatment and more commonly in people with higher percentage of weight gain. Further longitudinal research is needed to understand the effects of TB disease and treatment on insulin resistance, impacted immune pathways, and long-term DM complications.

0814

TUBERCULOSIS CARE SERVICES AMIDST THE EBOLA VIRUS DISEASE EPIDEMIC (2014-2015) IN LIBERIA: TIME SERIES-ANALYSES FOR 2013-2017

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Tuberculosis is a major public health problem in Liberia. To estimate the magnitude of the impact of the EVD outbreak and establish trends of TB care services in Liberia, we conducted an interrupted time-series analysis, using five years of routinely collected health information system data. We analyzed three TB care services indicators before, during, and after the EVD outbreak from January 2013 to December 2017. We used a segmented linear regression model to generate estimates and predictions for trends in services over three time periods. We found that the number of presumptive TB cases significantly declined at the start of the EVD outbreak with an estimated loss of 3222 cases (p= 0.014, 95%CI: -5691, -752). There was also an estimated loss of 709 cases per quarter post-EVD (p = 0.032, 95%CI: -1346, -71). The proportion of smear-positive to presumptive cases increased by 1.45% over the post-EVD period (p= 0.011, 95%CI: 0.38%, 2.5%). The proportion of treatment success to TB cases evaluated also increased significantly by 3.3% quarterly over the post-EVD period (p = 0.013, 95%CI: 0.82%, 5.79%). These findings suggest that the EVD outbreak (2014-2015) negatively affected TB care services with a decline in the number of presumptive cases. While the numbers of presumptive cases continue to decline, there has been an improvement in case ascertainment and successful treatment outcomes of TB cases evaluated. Health care delivery needs to be maintained in future outbreaks to sustain chronic infectious disease services, and the national TB control program needs newer strategies to maintain post-EVD progress and address current gaps in control activities.

0815

TUBERCULOSIS INCIDENCE AND ASSOCIATED FACTORS AMONG HOUSEHOLD CONTACTS OF TUBERCULOSIS PATIENTS IN THE 100 MILLION BRAZILIAN COHORT

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Using the '100 Million Brazilian Cohort', we estimated the incidence of Tuberculosis (TB) in a group of 92,153 household contacts of TB patients and investigated the factors related to this occurrence. The 100 Million Brazilian Cohort comprises individual socioeconomic and demographic data on over 114 million individuals and their applying for social benefits in Brazil (2001-2015) linked to nationwide mortality data (2001-2015) and TB registries (2006-2013). We selected new applicants to the cohort between January 1, 2006, and December 31, 2013. We defined the first case of TB in each household as the primary case. All household contacts were followed up from the date of diagnosis of the primary case until the detection of a subsequent case, the end of the cohort or death, whichever date comes first. Than we compared the demographic, socioeconomic and the primary case clinical characteristics of household contacts that did or did not developed TB subsequently to the exposure with a primary case using multilevel mixed-effects logistic regression model allowing for the municipality- and household-level random effects. We studied 92,153 household contacts of 32,504 primary cases. Household contacts were young (mean age= 18.8 years [sd=17.6] and woman (53.6%). During the eight years of follow-up (median=2.5 years), the incidence rate of TB among household contacts was 523.5 (95% CI=496.6-551.9) per 100,000 person-years at risk and 817.1 (95% CI=692.3-964.3) per 100,000 person-years at risk among children younger than 5-years. Sixty percent of cases were detected within one year after the detection of the primary case. Household contacts of primary cases with pulmonary TB had higher odds of developing the disease (OR=3.01; 95% CI=2.32-3.90). The high

incidence of TB among household contacts and the high number of cases detected after the first year of follow-up suggests the need to strengthen contacts tracing policies in Brazil that, along with chemoprophylaxis for latent TB, is key to reach TB control.

0816

THE IMPACT OF COVID-19 ON THE CIRCULATION OF RESPIRATORY VIRUSES AMONG UNITED STATES MILITARY BENEFICIARIES AT A MEDICAL TREATMENT FACILITY IN HAWAII

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Viral respiratory tract infections are a leading cause of morbidity worldwide. Hawaii implemented significant public health measures to include travel restrictions, mask-mandate, stay-at-home orders, and mandatory pre-travel testing to limit transmission, resulting in one of the lowest COVID-19 rates in the nation. This study aimed to evaluate the prevalence and etiology of respiratory viral infections during the ongoing COVID-19 pandemic among military health system beneficiaries on Oahu, HI and the impact of public health measures on viral transmission and circulation on the island. We retrospectively analyzed respiratory viral specimens collected from FilmArray BioFire or Cepheid GeneXpert Infinity tests from January 2020 to February 2021 at our institution. Patient demographic, specimen information, and encounter data were extracted from the electronic medical record. Descriptive statistics were calculated for the demographic characteristics and respiratory viruses detected. A total of 5,656 respiratory viral panels (BioFire and Cepheid) were performed during the study period with 983 positive specimens identified including 50 co-infections. Among the positive tests the most common pathogen detected was influenza A (40.1%), followed by rhinovirus (35.3%), influenza B (12.6%), adenovirus (6.3%), and RSV (6.1%). Viral distribution differed strongly with age. Circulation of respiratory viruses other than rhinovirus/enterovirus and adenovirus largely disappeared after SARS-CoV-2 onset and decreased activity continued throughout the study period. Public health mitigation measures, environmental persistence of rhinovirus/enterovirus and adenovirus, and viral interactions could all be drivers of this observation. The nature of how these viruses are transmitted, severity of disease, functional status of infected patients, and age of the population affected may have contribute to the distribution of pathogens identified. Further research is warranted to explore the prevalence, clinical presentation, and strain type among the respiratory viral pathogens and their impact on public health policies.

0817

"PEOPLE LISTEN MORE TO WHAT ACTORS SAY": A QUALITATIVE STUDY OF TUBERCULOSIS-RELATED KNOWLEDGE, BEHAVIORS, STIGMA, AND POTENTIAL INTERVENTIONS IN PUDUCHERRY, INDIA

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To capitalize on advances in tuberculosis (TB) diagnostics and therapeutics, and maximize the likelihood of the best TB outcomes, it is critical that people living with active TB (PLWATB) engage in care. One major challenge is stigma, which occurs when PLWATB experience discrimination or rejection due to their disease status; this can lead to delays in diagnosis and treatment. Understanding TB stigma is particularly important in India, which shoulders a quarter of the worldwide TB burden. We aimed to understand TB stigma (anticipated, enacted, and internalized) and collect views on potential interventions to reduce stigma from PLWATB, their household members (HHM), and other key stakeholders in Puducherry and Tamil Nadu, India. We conducted 47 in-depth interviews (IDI) with PLWATB (n=23) and their HHM (n=24), and eight focus group discussions (FGD): two each with PLWATB, HHM, healthcare workers, and key informants, such as community leaders and outreach workers. A total of 54 individuals participated in the FGDs. Overall, 38 (80.9%) of IDI participants reported misconceptions about TB transmission, though most (70.2%) were aware that TB is curable. Participants reported high levels of anticipated stigma; 59.6% of PLWATB chose to hide their disease to avoid stigma in their community. They also reported experiencing enacted stigma from community members (e.g. neighbors), employers, and healthcare workers (who were often described as "scolding"). Participants suggested many interventions, including celebrity advocacy (to raise awareness through social media) and school-based programs to increase community knowledge and reduce enacted stigma. They also supported interventions to reduce internalized stigma among PLWATB, including support groups and counseling. These findings have the potential to inform future interventions to reduce TB-related stigma in India, which could increase engagement in care and result in higher TB treatment success.

0818

SYMPTOMATIC GIARDIA LAMBLIA INFECTIONS IN NICARAGUA, EPIDEMIOLOGY AND IMPACT ON THE GROWTH OF YOUNG CHILDREN DURING THE FIRST THREE YEARS OF LIFE

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Giardia lamblia infections cause a high burden of disease in children in low- and middle-income countries. Between June 2017 and July 2018, we enrolled 444 infants from León, Nicaragua in a population-based birth cohort. Clinical and epidemiological, socioeconomic and anthropometric data were collected monthly, while household surveillance for acute gastroenteritis (AGE) was done weekly. Here we present preliminary data on analysis of *Giardia lamblia* during the first 36 months of life of 184 infants, including parasites detected by optical microscopy of wet mounts or qPCR. The one hundred and eighty-four children experienced 340 AGE episodes, where *G. lamblia* was detected in 25 (7.35%) AGE stool samples. Children with AGE episodes in which *G. lamblia* was detected were significantly less likely to receive care in primary care settings as compared to AGE episodes due to other etiologies (p=0.01, OR: 0.2, 95% CI: 0.07, 0.8), even when microscopy results were available to families. Similarly, children with *G. lamblia* were less likely to receive zinc supplementation (p=0.13, OR: 0.4, 95% CI: 0.1, 1.3). In the first year of life, *G. lamblia* was detected in 2.04% (4/196) of AGE stools. By age two, frequency of detection increased to 9.82% (11/112). While by age three, *G. lamblia* was detected in almost 1/3 of AGE stools 31.25% (10/32). The LAZ score decreased after 3 months (ΔLAZ: -0.40), at 2 years (ΔLAZ: -0.32) and 3 years (ΔLAZ: -0.84) after a *G. lamblia* episode. A similar pattern was observed for difference in WAZ after 3 months (ΔWAZ: -0.28), at 2 years (ΔWAZ: -0.32) and 3 years (ΔWAZ: -0.13). Comparing *Giardia lamblia and* other AGE etiologies*,* Reduction in WAZ at 3 months post-episode greater in children with *Giardia Lamblia* as compared to children AGE of another etiology (p=0.021). In summary, we observed a negative impact on ΔLAZ and ΔWAZ after an AGE episode associated with *Giardia lamblia*.

0819

CONTRIBUTION TO THE EPIDEMIOLOGICAL STUDY OF TOXOPLASMOSIS IN BEEF HANDLERS IN SLAUGHTERHOUSES ON THE ATLANTIC COAST OF COLOMBIA

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Toxoplasma gondii infections are very common in humans and food animals. Raw beef may contain viable T. gondii tissue cysts and therefore handling them may pose a risk of T. gondii infection to slaughterhouse workers. 686 serological tests were carried out that represented the universe of the existing population in 16 municipal slaughterhouses belonging to the seven departments of the Atlantic coast of Colombia, with the aim of determining the prevalence of reactors for T. gondii, using indirect hemagglutination technique (IHA) and determine the relationship between the seroreaction found with the degree of risk and the work time of the handler in the slaughter process. The serological study revealed a general prevalence of 77.69% (range: 66.3 - 99-6%) that exceeds the figures of 40.0 - 50.0% indicated for humans worldwide. In particular, prevalences were found according to departments, corresponding to: Córdoba (78.9%), Sucre (83.1%), Bolívar (78.9%), Atlántico (0.8%), Magdalena (70.7%), Cesar (75.0%) and Guajira (74.3%). The interpretation of the serological reaction showed a trend for high titers (greater than and equal to 1: 1280) (33.8%) and lower values for intermediate titers (Range: 1: 320 - 1: 640) (27.8%) and low (Range: 1:80 - 1: 160) (16.0%). The relationship of seropositivity for working times of less than 4 years, 4 -8 years and older than 8 years, did not show statistical significance, unlike the marked significance ($p < 0.05$) for the seroreaction relationship - risk grade III (viscera wash). The prevalence found, when relating it in a large proportion to handlers with high-risk trades and with titles equal to or greater than 1: 1280, added to the unwanted hygienic-locative conditions, the lack of education, little professional supervision and inadequate protection of operators, requires epidemiological surveillance for the population studied.

0820

A FIRST REPORT ON EXPERIENCE IN MANAGING INFANTS WITH CONGENITAL TOXOPLASMOSIS IN ETHIOPIA: A REVIEW OF EVALUATION AND TREATMENT IN RESOURCE-LIMITED SETTINGS

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Congenital toxoplasmosis is a major sequela of untreated primary maternal infection. With or without symptoms, untreated infections eventually lead to multiple neurologic complications. Despite the high *Toxoplasma gondii* seroprevalence in the Ethiopian population, there are no reports of newborns diagnosed and treated for congenital toxoplasmosis. The clinical presentation, evaluation, and management of three infants diagnosed with congenital toxoplasmosis in our clinic are described. Two were symptomatic at birth. All three had confirmed diagnoses using *Toxoplasma* serologic tests. Two completed their treatment with one infant developing complications of strabismus and seizure disorder. There is little experience in managing congenital toxoplasmosis in Ethiopia due to constraints in diagnostics and therapy. The description of this first such report underscores the need for risk assessment and evaluation during antenatal care to obtain favorable fetal outcomes.

0821

MULTIPLEX ANTIBODY BEAD ASSAY DETECTS EARLY AND PERSISTENT CHRONIC BABESIA MICROTI INFECTIONS IN BLOOD DONORS

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Babesia microti, a major causative agent of human babesiosis, represents an emerging public health concern in United States and globally. In the US, the number of reported cases of babesiosis nearly doubled between 2011 and 2018. Our goal was to develop a test to effectively detect window period cases (prior to seroconversion) and persistent chronic infections. To this end, we developed a multiplex assay using the Luminex bead-based platform for detection of antibody specific for four immunodominant *B. microti* antigens that we identified previously—BmSERA, BmPiβS, BmMCFRP1, and BmBAHCS. Assay sensitivity and specificity were determined in plasma samples collected from blood donors as part of an investigational study by the American Red Cross. Plasma samples were stratified based on the results of *B. microti* detection assays (IFA and PCR). BmBAHCS was the most sensitive antigen in detecting *B. microti*-specific antibody in donor plasma samples tested so far. BmBAHCS-coated beads (single antigen) yielded positive results with 98% (54/55) of IFA+ samples. Multiple-antigen coated beads (all four antigens) were less sensitive compared to IFA, detecting 87% (151/174) of IFA+ samples. On the other hand, multiple antigen-coated beads detected 46% (6/13) of PCR+ window period cases that were not detected by IFA, whereas BmBAHCS-coated beads detected 16.7% (2/12) of window period cases. Thus, our single antigen assay is comparable to IFA in identifying *B. microti*-exposed blood donors, and the four antigen combination improves detection of early antibody responses undetected by IFA. Additional development is required, but this test could potentially be adapted as a high throughput multiplex antibody bead assay to identify *B. microti* exposed individuals in endemic areas.

0822

UNEXPECTED CONSEQUENCES OF A PANDEMIC ON CRYPTOSPORIDIOSIS INFECTIONS IN NEW YORK STATE

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Cryptosporidiosis is an infectious disease transmitted through the fecal oral route and is caused by the pathogenic parasite, *Cryptosporidium*. *Cryptosporidium* infections can be contracted by drinking or contact with contaminated water, eating contaminated food, or through fomites. We examined whether novel Coronavirus 2020 guidelines of decreased human contact and social gatherings might have impacted cryptosporidiosis infections. Since most *Cryptosporidium* specimens are submitted during the summer months when travel, gatherings and outdoor activities are highest we focused on cases received between May and September. The *Cryptosporidium* species was determined for specimens that were PCR positive. Results showed that *C. parvum* infections, which are mainly acquired through agricultural influences, slightly decreased between 2019 and 2020 but were not significantly different. However, there was a significant decrease in *C. hominis* infections ($p<0.0053$) which are typically spread from human-to-human, and a significant increase in infections from *Cryptosporidium* sp. found in the environment ($p<0.0001$). For infections caused by species other than *C. parvum* and *C. hominis*, the most common was *Cryptosporidium* chipmunk genotype for both 2019 and 2020, with 7 and 29 cases respectively. Other identified species include *C. ubiquitum*, *C. felis*, and *Cryptosporidium* skunk genotype. When demographics of patients were examined for both years, spatial and temporal information suggests that human-to-human transmission of *Cryptosporidium* sp. typically found in the environment was unlikely. This

data suggests that the 2020 guidelines for decreased human interaction likely contributed to the decreased human-to-human cryptosporidiosis infections.

0823

THE EFFECT OF TEMPERATURE ON PERSISTENCE OF A SARS-COV-2 SURROGATE ON MEAT PRODUCTS

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COVID-19, the disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was declared a pandemic by the World Health Organization (WHO) in March 2020. Multiple pathways of SARS-CoV-2 transmission have been examined and the role of contaminated foods as a source of SARS-CoV-2 exposure has been suggested. As many cases of SARS-CoV-2 have been linked to meat processing plants, it may be that slaughterhouse workers or meat processing plant procedures transfer viral particles during meat processing, storage or transport. Because of the potential for contamination of foods such as fish, meat and poultry, the goal of this study was to evaluate the survival of a lipid enveloped RNA bacteriophage, phi 6, as a surrogate for SARS-CoV-2 survival under various meat and fish cold storage conditions over 60 days.

0824

FECAL CONTAMINATION IN CHILD PLAY SPACES AND ON CHILD HANDS IS ASSOCIATED WITH SUBSEQUENT ADVERSE CHILD DEVELOPMENTAL OUTCOMES IN RURAL DEMOCRATIC REPUBLIC OF THE CONGO (REDUCE PROGRAM)

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The objective of the Reducing Enteropathy, Undernutrition, and Contamination in the Environment (REDUCE) program is to identify exposure pathways to fecal pathogens that are significant contributors to morbidity among young children in the Democratic Republic of the Congo (DRC), and on developing and evaluating scalable interventions to reduce fecal contamination from these pathways. This prospective cohort study of 270 children under 5 years of age was conducted in rural South Kivu, DRC to investigate the association between *Escherichia coli* in hand rinse, soil, food, object, surface, stored-water, and water-source samples and child developmental outcomes. Child developmental outcomes were assessed by communication, fine motor, gross motor, personal social, problem solving, and combined scores measured by the Extended Ages and Stages Questionnaire (EASQ) at a 6 month follow-up. Children with E.coli present in the soil in their play spaces had significantly lower combined EASQ z scores (coefficient: -0.38 (95% Confidence Intervals (CI): -0.73, -0.03)). E.coli on children's hands was associated with lower communication EASQ z scores (-0.37 (95% CI: -.0.10, -0.01), and E.coli in stored drinking water was associated with lower gross motor EASQ z scores (-0.40 (95% CI: -0.68, -0.12). In the REDUCE cohort study, E.coli in child play spaces, on children's hands, and in stored-drinking water was associated with lower developmental outcomes (communication, gross motor, fine motor, and problem solving skills). These results demonstrate the need for interventions to reduce fecal contamination in the household environment to protect the health of susceptible pediatric populations in rural DRC.

0825

ACCESS TO WATER SANITATION AND HYGIENE FACILITIES AT PRIMARY HEALTH CARE CENTERS IMPACTING MOTHER'S PARTICIPATION IN A GROUP SESSION: EXPERIENCES FROM RINEW-G STUDY IN BANGLADESH

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There are many interrelated ways to improve child cognitive development and growth such as good nutrition, clean home environment, and parent's interacting positively with their children. We integrated maternal and child nutrition, maternal mental health, water, sanitation, hygiene and reduction of lead exposure with child stimulation to learn about the best ways to deliver the intervention package. The intervention was delivered from mid 2019 by the government frontline health workers in Chatmohar sub district of northern central Bangladesh. This package offered 24 sessions to the mother-baby group and four sessions to pregnant women. Sessions were delivered by the health workers in the sub-district health complex, union health and family welfare centers and community clinics. We conducted 12 interviews and four focus groups with pregnant and lactating mothers and explored the facilitators and difficulties to participate in the group sessions. We also conducted spot checks to examine WASH situation in all (n=44) health care facilities. Mothers reported that due to lack of functional toilets for visitors' use, after attending 1 or 2 sessions, many of them did not attend any subsequent sessions. Lack of water for both drinking and toilet use was another reason for them to leave the sessions earlier. Mothers faced problems with cleaning up if their babies defecated or vomited during the sessions. Spot checks revealed that 84% of the facilities had functional toilets but only for staff use, only 30% had drinking water facility, and 20% had handwashing provision with soap and water. Findings suggested that, improved water, sanitation and hygiene facilities is one of the important pre-conditions to increase mothers' participations in the group sessions in the health care centers. Improvement of WASH facilities could increase participation and acceptance when integrated intervention or other interventions delivered through group sessions.

0826

EPIDEMIOLOGIC FEATURES OF ACUTE PEDIATRIC DIARRHEA IN MANAGUA, NICARAGUA FROM 2011 TO 2019

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Diarrhea remains a leading cause of death in children in low- and middle-income countries, including Nicaragua, but little is known about diarrhea incidence in cohort settings in Central America. The purpose of this study was to determine the incidence, risk factors, long-term trends, and seasonality of diarrhea in children aged 2-14 years old in Managua, Nicaragua, as the population underwent a demographic transition. From 2011 to 2019, we examined medically attended diarrheal episodes among 6,485 children who participated in a long-term prospective cohort study and presented for care in the study primary care facility. We performed a longitudinal analysis considering time-varying variables and intra-subject correlation. In addition, we analyzed the weekly incidence of diarrhea, applying seasonal trend decomposition to extract secular and seasonal patterns. The overall incidence rate of medically attended diarrhea was 156 per 1,000 (95% CI: 152 - 161). We observed a slight increase in the

incidence of diarrhea between 2011 and 2019. Age was the strongest predictor of the risk of diarrhea, and the incidence increased with every additional hour without running water supply per day in the household. Diarrhea in Managua behaved seasonally, with high peaks each year between May and July. Despite reductions in childhood mortality since 1990 in Nicaragua, diarrheal disease remains a major problem in Managua.

0827

FECAL CONTAMINATION ON THE HOUSEHOLD COMPOUND AND IN WATER SOURCES ARE ASSOCIATED WITH SUBSEQUENT DIARRHEA IN YOUNG CHILDREN IN URBAN BANGLADESH (CHOBI7 PROGRAM)

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A prospective cohort study was conducted to investigate the environmental and individual-level risk factors for diarrheal disease among 884 children under 5 years of age in slum areas of Dhaka, Bangladesh. Caregiver reports were collected on sociodemographic factors and hygiene behaviors. Diarrhea surveillance data was collected monthly based on caregiver-reported diarrhea for children in the past two weeks during the 12-month study period. Unannounced spot checks of the household compound were performed at 1, 3, 6, 9 and 12 months after enrollment to check for the presence of feces (animal or human), presence of animals in the child's sleeping space, assess child and caregiver hands for the presence of dirt, and to collect samples of the household's source and stored drinking water. Children with feces found on the household compound during spot checks had a significantly higher odds of diarrhea (Odds Ratio: 1.71; 95% Confidence Interval: 1.23, 2.38). Children residing in households with >100 colony forming units (CFU)/ 100 mL *E. coli* in source drinking water had a significantly higher odds of diarrhea (OR: 1.43; 95% CI: 1.06, 1.92). The presence of feces on the household compound and source drinking water with >100 CFU/ 100 mL *E. coli* were significant risk factors for diarrheal disease for children <5 years of age in slum areas of Dhaka, Bangladesh. These findings demonstrate the urgent need for comprehensive interventions to reduce fecal contamination on the household compound to protect the health of susceptible pediatric populations.

0828

EFFECTS OF A SCHOOL WATER, SANITATION, AND HYGIENE INTERVENTION ON CHILDREN'S NUTRITION, HYDRATION, HEALTH LITERACY, AND HANDWASHING: A MULTI-ARM CLUSTER-RANDOMIZED CONTROLLED TRIAL IN MANILA, PHILIPPINES

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Inadequate water, sanitation, and hygiene (WaSH) threatens the health of millions of children globally. Urban poor children suffer from disease due to inadequate school WaSH facilities and health education programs. But evidence is limited about which types of interventions are most effective in improving children's health in tropical megacities. Our study aimed to evaluate the impacts of a school-based WaSH intervention on children's nutrition, hydration, hygiene-related health literacy (HL), and handwashing (HW) in Metro Manila, Philippines. We conducted a cluster-randomized controlled trial in 15 public schools, assigning two schools to the control group (CG) and randomizing 13 schools to 1 of 3 intervention groups (IGs), IGA, IGB, and IGC, which received low-, medium-, and high-intensity health education, respectively. The intervention consisted of WaSH workshops for teachers, health education for children, hygiene supplies, and WaSH facilities repairs. We measured nutrition status via anthropometry; dehydration via urine test strips; HL via questionnaire; HW via observation. Our sample was 756 and 701 children at baseline and end-line (8 months later), respectively (retention rate ~93%). We used intention-to-treat analysis. At end-line, compared to the CG, IGA and IGC had significantly lower odds ratios (OR) for obesity and overweight, respectively. The intervention had no significant effects on stunting or thinness prevalence. Compared to the CG, IGB and IGC had significantly lower risks of dehydration. Compared to the CG, IGB had a greater knowledge of using HW to prevent infection. IGC had a significantly higher OR for observed HW after using the toilet/urinal. We conclude that the intervention reduced the risks of over-nutrition and dehydration and improved HL and HW. But focusing only on schools without addressing household WaSH may not be enough to reduce stunting and thinness risks. We are currently conducting sensitivity analyses and will present those results during the conference. Findings from our trial may be helpful for future WaSH interventions aimed at improving children's health in other tropical megacities.

0829

HOSPITALS IN DHAKA AND DISCHARGE OF WASTEWATER WITH HIGH SARS-COV-2 VIRAL RNA GENETIC LOADINGS: AN ASSESSMENT OF POTENTIAL ENVIRONMENTAL HEALTH RISK

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Hospitals in low-and middle-income countries are frequently the center of epidemics and emerging infectious diseases. Significant health hazards result due to lack of systematic monitoring of wastewater (WW) discharges from hospitals containing a significant load of pathogens. We conducted a cross-sectional study to explore the WW management in Dhaka hospitals and analyzed the genetic materials of SARS-CoV-2 viral RNA discharged with the hospital WWs to the environment during COVID-19 pandemic between September 2020-January 2021. We selected 4 COVID and 2 non-COVID hospitals and conducted spot checks of the sanitation systems to explore availability of WW treatment facilities, and containments and collected 90 WW effluent samples (68 from COVID and 22 from non-COVID hospitals) and analyzed them for SARS-CoV-2 viral RNA gene copies using RT-PCR. Among 6 hospitals, no hospital had a WW treatment facility; 2 hospitals (1 COVID and 1 non-COVID) had containments (anaerobic baffled reactor); and 3 (2 COVID and 1 non-COVID) discharged WW effluents directly to environment. Average number of toilet users were 972/day (range:500-2272) in COVID and 3268/day (range:3063-3472) in non-COVID hospitals. All samples were positive with *E.coli,* with mean $\log_{10}$ concentrations=7.1/100mL. Overall, 67%(n/N=60/90) of samples were positive with SARS-CoV-2 viral RNA [66%(45/68) in COVID and 68%(15/22) in non-COVID] in selected hospitals. In COVID hospitals, highest SARS-CoV-2 viral RNA copies (18214 copies/mL; range:13-18214 copies/mL) were detected, and in non-COVID hospital 1829 copies/mL (range:30-1829 copies/mL) were detected from the WW effluents. The prevalence of the genetic materials of SARS-CoV-2 in both hospitals are however independent of the number of admitted

patients. High concentrations of *E.coli* and genetic loading of SARS-CoV-2 viral RNA were discharging through the hospital WW without treatment in the ambient water bodies of Dhaka, contributing to significant health hazards to neighboring communities. Appropriate technologies should be adopted by the hospitals for WW treatment to reduce growing health risk to the environment.

0830

"NON-PSEUDOMONAS" SWIMMERS' EAR, A CONDITION UNFAMILIAR TO MOST CLINICIANS

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Most cases of swimmers' ear are due to exposure to *Pseudomonas aeruginosa* found in fresh water. But other environmental, fresh or brackish water and salt water associated organisms may be responsible for some cases. Multiple species of salt water vibrio's (Vibrio alginolyticus, V. cholerae, V. fluvialis, V. cincinnatiensis, V. parahaemolyticus), fresh or brackish water *Aeromonas* species (Aeromonas caviae, Aeromonas hydrophila, Aeromonas veronii, Aeromonas sp.) and *Achromobacter* are the most frequently reported "non-Pseudomonas" species. Most reports of vibrio caused otitis externa are from coastal areas. All the reported swimmers' ear bacteria appear to be motile. In a study of clinical cases of infections due to *Achromobacter xylosoxidans* and *Stenotrophomonas maltophilia, ,*we found 23 cases of ear infections due to these 2 environmental organisms. Age range was 4-87 with 14 males and 10 females. Ten were children. Some had started swimming lessons recently. Most otitis externa was treated with ciprofloxacin ear drops. Antibiotic susceptibilities of these 2 organisms differ significantly from *Pseudomonas*, vibrio's and *Aeromonas* that are usually susceptible to quinolones, aminoglycosides, and other anti-Pseudomonas agents. Only 25% *Achromobacter* isolates were susceptible to ciprofloxacin with very poor susceptibility to gentamicin (5%) and aztreonam (1%). Most active drug for Achromobacter was meropenem (95%). All *Stenotrophomonas* were susceptible to trimethoprim/sulphamethoxazole. Patients with persistent or recurrent infections would benefit from antibiotic changes based on susceptibilities.

0831

BIOACTIVITY OF FOUR NIGERIAN WILD MUSHROOMS AGAINST SOME TYPED CLINICAL ISOLATES

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This study aimed at investigating the antimicrobial activity of some wild Nigerian mushrooms against selected typed clinical isolates. We collected wild mushrooms from an integrated organic farm in Ilesa, Southwest Nigeria. Crude methanolic extract of *Lentinus squarrosulus* Mont., *Termitomyces robustus* (Beeli) R. Heim, *Trametes ochracea* (Pers.) Gilb. & Ryvarden and *Xylaria hypoxylon* (L.) Grev. were screened singly and in different combinations for bioactivity against the selected bacterial and yeast isolates. The minimum inhibitory concentration (MIC) and chemical constituents of the extracts were studied following standard procedures. Overall, we obtained a total of 16 mushrooms belonging to 14 genera. The extracts showed varied clearance zones against at least one of the eight bacteria, and one yeast isolates tested when applied singly with the antimicrobial inhibitory zone ranging from 7.2 mm to 20.0 mm in *Staphylococcus aureus* (*T. ochracea* extract) and *Pseudomonas aeruginosa* (*L. squarrosulus* extract) respectively. Furthermore, the MIC ranged from 2.09 to 16.75 mg/mL. When combined, the blends were active against some Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus mirabilis*) but inactive against the Gram-positive bacteria and yeast. Except for *X. hypoxylon*, other extracts contained saponins, tannins and terpenoids. Our findings revealed that the wild mushrooms are potential antimicrobial agents against the tested isolates. Further development of the bioactive compounds into antimicrobial drugs is advocated.

0832

CORE MICROBIOME OF MALNOURISHED CHILDREN WITH ENVIRONMENTAL ENTERIC DYSFUNCTION

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Environmental Enteric Dysfunction (EED) is a subclinical acquired syndrome of altered gut function and poor absorptive capacity whose etiology is unknown. EED is postulated to be an important contributor to linear growth faltering in early childhood. The role of upper gastrointestinal (GI) bacterial communities in the pathophysiology of EED is poorly defined due to a paucity of studies where there has been direct collection of proximal small intestinal biological samples from undernourished children. In a two-year longitudinal follow-up study, we obtained duodenal aspirates and fecal samples from a subset of undernourished children (n=43) who underwent esophagogastroduodenoscopy for growth failure refractory to nutritional interventions. We analyzed duodenal and fecal microbiota composition from cases and fecal microbiota from age-matched healthy controls by performing amplicon sequencing on the V4-16S rRNA gene. All reads were processed using DADA2 and analyzed using the Bioconductor software packages 'Microbiome' and 'phyloseq'. We discovered a 'core' group of eight upper-intestinal bacterial amplicon sequence variants (ASVs) that were detected at >0.01% relative abundance in at least 80% of the aspirate samples, with *Streptococcus angionosus* being the most prevalent, followed by *Gemella haemolysans* and other Streptococcus species. A group of 12 bacterial ASVs with relative abundances >0.01% were detected in >50% of all fecal samples from both cases and healthy growing children. The top three taxa were *Escherichia-Shigella* sp., *Bifidobacterium breve* and a *Streptococcus* sp. In age-matched fecal specimens sampled monthly from healthy controls, we detected a high abundance of Bifidobacterium species in greater than 90% of samples. These observations provide a starting point for follow-up studies designed to examine the contributions of the small intestinal microbiota more directly to the pathogenesis of EED, including analyses that correlate the absolute abundances of the shared ('core') group taxa identified in the duodenal microbiota of subjects with EED with features of gene expression in duodenal mucosa.

0833

THE CRUCIAL ROLE OF ECONOMIC EVALUATIONS TO ASSESS THE IMPACT OF WATER, SANITATION AND HYGIENE ON REDUCING SCHISTOSOMIASIS BURDEN IN UGANDA

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Schistosomiasis is a debilitating disease caused by parasitic worms with over 240 million people infected. Individuals acquire infections when contacting contaminated fresh water through daily activities, such as fishing or bathing, and transmit it in areas with inadequate sanitation. This is the case in Uganda where, after a decade of mass drug administration (MDA), mean infection intensity is higher than at baseline in high endemic

areas. The WHO estimates that the potential reduction in schistosomiasis burden due to improvements in water, sanitation and hygiene (WASH) can reach 60-70%, and that they could hold the key to eliminating the disease.A recent discrete choice experiment in Uganda informed about the popularity of different WASH practices, infrastructure improvements and behavioural changes. We are using these data, combined with primary data from previous studies, to assess which combination of WASH interventions, on top of bi-annual MDA, is the most cost-effective to reduce schistosomiasis transmission in Mayuge (Uganda). We are building a Markov model to characterise the risk of schistosomiasis infection among the population of Mayuge during their lifetime, and follow a societal perspective to calculate the expected lifetime costs and benefits associated with each intervention. The WASH interventions aim at: (1) improving sanitation and reducing infection risk to others, such as public latrines and open defaecation fines; and (2) improving water access and reducing infection risk to oneself, mainly water sources for domestic chores and/or drinking and water access points. We will, then, compare them and calculate incremental cost-effectiveness ratios, as the 'extra cost per disability-adjusted life-year (DALY) averted', to decide which combination of interventions is the most cost-effective in these settings. This project will improve our understanding of WASH and will inform on the most cost-effective interventions needed to achieve schistosomiasis elimination. It focuses on using novel mathematical modelling tools to inform policy-makers on popular and sustainable WASH to complement MDA.

0834

WORLD HEALTH ORGANIZATION - HOUSEHOLD WATER TREATMENT TECHNOLOGY AND MICROBIAL LOG-REDUCTION VALUES

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Practitioners and researchers use household water treatment technology microbial removal ratings as a key tool and benchmark to assess innovative technologies in low-and-middle income countries. The current World Health Organization Drinking Water Quality Guidelines includes a log-reduction value (LRV) table based on bacteria, virus, and protozoa categories by household water technologies. Our team worked closely with WHO to update the current table to be included in the next (i.e., fifth) edition. We implemented a systematic, replicable, peer-literature review. Literature searches were conducted from 1997-2021 using a standardized search string query for target databases. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) best practices were followed for the review. Subject-matter experts were gathered in December 2020 to assess missing literature and discuss reported LRVs. Preliminary results included mean LRVs and 95% confidence intervals [CI] across bacteria (B), virus (V), and protozoa (P) categories for chlorination (n=21; B= 3.3 [2.5—4.1], V= 3.1 [1.9—4.2]), filtration (n=20; B= 3.5 [2.5—4.4], V= 2.9 [1.9—3.8], P= 3.0 [0.40—5.6]), coagulation/disinfection (n=6; B= 7.4 [6.5—8.4], V= 3.3 [-1.3—7.9], P= 3.1 [2.2—4.1]), solar (n=54; B= 4.2 [3.7—4.7], V= 2.9 [2.2—5.6], P= 3.3 [2.2—4.4]), UV (n=19; B= 4.3 [3.3—5.2], V= 2.7 [2.0—3.3], P= 2.7 [1.7—3.7]), and thermal water treatment (n=9; B= 1.8 [1.0—2.6]). Data was further disaggregated between field and lab-based studies. Updated LRVs indicate a variety of effective household water treatment options, but limitations exist based on technology performance, microbial target, and lab vs. field-based studies. Practitioners and researchers can use the updated microbial LRVs as a future benchmark, assessment of novel technologies, and international standard for household water treatment technologies.

0835

USING CHOICE MODELLING TO IDENTIFY POPULAR AND AFFORDABLE ALTERNATIVE INTERVENTIONS FOR SCHISTOSOMIASIS IN UGANDA

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Schistosomiasis is caused by a vector-borne parasite, commonly found in low- and middle-income countries. People become infected by direct contact with contaminated water, through activities such as bathing and fishing. Water becomes contaminated when human waste is not adequately contained. We elicit community preferences towards alternative water access, sanitation and hygiene interventions that would reduce individuals' risk of contracting, or transmitting, *Schistosoma mansoni*. We administered a discrete choice experiment to understand community preferences for improved WASH interventions. We compared interventions that target behaviours that put oneself at higher risk versus behaviours that mainly put others at risk. We used two payment vehicles to quantify what individuals are willing to give up in time and/or labour. Key findings indicate that new sources of potable water and fines on open defection are the highest valued interventions. A large portion of our sample ignored the payment vehicles, which is key for policy analysis.

0836

WATER TREATMENT BEHAVIOR AND SOCIAL NORMS AMONG WOMEN LIVING WITH YOUNG CHILDREN IN RURAL UGANDA

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Many households worldwide need to treat drinking water to prevent infection. Perceived norms of others' water treatment practices may influence individuals' personal water treatment use. This study assesses personal water treatment use and perceived peer norms among all women living with children under five years old in a water insecure setting in rural Uganda. We used data from women who were part of a whole-population study of 8 villages in Rwampara District, southwest Uganda. We recorded participants' self-reported household water treatment practice, the self-reported water treatment practice by participants' neighbors (within 0.2km) who were also study participants, and participant's perceptions about water treatment practice by most women living with young children in their village. A multivariable Poisson regression model estimated the association between individual water treatment practice and perceived peer norms, adjusting for neighbor behavior, perceived water source quality, having had diarrhea recently, and socioeconomic and demographic factors. Of 274 participants (78% response rate), 225 (82%) reported taking action to make their household drinking water safer, and 49 (18%) reported taking no action. Most [218 (80%)] reported boiling water. Across villages, the prevalence of participants who treated their drinking water ranged from 73-89%. Treating water to make it safer was the population norm in every village among women living with young children. However, 129 (47%) participants mistakenly thought most other women with young children in their village did not take any action to make household drinking water safer. Participants who misperceived the norm were less likely themselves to treat their household drinking water (adjusted relative risk = 0.81.67; 95% CI 1.68-0.97, p-value = 0.018), adjusting for other factors. Boiling household water to make it safe in this

setting is common. Future research should assess whether communicating accurate norms about water treatment practice can encourage greater uptake of water treatment practices.

0837

DETERMINATION OF GROUNDWATER QUALITY IN AND AROUND KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY (KNUST), KUMASI, GHANA

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In recent times, there has been a tremendous pressure on the provision of potable water to individuals due to a global increase in human population. The availability and accessibility of potable water has therefore become a critical and urgent problem worldwide, especially in developing countries. Groundwater is one of the major sources of potable water readily available and accessible to individuals, however, periodical monitoring is key to ensure groundwater is potable for consumption. To this end, this study was conducted at the Kwame Nkrumah University of Science and Technology (KNUST) and its environs between January and March, 2019 to determine the quality of groundwater accessed by students in various residences. Groundwater was sampled from eight residences each from two different sites namely Zone A (on-campus) and Zone B (off-campus). Groundwater sampled from the selected residential sites were analyzed for total hardness, pH and concentrations of cadmium (Cd) and lead (Pb). The results indicated that the total hardness of water obtained from the various residential sites (28.13 mg/L, ± 9.21) were far below WHO's standard, 500 mg/L ($p < 0.05$). In addition, only five (all on-campus) residences had the pH of groundwater falling within the WHO standard for pH of water (6.5-8.5). There were considerably high concentration of lead (Pb) in all the water samples obtained (0.28 mg/L ± 0.16), way above WHO standard of 0.01mg/L ($p < 0.05$), which is of great health concern. The concentration of cadmium (Cd) was below detection level (BDL) for all the residential sites. In conclusion, there is the need for regular monitoring of groundwater accessed by residents in the study area to ensure compliance with standards given the increase in population in the study area.

0838

QUANTIFYING THE CONTRIBUTION OF LOW-DENSITY AND ASYMPTOMATIC INFECTIONS TO PLASMODIUM VIVAX TRANSMISSION IN THE AMAZON

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Low-density and asymptomatic *Plasmodium vivax* infections remain largely undetected by malaria control programs in the Amazon. Quantifying their relative contribution to onward transmission is critical for designing regional strategies for malaria elimination. We did a systematic review and pooled analysis of individual data from population-based surveys that measured *P. vivax* prevalence by both microscopy and polymerase chain reaction (PCR). We modeled the relationship between parasite density and infectiousness to mosquitos using membrane feeding assay data. We examined: (1) how malaria transmission intensity and age relate to the proportion of *P. vivax*infections that are subpatent and asymptomatic, and (2) how parasite density relates to the risk of clinical manifestations and *P. vivax* transmission to mosquitoes in the Amazon. Seventy-nine pairs of prevalence measurements from Brazil, Peru, and French Guiana were analyzed. PCR detected 5-fold more infections than microscopy, with similar microscopy detection rate (mean, 28%) in high and low prevalence settings. Asymptomatic and subpatent infections predominated over symptomatic and patent infections across all age groups in six studies with individual-level data. We used parasitemia measurements by quantitative PCR to characterize site-specific and age-dependent parasite density thresholds above which most *P. vivax* infections were symptomatic. We estimate that parasite carriers missed by microscopy are the source of only 12% to 24% of mosquito infections, while asymptomatic carriers contribute 27% to 79% of community-wide *P. vivax* transmission. In conclusion, microscopy may suffice to identify the infectious reservoir of *P. vivax* to be targeted by active case detection across the Amazon.

0839

USING SOURCE OF INFECTION DATA TO TARGET MALARIA ELIMINATION IN THAILAND

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With the adoption of its National Malaria Elimination Strategy in 2016, Thailand's national malaria program enhanced its long-standing case investigation efforts. The revised process continues to collect minimal essential data on human movement and travel history but adds details on source of infections. As incidence further declines, Thailand is assessing these data to understand their utility in identifying remaining hotspots and reservoirs that may impede elimination. This study examined routine surveillance data from fiscal years 2016 through 2020 for associations between source of infections and at-risk populations. Among 50,178 confirmed cases recorded in the national database, 34,189 (68.1%) had a case investigation form with complete travel history on file and were included in the analyses. Source of infection was categorized as home (no travel outside of focus or to forested/agricultural areas; 56.5%), outside (domestic travel either outside of focus or to forested/agricultural areas; 28.2%), or imported (travel outside of the country; 15.3%). Over the study period, home transmission decreased dramatically (66.0%), whereas outside and imported cases saw smaller absolute gains. Among patients with outside infections, for which fewer interventions are effective, 53.7% reported an overnight forest stay and 44.3% an overnight farm stay. Outside transmission was associated with young adult and adult groups (15-59 years), males, non-Thai migrants, and mixed species infections ($p<0.01$). Although incomplete data was a challenge at the start of the study period, 90.8% of case investigations were completed for the final year, showing great improvement in reporting. The results align with other surveillance and research findings on key populations prone to outside transmission, validating the utility of source of infection data. Implementers are optimistic that improved case investigation processes, and perhaps refined data highlighting the most useful details on source of infections, will support the program in protecting at-risk groups and in targeting its strategies for elimination and prevention of reintroduction.

0840

TRAINING ISOLATED POPULATIONS IN THE AMAZON TO SELF-DIAGNOSE AND SELF-TREAT FOR MALARIA: RESULTS OF THE MALAKIT OPERATIONAL RESEARCH STUDY

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Gold miners are currently a key reservoir for malaria in the Guiana shield, with a risk of emergence of antimalarial resistance linked to improper use of artemisinin-based combination therapy (ACT). The remoteness of the mines, and regulatory as well as political issues hinders their access to health care. A large-scale operational research called Malakit was hence implemented at the French Guiana (FG)-Brazil and FG-Suriname borders in order to improve access to malaria testing and treatment in illegal gold mining areas in the Amazon forest. This operational research evaluated the efficacy of a combination of training and distribution of kits for self-diagnosis and self-treatment to gold-miners, at strategic border staging areas. Evaluation relied on questionnaires at inclusion and follow-up visits, and cross-sectional pre/post studies. The primary outcome was the proportion of participants of reporting the proper use of the ACT after a malaria diagnosis and the secondary outcomes assessed the use of the kit and the impact on malaria prevalence.From April 2018 to March 2020, 3,733 persons participated in the Malakit intervention. Of the 631 participants who attended a follow-up, 223 reported having used a malakit: 71.7% [65.8-77.7] had used it correctly. Among the 1,098 participants of the pre/post studies, the odds-ratio of using both a test and treatment (using a malakit or turning to the healthcare system) was 1.8 (IC95% [1.1-3.0]) after the intervention relative to pre-intervention. Treatment adherence was higher when the patient reported receiving care in a health center (91% [89-94]) or using a malakit (81% [67-96]) than with unregulated self-medication (65% [60-70]) (OR=5.4 [3.7-7.8]). The Malakit project would have increased the decline in malaria prevalence in the region by 47%.This innovative international project showed that people with low educational level can manage their malaria symptoms from diagnosis to treatment with appropriate training tools, and change their behavior. This new malaria control strategy could be transferred to other regions facing similar situation.

0841

EFFECTIVENESS AND COST-EFFECTIVENESS OF REACTIVE, TARGETED INDOOR RESIDUAL SPRAYING FOR MALARIA CONTROL IN LOW-TRANSMISSION SETTINGS: A CLUSTER-RANDOMISED, NON-INFERIORITY TRIAL IN SOUTH AFRICA

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Increasing insecticide costs and constrained malaria budgets could make universal vector control strategies, such as indoor residual spraying (IRS), unsustainable in low-transmission settings. We investigated the effectiveness and cost-effectiveness of a reactive, targeted IRS strategy. This cluster-randomised, open-label, non-inferiority trial compared reactive, targeted IRS with standard IRS practice in northeastern South Africa over two malaria seasons (2015-17). In standard IRS clusters, programme managers conducted annual mass spray campaigns prioritising areas using historical data, expert opinion, and other factors. In targeted IRS clusters, only houses of index cases (identified through passive surveillance) and their immediate neighbours were sprayed. The non-inferiority margin was 1 case per 1000 person-years. Health service costs of real-world implementation were modelled from primary and secondary data. Incremental costs per disability-adjusted life-year (DALY) were estimated and deterministic and probabilistic sensitivity analyses conducted. Malaria incidence was 0·95 per 1000 person-years (95% CI 0·58 to 1·32) in the standard IRS group and 1·05 per 1000 person-years (0·72 to 1·38) in the targeted IRS group, corresponding to a rate difference of 0·10 per 1000 person-years (-0·38 to 0·59), demonstrating non-inferiority for targeted IRS. Per additional DALY incurred, targeted IRS saved US$7845 (2902 to 64 907), giving a 94-98% probability that switching to targeted IRS would be cost-effective relative to plausible cost-effectiveness thresholds for South Africa ($2637 to $3557 per DALY averted). Depending on the threshold used, targeted IRS would remain cost-effective at incidences of less than 2·0-2·7 per 1000 person-years. Findings were robust to plausible variation in other parameters. Targeted IRS was non-inferior, safe, less costly, and cost-effective compared with standard IRS in this very-low-transmission setting. Saved resources could be reallocated to other malaria control and elimination activities.

0842

GAMETOCYTE PERSISTENCE, INFECTIVITY AND ASSOCIATIONS WITH HRP-2 LEVELS AFTER PYRONARIDINE-ARTESUNATE AND DIHYDROARTEMISININ-PIPERAQUINE WITH AND WITHOUT SINGLE-LOW DOSE PRIMAQUINE: A SINGLE-BLIND RANDOMIZED CLINICAL TRIAL IN OUELESSEBOUGOU, MALI

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Achieving malaria elimination may depend on the transmission-blocking capabilities of anti-malarial drugs. The World Health Organization (WHO) recommends that a single low dose (SLD) of primaquine (PQ) be added to artemisinin combination therapy (ACT) to reduce *P. falciparum* transmission. The transmission blocking efficacy of ACTs alone is unclear. We aimed to determine i) the effectiveness of pyronaridine-artesunate (PA) and dihydroartemisinin-piperaquine (DP) with and without 0.25mg/kg PQ for reducing transmission to mosquitoes, and ii) the detectability of gametocyte derived HRP2 protein following treatment. We conducted a four-arm, single-blind, randomised clinical trial at the Ouelessebougou Clinical Research Unit of the Malaria Research and Training Centre (MRTC) of the University of Bamako, Mali. Participants were male and non-pregnant females aged 5-50 years with asymptomatic *P. falciparum* mono-infection and gametocyte carriage (n=100). Allocation to four treatment groups (PA, PA+PQ, DP, and DP+PQ) was randomised in a 1:1:1:1 ratio. Individuals were followed for up to 49 days post-treatment with regular blood sampling and mosquito feeding assays. Before treatment, 66% (66/100) of individuals were infectious to mosquitoes, infecting an average of 15.8% mosquitoes (IQR 5.41-31.94). In infectious individuals the median percent reduction in mosquito infection rate after 48 hours

was 100% (IQR 100-100) in the PA+PQ (n=18) and DP+PQ (n=15), arms, compared to -8.7% (IQR -54.8-93.2) and 50.4% (IQR 13.8-70.9) in the PA (n=17) and DP arms (n=16) respectively. Individuals continued to infect mosquitoes up to 28 days after PA and DP treatment without PQ. Gametocyte density measures, sex ratio, and the detectability of PfHRP2 protein by standard RDT, ultra-sensitive RDT (uRDT), and quantitative assays were also analysed. Our data strongly support the use of SLD PQ as an effective supplement to PA for blocking *P. falciparum* transmission, may have implications for uRDT based parasite surveillance, and are of immediate relevance to areas aiming to eliminate malaria or constrain the spread of drug resistant parasites strains.

0843

USE OF QPCR AND SEROLOGY TO INFORM TARGETING MALARIA ELIMINATION STRATEGIES IN LOW BURDEN SETTINGS IN BOTSWANA

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Botswana reoriented its malaria program towards elimination in 2010. Between 2013 and 2016, 78% of the 1532 locally infected cases were focalized within three districts in Northern Botswana. However, the surveillance system had gaps that hindered a full understanding of the geographic limits and drivers of transmission. To address these surveillance gaps, the National Malaria Programme (NMP) and Clinton Health Access Initiative (CHAI) conducted a household parasite survey comprising 7459 individuals from 5503 households during the peak transmission season from February to April 2018 in Okavango, Ngami and Chobe districts. In each household, a structured questionnaire was administered and two individuals (one >5 years and one ≤5 years of age) were randomly selected to receive a malaria Rapid Diagnostic Test (RDT), and provide blood spots on filter paper for quantitative polymerase chain reaction (qPCR) and ELISA serology testing. Out of the 7227 samples, only five (0.07%) tested positive by RDT, four of which were confirmed to be positive by qPCR. Three additional low-level infections were detected by qPCR. Preliminary serology results indicate that 954/7227 (13%) participant samples were seropositive based on a cutoff determined by a finite mixture regression model. Controlling for age and vector control, unemployed individuals were significantly less likely to be seronegative than those who reported working in agriculture or forestry (OR=0.37, p=0.002). Overall, only 33 out of 1721 (<2%) of the under 5 year old participants were seropositive, with 4% with moderate antibody response (>0.5 OD), suggesting low recent infections. Both qPCR positives and recent past infections were focalized to areas where the surveillance system also detected relatively high local cases and confirmed findings from routine data that cases declined over time and that farmers have the most exposure. These similarities indicate that the surveillance system is strong enough to inform malaria elimination. The results of the survey are being used to target health promotion activities to at-risk groups, and to improve targeting and uptake of vector control.

0844

IMPROVING ACCESS TO MALARIA DIAGNOSIS AND TREATMENT IN BORDER COMMUNITIES AND MOBILE AND MIGRANT POPULATIONS

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The SADC Malaria Elimination Eight (E8) is a coordinated, eight-country initiative in Southern Africa aiming to achieve malaria elimination by 2030. Countries that have reduced malaria to low levels face the challenge of persistent importation from neighbouring countries with higher rates of malaria infection. In 2017, the E8, in collaboration with National Malaria Control Programs (NMCPs) established 46 border health facilities on 5 key international borders between E8 countries. These services aimed to improve access to timely diagnosis and treatment of malaria for border communities and mobile and migrant populations (MMPs) who frequently cross the border. To evaluate the impact of these facilities, a quasi-experimental, non-randomized, matched comparison of seven pairs of sites (intervention and control) in was conducted in the four low-burden, so-called frontline countries. In second-line countries, cross-sectional surveys of border residents living near border post were carried out. Studies in second-line countries were descriptive and showed that there were reasonably high levels (>70%) of timely treatment-seeking and access to diagnosis when experiencing fever and that all those who did test positive for malaria reported receiving treatment. However, up to 40% in some settings did not receive a blood test when experiencing fever. Residents reported good access to healthcare in terms of distance and time to facility. Although residents reported access to IRS and LLINs, a proportion of households did not own any nets, even in sites where this was the primary form of vector control. Knowledge about malaria symptoms and prevention was high and bed nets were overwhelmingly regarded as an effective method of preventing malaria. Border residents travelled frequently both within their own country and across borders; sometimes to seek healthcare. Results identified a gap in the provision of malaria prevention for travelers who often slept outside whilst travelling without any protection against mosquitoes. Studies in front line countries will provide an analytical comparison between intervention and control sites.

0845

DESCRIBING PREDICTOR RISK FACTORS FOR LATE-RELAPSING HEPATITIS AFTER YELLOW FEVER

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Despite intense study, yellow fever (YF) is poorly understood in humans. Previous studies reinforced the occurrence of late-relapsing hepatitis as a possible late clinical manifestation of YF, with the persistence of symptoms (fatigue, weakness, and jaundice) and slightly abnormal levels of liver and renal injury markers. During the YF 2017-2018 outbreak in Brazil, 302 confirmed YF cases were admitted to Hospital Eduardo de Menezes (HEM), Belo Horizonte, Brazil. Following discharge, patients were advised to return at 15-, 30-, 45- and 60-days post symptom onset (DPS) for clinical and laboratory examination and serum sample collection. For the first two visits, the patients presented with normal or slightly elevated liver and renal enzymes and did not report any signs or symptoms related to YF. However, from 30 up to 60 DPS, 12% of patients (n=38) exhibited a rebound of elevated transaminase (>500IU/L) and bilirubin levels. The patients showed general clinical worsening: a majority reporting myalgia (92%), arthralgia (92%), weakness (61%), headache (24%), and jaundice (24%). Eight patients needed to be re-hospitalized. Serum samples collected during follow-up were tested by RT-qPCR and were all negative. Other etiologies of hepatitis were ruled out by PCR or serology, including dengue, Zika, chikungunya, hepatitis A, B, C, HIV, and other viruses that can cause hepatitis. Tests for autoimmune hepatitis were also performed and were negative. Univariate analysis of risk ratios did not identify any significant demographic, clinical, or image exams risk factors for late-relapsing hepatitis after YF (LHep-YF) present during the acute (inpatient) phase of YF. AST and ALT values during LHep-YF were higher in the group that presented this clinical picture than in the other group (P=0.003). These results provide new data on the clinical course of YF and highlight the need for extended patient follow-up. Further studies to investigating the viral persistence and immune responses causing LHep-YF are needed.

0846

INFECTION WITH CHIKUNGUNYA VIRUS CONFERS CROSS-PROTECTIVE NEUTRALIZING ANTIBODIES AND MEMORY B-CELLS THAT REMAIN IN CIRCULATION FOR DECADES FOLLOWING NATURAL INFECTION IN BOTH ENDEMIC AND NON-ENDEMIC TRANSMISSION SETTINGS

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Chikungunya virus (CHIKV) a mosquito-borne alphavirus, is an important viral pathogen that causes acute febrile illness often with arthralgia than can persist for months to years, posing a significant public health risk. Here we describe the antibody-mediated immune response following CHIKV infection, upon which, naïve host B-cells expand, produce, and secrete CHIKV-specific antibodies (Abs) that recognize viral antigens, namely structural proteins E1 and E2. After viral clearance, some of these B-cells become long-lived plasma cells (LLPC) which constitutively secrete CHIKV-Abs, while others become CHIKV-specific memory B cells (MBCs) that remain in circulation, quiescent, but poised to respond and expand on repeat infection. This MBC "founder" population is expected to play a critical role in broader alphavirus immunity that is established following repeat exposure. Using a panel of 15 convalescent immune samples collected 1 to 24 years post infection from endemic (n=5) and non-endemic (n=7) subjects with suspected or confirmed CHIKV infection, 50% neutralization titers were determined against a panel of alphaviruses, including the Semliki Forest complex, CHIKV, Mayaro (MAYV), Ross River Virus (RRV), Una (UNAV) and Venezuelan Equine Encephalitis virus (VEEV), part of the VEEV complex. To determine MBC frequency, peripheral blood mononuclear cells (PBMCs) from CHIKV immune donors were evaluated by limiting dilution assay, where PBMCs are serially diluted and stimulated to become Ab-secreting cells. The resulting Abs are assessed for homotypic and heterotypic-specificity by ELISA using whole CHIKV and MAYV.Using these approaches, we identified neutralizing Abs and long-lived MBCs with broad potency against members of the Semliki Forest complex, however cross-neutralization did not extend to the VEEV complex, suggesting that broadly protective Abs may be complex restricted. Subsequent experiments seek to identify the predominant epitope responsible for this cross protection in both LLPC and MBC populations. These results will provide insight into the MBC founder population following alphavirus infection.

0847

LONG-TERM SEQUELAE OF CHIKUNGUNYA AMONG CHILDREN AND ADULTS IN MANAGUA, NICARAGUA, 2014-2018

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Chikungunya virus (CHIKV) infections cause debilitating arthritis in many symptomatic individuals, often presenting as acute, self-limited pain but occasionally manifesting as chronic arthralgia for months or years post-infection. Relatively little is known about pediatric chikungunya, how the disease differs between children and adults, and the relationship between longitudinal antibody titers and the risk of chronic arthralgia. To characterize the long-term sequalae of chikungunya, we recruited 782 patients (0-9 years old [y/o], 294; 10-15 y/o, 310; 16-21 y/o, 51; 22-35 y/o, 57; 35+ y/o, 53) with rRT-PCR-confirmed symptomatic CHIKV infections in Managua, Nicaragua, during two sequential chikungunya epidemics (2014, 2015). Participants were followed up at 15 days and 1, 3, 6, 12, and 18 months post-illness, and we analyzed longitudinal clinical data from 2014 to 2018. Participants were tested for anti-CHIKV IgM antibodies, and anti-CHIKV antibody titers of pediatric patients were measured annually by CHIKV inhibition ELISA (iELISA). Multivariable analysis indicated that individuals 35+ y/o had the highest odds of pain, relative to the baseline group of ages 10-15 (odds ratio (OR) 7.5, 95% CI: 4.6-12.3, p-value <0.001), and males had lower odds of pain than females (OR 0.6, 0.5-0.9, p-value = 0.004). For both children and adults, pain was most commonly localized in the legs (29.2%), followed by hands (22.5%) and torso (14.4%). Compared to the 10-15 y/o group, 0-9 y/o children had significantly lower odds of wrist pain (OR 0.5, 0.3-0.8, p-value = 0.006) while patients 15+ y/o had significantly higher odds of pain. Individuals younger than 15 years had a higher prevalence of chronic (>3 months) than acute (6 days-3 months) pain; the opposite trend was observed among adults. Further, we observed that higher iELISA antibody titers and positive convalescent IgM tests correlated with a higher risk of arthralgia, suggesting a potential relationship between antibody titer levels and disease severity. In sum, differences in the presentation of pain were observed across age, sex, and antibody titers in our longitudinal chikungunya cohort.

0848

CHIKUNGUNYA: PHASE 3 CLINICAL DEVELOPMENT OF A SINGLE-SHOT LIVE-ATTENUATED VACCINE

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Chikungunya is a mosquito-transmitted outbreak disease with potentially debilitating consequences and no available causative treatments or preventative vaccines. VLA1553 is a live-attenuated chikungunya virus (CHIKV) vaccine candidate designed for active immunization as a

prophylactic measure for travelers to endemic areas or areas at risk for an upcoming outbreak, as well as for the general population living in endemic regions. A double-blinded, multicenter randomized, pivotal phase 3 study enrolling approximately 4,060 adult volunteers randomized in a 3:1 ratio to receive the live-attenuated CHIKV vaccine candidate VLA1553 or placebo is currently ongoing (NCT04546724). VLA1553 or placebo were administered as a single intramuscular immunization. The primary objective of the study is to evaluate the immunogenicity and safety of VLA1553 28 days after immunization. Subjects in this study were stratified into two age strata from 18 to 64 years and 65 years of age or above. Immunogenicity evaluations include the seroprotection rate as primary endpoint. This seroprotection rate is based on neutralizing CHIKV antibody titers higher than a surrogate threshold of protection agreed with the FDA. To date, recruitment is completed and 4,131 volunteers have been vaccinated. The study is progressing and primary endpoint analysis on immunogenicity up to Day 29 will be available mid 2021.

0849

LONG-TERM SAFETY AND IMMUNOGENICITY OF CHIKUNGUNYA VIRUS-LIKE PARTICLE VACCINE IN CHIKUNGUNYA SEROPOSITIVE INDIVIDUALS

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Chikungunya virus (CHIKV) causes acute febrile illness accompanied by debilitating joint pain that may progress to chronic manifestations including arthralgia. Since outbreaks on the Indian subcontinent in 2005 and 2006, the geographic distribution of CHIKV has expanded to >100 countries. A safe, effective vaccine with durable protection against chikungunya disease is urgently needed. The long-term safety and immunogenicity of an investigational chikungunya virus-like particle (CHIKV VLP) vaccine in individuals with pre-existing CHIKV immunity who live in CHIKV-endemic areas is unknown. VRC704 was a multi-center, randomized, placebo-controlled, double-blind Phase 2 study to evaluate the safety and immunogenicity of a two-dose regimen of CHIKV VLP vaccine (CHKVLP059; 20 µg CHIKV VLP administered by intramuscular injection 28 days apart) in healthy adults 18-60 years of age in the Caribbean (N=400). Study criteria required that subjects were seronegative. Despite screening negative via IgG/IgM ELISA at the time of inclusion, 39 CHIKV VLP vaccine and 43 placebo recipients from sites in Haiti and the Dominican Republic were retrospectively found to have pre-vaccination CHIKV neutralizing antibodies at the time of enrollment. This study is a post-hoc analysis comparing the safety and immunogenicity of CHIKV VLP vaccine to placebo in these seropositive subjects for 72 weeks post-vaccination. Two CHIKV VLP vaccine recipients and one placebo recipient had serious adverse events (SAEs) during the 72-week follow-up period which were considered unrelated to vaccination. Unsolicited AEs were monitored for 4 weeks after each dose; no difference was seen between the CHIKV VLP vaccine and placebo groups. Baseline seropositive vaccine recipients had a geometric mean titer (GMT) of approximately 2000 at baseline with 3.7 and 4.2 geometric mean fold rises (GMFR) at 4 weeks after the first dose and 12 weeks after the second dose, respectively. The GMFR remained >3 for 72 weeks post-vaccination. These data support further evaluation of long-term safety and immunogenicity of the CHIKV VLP vaccine in individuals with pre-existing immunity to CHIKV.

0850

MODELING THE KINETICS AND DURABILITY OF THE HUMORAL RESPONSE TO A CHIKUNGUNYA VIRUS-LIKE PARTICLE VACCINE

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Chikungunya is a globally recognized infectious disease transmitted by *Aedes* mosquitoes. It causes disruptive epidemics of febrile illness characterized by acute symptoms such as incapacitating joint pain. Long-term chronic symptoms including arthralgia are common. Using data from two Phase 2 immunogenicity studies in populations based in the United States and Caribbean, we model antibody levels after immunization with a chikungunya virus-like particle (VLP) vaccine to characterize kinetics and duration of the humoral immune response. Depending on the study, blood samples were taken at roughly weekly intervals through Week 8 and at additional timepoints through 2 years post-immunization. Neutralizing antibody titers were determined using a luciferase-based virus neutralization assay and measured with a stringent 80% cutoff (NT_{80}). We use time series analyses to evaluate variability in the magnitude of the neutralizing response, and mechanistic modeling to estimate parameters associated with the humoral immune response and to estimate the long-term durability of antibodies. Neutralizing antibody titers were available from 528 study participants receiving at least one dose of vaccine. The induced neutralizing antibody response was rapid, with up to 80% of study participants mounting at least a 4-fold rise in neutralizing antibody titers by 1 week, and 89-100% by 3 weeks post-immunization. Antibody titers subsided by week 26 but stabilized ≥4-fold above baseline in ~80-90% of vaccinees through 104 weeks of follow-up. There was a ~6% drop in geometric mean titer (GMT) between weeks 78 and 104. The magnitude of response varied by dose and dosing regimen, though kinetics remained similar. A single alum-adjuvanted dose induced a ≥4-fold rise in >80% of recipients by 1 week and the highest neutralizing antibody response at 26 weeks. The rapid immune response after a single dose of the VLP vaccine would support its utility for travelers to chikungunya affected regions and outbreak response. Ongoing modeling will further characterize the long-term antibody dynamics to estimate the duration of immunity and the timing of revaccination.

0851

SCALE UP OF JAPANESE ENCEPHALITIS VACCINATION IN IMMUNIZATION PROGRAM OF BIHAR AMIDST COVID-19 PANDEMIC IN 2020 IN INDIA, THROUGH EFFECTIVE PARTNERSHIPS

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Bihar which is the third populous State of India has been witnessing Japanese Encephalitis outbreaks regularly since 2005. The most effective immunization strategy implemented in JE endemic areas is a one-time campaign targeting children (1-15 years), followed by integration of JE vaccine into routine immunization program, for the new cohort. In late 2019, decision was taken by Bihar state to undertake JE vaccination campaign in 14 newly identified JE endemic districts in State and PATH was envisaged to provide technical assistance in planning and implementation of these campaigns to the government. JE vaccination campaign launched in January 2020 in two districts initially, was staggered due to COVID-19 pandemic. Campaign was resumed in a phased manner in other 9 districts from June 2020. PATH with support from Bill & Melinda Gates Foundation provided technical assistance to state Govt. at multiple levels, in training 262 medical officers and ~ 24,000 health workers via online platform and

classroom sessions, maintaining COVID appropriate safety protocols. In the backdrop of school shut down due to pandemic, leveraged routine immunization platform for mobilizing eligible children for JE vaccination. Over the next few months due to worsening pandemic situation, affecting health workers and burdening the health system, progress of JE campaign was hindered. Coverage achieved was 73% by November 2020, that was ramped-up, through special staggered initiatives under state program officer, supported by PATH project team. Initiatives like prioritization of low performing pockets, line-listing of unvaccinated children, liaising with related government departments (e.g., education, social welfare and rural development) were adopted to accelerate mobilization of eligible children to nearest vaccination sites. Vaccination campaign in 11 districts was completed in January 2021 with net coverage of >90 % where > 9 MM children were vaccinated alongside COVID response, underway. Effective coordination, close collaboration with all partners and stakeholders and using RI platform has helped in achieving the desired JE Vaccination coverage.

0852

ROLE OF PLASMODIUM FALCIPARUM KELCH 13 PROTEIN MUTATIONS AND FALCIPAIN 2A HAPLOTYPES IN ARTEMISININ RESISTANCE

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Mutations in the *Plasmodium falciparum* Kelch 13 (PfK13) protein are associatedwith artemisinin resistance. PfK13 is essential for asexual erythrocytic development,but its function is not known. We tagged the PfK13 protein with green fluorescentprotein in *P. falciparum* to study its expression and localization in asexualand sexual stages. We used a new antibody against PfK13 to show that the PfK13protein is expressed ubiquitously in both asexual erythrocytic stages and gametocytesand is localized in punctate structures, partially overlapping an endoplasmicreticulum marker. We introduced into the 3D7 strain four PfK13 mutations (F446I,N458Y, C469Y, and F495L) identified in parasites from the China-Myanmar borderarea and characterized the *in vitro* artemisinin response phenotypes of the mutants.We found that all the parasites with the introduced PfK13 mutations showed highersurvival rates in the ring-stage survival assay (RSA) than the wild-type (WT) control,but only parasites with N458Y displayed a significantly higher RSA value (26.3%)than the WT control. After these PfK13 mutations were reverted back to the WT infield parasite isolates, all revertant parasites except those with the C469Y mutationshowed significantly lower RSA values than their respective parental isolates. Althoughthe 3D7 parasites with introduced F446I, the predominant PfK13 mutation innorthern Myanmar, did not show significantly higher RSA values than the WT, theyhad prolonged ring-stage development and showed very little fitness cost in *in vitro*culture competition assays. In comparison, parasites with the N458Y mutations alsohad a prolonged ring stage and showed upregulated resistance pathways in responseto artemisinin, but this mutation produced a significant fitness cost, potentiallyleading to their lower prevalence in the Greater Mekong subregion.

0853

THE IMPORTANCE OF THE PARASITE PROTEASOME IN ARTEMISININ RESPONSE

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Artemisinin (ART)-based combination therapies (ACTs), the recommended first-line treatment for *falciparum* malaria, are largely responsible for the nearly 50% decline in malaria-related deaths in the last two decades. Alarmingly, resistance to ACTs is widespread in Southeast Asia (SEA), and has been detected in greater Asia, Africa, and South America. The most prevalent ART-resistance conferring kelch 13 (K13) haplotype in SEA is C580Y. ART causes widespread protein damage in parasites, resulting in accumulation of ubiquitinated proteins which are then degraded by the parasite proteasome. We hypothesized that the proteasome is critical for parasite ART-response and mutations that decrease proteasome catalytic activity reduce the ability of parasites to recover following ART-induced protein damage. We have previously generated β2 and β5 proteasome mutants in Cam3.II strain parasites of Cambodian origin that express $K13^{C580Y}$. In response to dihydroartemisinin (DHA), these proteasome mutants exhibit decreased β2 and β5 catalytic activity. In addition, relative to their parent, DHA-treated β2 proteasome mutants display increased accumulation of K48-linked ubiquitinated polypeptides and increased activation of the unfolded protein response, signifying a reduced capacity to restore proteostasis. Lastlly, we observe that β2 proteasome mutants display increased sensitivity to DHA and the related endoperoxide OZ439. This increase in sensitivity was observed not only at early ring stages, where ART resistance is classically observed, but also at trophozoite stages and in asynchronous cultures. The sensitivity throughout the intraerythrocytic development is likely due to the necessity of the parasite proteasome throughout its lifecycle. These data demonstrate the importance of the parasite proteasome in response to endoperoxides.

0854

ASSESSING FEASIBILITY OF A STRATEGY DEPLOYING MULTIPLE FIRST-LINES ARTEMISININ-BASED COMBINATION THERAPIES FOR UNCOMPLICATED MALARIA IN THE HEALTH DISTRICT OF KAYA, BURKINA FASO

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The emergence and spread of resistance to artemisinin derivatives is a serious threat to malaria control. Thus, the development of new strategies to rapidly mitigate this threat is crucial. This study aimed at assessing for the first time in a structured way, the feasibility of deploying simultaneously three artemisinin-based combination therapies (ACTs) at health facility (HF) level for the management of uncomplicated malaria cases in Burkina Faso, with the hypothesis that this strategy is feasible and can be successfully managed by health workers (HWs). For this pilot, the MFT strategy consisted of segmenting the populations in three groups and allocating one ACT to each. Malaria patients under 5 years received pyronaridine-artesunate, 5 years and above, dihydroartemisin-piperaquine, and pregnant women, artemether-lumefantrine (AL), respectively at HF level. The community case management of malaria continued with no change using AL. Over 12 months from December 2019 to December 2020, MFT was deployed in 40 public primary HF of Kaya health district. All HWs (278) and drug store managers (50) were trained before initiating the deployment of MFT. A monitoring plan was implemented. HF-based survey, interviews (27) and focus group discussions (10) were carried out as part of the evaluation of MFT deployment. 182,247 and 4,955 malaria cases were seen at HF and community level respectively and treated with ACTs. No serious adverse drug reaction was reported. Of the 2018 suspected malaria cases randomly selected at HF level for records review, 44.8% and 4.7% of the cases were under 5 and pregnant women, respectively. 78.8% were tested for malaria and 98.3% of positive tests were treated. 81.9% of patients were assigned the correct treatment for their segment (p=0.002). The overall HWs compliance level was 73.2 % and was associated with segments of the population (p<0.001). Participants interviewed had a good knowledge

of the MFT implementation strategy and a favorable opinion. The pilot study showed that implementing a MFT strategy is operationally feasible and well accepted by the HWs. The scale-up of this MFT strategy could be considered.

0855

MEASURING GROWTH, RESISTANCE, AND RECOVERY AFTER ARTEMISININ TREATMENT IN A SINGLE SEMI-HIGH-THROUGHPUT ASSAY

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Artemisinin (ART) resistance has spread throughout Southeast Asia and mutations in Kelch13, the molecular marker of ART resistance, have been identified in Africa. Effective *in vitro* assays for resistance are crucial for monitoring populations for the emergence and spread of resistance around the world. The Ring Stage Assay (RSA) is a time consuming and labor-intensive *in vitro* measure of resistance. Previously, we developed a modified RSA that improved correlation with patient clearance half-life and significantly reduced experimental noise and the labor intensiveness of RSA. This also allowed the higher throughput needed to quickly phenotype large numbers of parasites in less time. Here we report an extension and refinement of this assay to include measurements of parasite growth and parasite recovery after treatment with ART or other drugs. Growth is assayed by parasite multiplication rate using untreated control samples at 6h post-treatment and 96h post-treatment. Growth measures can reveal fitness defects and illuminate relationships between proliferation rates and drug resistance. Resistance is inferred at 120h as a differential between treated and untreated parasites in our assay; this is then then combined with an additional 192h post-treatment sample to generate a recovery profile. This serves as a novel and complementary phenotype to resistance that quantifies a parasite's ability to tolerate drug exposure, a hallmark of parasite response to ART. Recovery from drug is a crucial step to evolving high level drug resistance. This trio of readouts offer a more comprehensive phenotypic assessment of clinical isolates and parasite progeny populations for the effects of ART, piperaquine and other drugs.

0856

THE PLASMODIUM FALCIPARUM ELONGATION FACTOR 2 INHIBITOR M5717 IS EFFICACIOUS AGAINST MALARIA LIVER STAGE: A CONTROLLED LIVER-STAGE MALARIA INFECTION STUDY IN HEALTHY SUBJECTS

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M5717 is an anti-malaria compound targeting PeEF2 and is *in vitro* equipotently active against all stages of the parasite. As such, M5717 has the potential to be developed in combination as single dose treatment for chemoprevention for malaria. M5717 was well tolerated up to a dose of 1800 mg in a previous Phase I single dose ascending study (SAD) and demonstrated anti-malarial activity in a blood stage controlled human malaria infection model. Now a Phase Ib, randomized, double blind, sequential, adaptive dose-finding study was performed in healthy male and female volunteers to evaluate the chemoprophylactic effect and define an exposure-response relationship. Each participant was inoculated with 3200 sporozoites by direct venous inoculation and treated with M5717/placebo administered as an oral suspension two hours later. Blood parasitemia was measured by qPCR on a regular basis until reaching a threshold of ≥ 100 asexual Pf/mL, when subjects were treated with rescue medication (atovaquone/proguanil). PCR negative subjects received rescue medication at Day 28 after inoculation. The study included several cohorts and each cohort consisted of 1 to 3 groups of 4 subjects, of which 3 received M5717 free base and 1 received placebo. The first cohort included one group at the protocol defined dose of 200 mg. The dose and the number of groups in the subsequent cohorts were decided by a safety monitoring committee based on observed efficacy, pharmacokinetic (PK) parameters and variability. The initial cohorts followed a dose de-escalation pattern and the effect of doses ranging from 200 to 30 mg was evaluated. M5717 was well tolerated and the safety profile confirmed the data from the SAD study. Preliminary data showed that M5717 can provide full protection against malaria. PK evaluations will be performed to define the relevant parameter predicting liver stage efficacy. All together the data warrant further development of M5717 as part of a chemoprevention combination for malaria.

0857

A 2-PART STUDY TO EVALUATE THE ASEXUAL BLOOD STAGE ANTIMALARIAL ACTIVITY, AND THE TRANSMISSION BLOCKING ACTIVITY, OF A SINGLE ORAL DOSE OF TAFENOQUINE IN HEALTHY SUBJECTS EXPERIMENTALLY INFECTED WITH PLASMODIUM FALCIPARUM

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Tafenoquine (TQ) is a long-acting 8-aminoquinolone that has activity against hepatic stage schizonts, and *Plasmodium vivax* hypnozoites, as well as less well-characterised activity against blood stage parasites and gametocytes. However, like primaquine, TQ causes haemolysis in G6PD deficient individuals when used at currently recommended doses, thereby limiting its use as a component of mass drug administration. To define the lowest effective dose of TQ, we have undertaken a 2-part study in healthy volunteers experimentally infected with blood-stage *P. falciparum* aiming to investigate the minimum single dose of TQ that would effectively clear blood-stage *P. falciparum* parasitemia (Part 1), and prevent transmission of *P. falciparum* to mosquitoes (Part 2). In Part 1, 4 participants were inoculated with *P. falciparum* and treated on day 8 with a single 300mg dose of TQ. Although parasite clearance occurred in all 4 participants, recrudescent parasitemia occurred in 3 (18, 26 and 28 days post TQ dosing) requiring treatment with artemether-lumefantrine (AL). Based on PK/PD modelling, in subsequent cohorts participants were treated with TQ 200mg (n=3), 400mg (n=2) or 600mg (n=3). None of the participants in the 200mg achieved complete parasite clearance, and all required treatment with AL. All participants in the 400mg or 600mg dose group achieved complete parasite clearance and did not recrudesce by day 46. PK/PD modelling is underway to define the PK/PD parameters necessary for prediction of the efficacious dose, and results will be presented. For part 2 of the study, participants will be inoculated with blood-stage *P. falciparum*, and treated on day 9 with piperaquine to clear asexual parasitemia while allowing development of gametocytemia. TQ 50mg will be administered on day 24, with enriched membrane feeding assays conducted immediately before, and 1, 3 and 7 days post-TQ. The proportion of volunteers infective to mosquitoes (as measured by detection of oocyts and sporozoites) before and after TQ will be reported.

0858

ROSIGLITAZONE ADJUNCTIVE THERAPY FOR CHILDREN WITH SEVERE MALARIA

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Despite the widespread use of effective anti-malarials, the case fatality rate of severe malaria (SM) remains unacceptably high. Adjunctive therapies that target the host response to malaria infection may decrease mortality over that of anti-malarial agents alone. Rosiglitazone, a peroxisome proliferator-activated receptor-gamma agonist, has been shown to act on pathways implicated in the pathogenesis of SM including reducing inflammation and enhancing endothelial stability, as determined by decreased levels of angiopoietin-2, a surrogate maker of malaria severity and mortality. To test the hypothesis that rosiglitazone plus IV artesunate would improve outcome in SM over IV artesunate alone, we conducted a randomized, double-blind placebo-controlled trial in a rural hospital in Manhiça, southern Mozambique. The primary objective was to determine whether supplemental rosiglitazone (0.045mg/kg/dose) twice daily for 4 days, in addition to standard of care anti-malarial treatment (IV artesunate), accelerates the rate of decline in angiopoietin-2 from admission levels in children with severe malaria compared to standard of care anti-malarial treatment plus placebo. We have previously determined the safety and tolerability of rosiglitazone in children with uncomplicated malaria before proceeding to examine its efficacy in children with SM. Children with SM were enrolled between March 2016 and December 2019. We screened 203 children for eligibility and 180 children were enrolled: 91 children were assigned to rosiglitazone and 89 to placebo (all received parenteral artesunate). 85 were females (47.2%) and 95 were males (52.8%). 141 out 180 were younger than 5 years (78.3%) and 39 out of 180 were older than 5 years (21.6%). Secondary objectives included: comparing clinical outcomes between groups and quantifying the change in levels of biomarkers of disease severity and host response associated withendothelial activation, inflammation, coagulopathy, and neuro-protection. Analysis of primary and secondary objectives is currently underway. We will present the results of this adjunctive

0859

EXPLORING COVID-19 DISEASE BURDEN THROUGH SEWAGE AND DRAINAGE SYSTEM OF DHAKA CITY, BANGLADESH

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SARS-CoV-2 virus, the causative agent of COVID-19, is excreted in stool of both symptomatic and asymptomatic cases, and SARS-CoV-2 RNA can be detected in wastewater samples from the sewer system. Therefore, the concentration of SARS-CoV-2 RNA in wastewater can be used as a predictor of COVID-19 infection in specific catchment areas using wastewater-based epidemiology. Sewage lifting stations (SLS) and sewage treatment plants (STP) are important infrastructural components of the municipal sewage network of Dhaka city and are responsible for wastewater management for 20-25% of the population. Additionally, storm sewage pumping stations (SSPS) are a major part of the drainage system where both SLS and SSPS receive wastewater and grey water from the community and pump them towards the STP and river. We conducted a cross-sectional study from October 2020 to April 2021 on 14 active SLS, four SSPS, and the STP of Dhaka city to explore developing an early warning system using SARS-CoV-2 viral load in wastewater to indicate trends in COVID-19 confirmed clinical cases through a wastewater surveillance system. A total of 42 sewage and drainage samples (28 SLS, 4 SSPS, 5 STP inlets and 5 STP outlets) were analyzed and quantified by RT-PCR. Overall, 48% (20/42) of samples were positive for SARS-CoV-2 RNA: 54% (15/28) of SLS samples, 75% (3/4) of SSPS samples, and 40% (2/5) of samples from the STP inlet were positive. The highest concentration of SARS-CoV-2 genome equivalent copies (GEC) were detected in SSPS samples [median=111 GEC/mL (range=47-482)]. Median viral load in SLS samples was 70 GEC/mL (range=12-140), and 25 GEC/mL (range=15-45) in STP samples. Pearson's correlation coefficient analysis suggested that concentration of GEC detected in wastewater was significantly correlated with the number of clinically reported cases in the corresponding catchment area of Dhaka city on the day of sample collection (P=0.058). This study suggests that wastewater surveillance could be a helpful tool in tracking the circulation and accumulation of SARS-CoV-2 in sewage and drainage systems and could complement clinical surveillance of the COVID-19 burden in Dhaka.

0860

PREVALENCE OF CIRCULATING SARS-COV-2 IN WASTEWATER FROM NICARAGUA

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Wastewater-based monitoring for SARS-CoV-2 by real-time PCR (RT-qPCR) is a useful tool for epidemic surveillance. In Nicaragua, the first COVID-19 case was reported on March 18, 2020. In this study, the levels of SARS-CoV-2 transmission at community level was investigated for 10 months (May 2020 - March 2021) by detection of SARS-CoV-2 genes in wastewater. Weekly samples (50ml) were collected in 2 of the 4 wastewater sedimentation treatment plants in León, designated as plant A and plant B. SARS-CoV-2 RNA was purified from 350 µL of clarified sewage samples, by using an in-house silica/guanidium thiocyanate method. The presence of the SARS-CoV-2 E-gene in the sewage RNA eluate was by RT-qPCR and confirmed with a secondary RT-qPCR for the RdRp gene. Following the first screening, all RNA eluates were diluted 1:100 and re-tested for possible presence of RT-PCR inhibitors. In both treatment plants, temperature ranged between 28.9 (SD: 1.5, Min: 28, Max: 34) and 30.3ºC (SD: 1.7, Min: 25, Max: 36) and pH between 8 (SD: 0.2, Min: 8, Max: 9) and 10 (SD: 0.3, Min: 8, Max: 10). The weekly precipitation mean during the period of sampling was 50.4 mm (SD: 50.7, Min: 0, Max: 193) with the highest peak in the months of August and November. A total of 61.7% of the samples were positive in both plants, with 67.5% positive in plant A (mean Ct = 32.9, SD 2.2, Min: 28, Max: 38) and 56.1% in plant B (mean Ct = 34.08, SD: 0.936, Min: 33, Max: 36). There was a 80% correlation between E- and RdRp-gene detection, of notice the Ct values in the RdRP RT-qPCR were > 33. There was no correlation between SARS-CoV-2 detection and sewage's temperature, pH, or precipitation. This study shows that SARS-CoV-2 genes can be detected in sewage without the need of RNA concentration steps and the mean viral concentration was estimated to 7.59 $\log_{10}$ genome equivalents/L (SD: 0.52, Min: 6.32, Max: 9.17), data differ with other studies that report lower viral concentration, suggesting high transmission of SARS-CoV-2 at community level.

0861

SPATIAL AND TEMPORAL PATTERNS OF SAPOVIRUS GASTROENTERITIS IN CHILDREN UNDER THREE YEARS OF AGE IN LEÓN, NICARAGUA

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Sapovirus is a common cause of pediatric acute gastroenteritis but there is limited data about the incidence and environmental factors that facilitate transmission. We analyzed the spatial and temporal distribution of sapovirus gastroenteritis and measured the association with rainfall in a cohort of children (0-3 years of age) in León, Nicaragua. In a prospective cohort study, newborns received weekly household surveillance for gastroenteritis from 2017-2020. Diarrheal stool samples were examined using RT-qPCR testing for the presence of sapovirus. We used dwelling characteristics (construction of floor, wall, and ceiling, water source, sanitation, and overcrowding) and household income and education to create a poverty index (extremely poor, poor, and not poor). Participants' homes were georeferenced using Epicollect and to create maps showing the clusters density of sapovirus episodes using ArcGIS 10.7. Tropical Rainfall Measuring Mission Multi-Satellite Precipitation Analysis data from NASA were used to calculate average rainfall and extreme rainfall days by epidemiological week. Of 1,258 gastroenteritis episodes, 110 (8.74%) among 96 children were attributed to sapovirus during 3-year of surveillance. Sapovirus was more frequently observed in semi-urban areas with 57% of children living in families categorized as poor or extremely poor and neighborhood with low sanitation conditions. Sapovirus gastroenteritis was associated with rain, being more frequently detected at the beginning (June) and end (October) of the rainy season. Extreme rainfall in preceding weeks could predict sapovirus episodes; for instance, if extreme rainfall were observed at week 1, the probability (OR) of detecting sapovirus at week 3 was of 1.94 (95% CI [1.28,2.97]) and at week 4 was 2.04 (95% CI [1.32,3.23]) among children with observed gastroenteritis. Sapovirus was more frequently observed in semi-urban areas with high poverty index. Ecological and environmental factors, sanitation infrastructure, and seasonal climate should be further explored to understand how these conditions may affect disease progression over time.

0862

HIGH LEVELS OF EXTENDED SPECTRUM BETA-LACTAMASE (ESBL) CONTAMINATION ARE FOUND IN MALAWIAN HOUSEHOLDS WITH BASIC WATER, SANITATION AND HYGIENE (WASH) ACCESS

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Low-middle income countries face the greatest burden of antimicrobial resistance (AMR), and WASH factors play an important role in the environmental dispersal and transmission of AMR. We recruited 300 households in urban, peri-urban and rural sites in southern Malawi between 2018-2020 and completed up to 4 visits at each household over a 6-month period. Samples of food, water, environment, co-located animal stool and human stool were taken at each household visit, to assess the prevalence and flux of ESBL bacteria in humans, animals and the environment. The broader environment of each household was also sampled including drainage and river water. 11,719 samples were cultured, which identified a high prevalence of ESBL *E. coli* (18.0%), and ESBL *K. pneumoniae* (8.9%) in all sample types, from each site, especially river water (67.2%), human stool (41.7%) and animal stool (29.3%). Evaluation of water sanitation and hygiene practice (WASH) illustrated low availability of hand washing facilities at the households, poor animal waste management practices and high levels of interactions between humans and animals with the local riverine environment. In order to reduce transmission of ESBLs between the human, animal and environmental nexus, a one health approach is required which focusses on the need for human and animal faeces management and containment at household and community level to reduce the risk of more widespread environmental exposure. This should be complemented by both infrastructural and behavioural improvements in hygiene practices to reduce faecal oral transmission. Further genomic assessment of these samples will identify the interaction between human, animal and environmental compartments, their relationship to locally circulating clinical strains in blood stream infections and key ecological niches of AMR.

0863

CHILD MOUTHING OF SOIL AND CONTAMINATED FOMITES AND UNIMPROVED SANITATION ARE ASSOCIATED WITH SUBSEQUENT POOR CHILD DEVELOPMENTAL OUTCOMES IN URBAN BANGLADESH (CHOBI7 PROGRAM)

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Millions of young children annually in low and middle income countries are not meeting their developmental potential. Water, sanitation, and hygiene(WASH) program delivery presents a potential approach to improve child developmental outcomes by reducing enteric infections which can lead to malnutrition and poor child growth. This prospective cohort study of 224-children under 5-years was conducted in urban Dhaka, Bangladesh to investigate the association between household WASH infrastructure and behaviors and subsequent child developmental outcomes. Developmental outcomes were assessed by communication, fine motor, gross motor, personal social, problem solving, and combined developmental scores measured by the Extended Ages and Stages Questionnaire(EASQ) at a 12-month follow-up visit. Children who put soil in their mouth at the majority of surveillance visits had significantly lower combined EASQ Z scores(coefficient: -0.53 (95% Confidence Intervals (CI): -0.83, -0.22)) at the 12-month follow-up visit. Children who put visibly dirty objects in their mouths at the majority of visits had significantly lower combined EASQ Z scores(-0.50 (95% CI: -0.79, -0.22)). Children in households with unimproved sanitation had significantly lower combined EASQ Z scores(-0.63 (95% CI: -1.11, -0.16)). Children found to put soil and visibly dirty objects in their mouths at the majority of household visits, and those that resided in households using unimproved sanitation had lower subsequent cognitive developmental outcomes. These findings demonstrate the importance of interventions targeting child mouthing behaviors and sanitation infrastructure in households to reduce exposure to fecal pathogens and improve child cognitive developmental outcomes for susceptible pediatric populations.

0864

EXPLORING POOR WATER, SANITATION, AND HYGIENE AS FACTORS RELATED TO LEPROSY TRANSMISSION IN MINAS GERAIS, BRAZIL

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While leprosy is associated with poverty, it is not completely clear which factors, such as substandard and crowded housing conditions, unsafe drinking water, poor sanitation, or limited access to health care are driving this association. Given data suggesting that water and contaminated environments could be reservoirs for *Mycobaterium leprae* and that co-infections with water, sanitation, and hygiene (WASH)-associated helminths may increase susceptibility to leprosy, we aimed to explore the association of WASH factors with leprosy. A case-control study was conducted in the municipalities in and near Governador Valadares, Minas Gerais, Brazil, between June 2016-December 2018. Individuals ages three years or older were recruited as cases or community-matched controls. Cases were diagnosed by dermatologic experts with confirmatory skin slit smears for the bacillary index. Questionnaires were administered on socioeconomic status, education, occupation, and WASH factors. Descriptive and statistical analyses were conducted to identify WASH associations with leprosy. Seventy-nine cases of leprosy and 81 controls (non-household contacts) were recruited of which 52.5% were male. 75.2% (n=112) of the participants had piped water as their water source, 54.5% (n=85) acknowledged they did not treat water. Multivariate logistic regression revealed an association with larger household sizes with leprosy (aOR = 1.34; 95% CI 1.07, 1.68), and an unexpected positive association with the presence of a piped sewer system (aOR=4.67; 95% CI 1.51, 14.45). In a contrast, those with leprosy were less likely to have household plumbing (versus a well or unimproved sources) (aOR=0.39; 95% CI 0.13, 1.18) or to treat their water (aOR=0.52; 95% CI 0.25, 1.06), although these did not reach statistical significance. These associations suggest that WASH factors could be related to leprosy and supports other emerging research in this field. Still, there is a need for further research on the association of WASH and leprosy disease, more specifically the potential mechanisms of bacterium exposure and viability of *M. leprae* in the environment.

0865

THE STATUS AND IMPACT OF WATER, SANITATION, AND HYGIENE RESOURCES ON SOIL TRANSMITTED HELMINTHS IN OGUN STATE, NIGERIA: AN IMPETUS FOR DEVELOPING A CROSS-SECTORAL FRAMEWORK

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The recent call on WASH implementers to maximize delivery of interventions for soil-transmitted helminths (STH) control or elimination requires the development of a cross-sectoral framework. To support such effort, there is need to document in real-time, the status of WASH resources and their impact on STH transmission. We therefore assessed the status of WASH and STH in 1,499 households across 33 spatially selected communities in Ogun State, Nigeria where such data are lacking. We identified four sub-specific components of WASH and objectively assessed metrics related to availability, accessibility, functionality and adequacy using a newly developed scoring tool. Fresh stool samples were also collected from household members and examined for helminths ova using concentration method. Data obtained were used to estimate prevalence and odds of exposures. We found three most common STH species; *Ascaris lumbricoides* (*Al* - 13.6%), Hookworms (Hk - 4.6%) and *Trichuris trichiura* (Tt- 1.7%). The percentage of those who had access to improved water facilities within their household (76%) was higher compared to sanitation (46%) and handwashing facilities (11%). Personal hygiene metrics also showed that open defecation (48%), walking barefoot (32%), dirty fingernails (40.6%), pica (39%) and sucking fingernails (10%) were common. Furthermore, the availability of improved water source reduced the odds of *Tt* (OR=0.18), while access to any type of water facility within the household premises increased the odds of *Al* (OR=1.72), *Tt* (OR=4.43) and Hk (OR=1.92). Availability of handwashing facilities reduced the odds of *Al* (OR=0.35). There were no significant relationship between sanitation and STH prevalence, However, sucking of nails and open defecation increased the odds of *Al* (OR=2.63) and Hk (OR=3.87, OR=1.95) respectively. This study has identified provision of improved water, handwashing facilities and hygiene education as drivers of reduced STH transmission. We believe these results can guide state-wide WASH intervention delivery, even in resource challenged areas with the potential to maximize impact on STH control.

0866

VECTOR COMPETENCE OF THE INVASIVE ASIAN LONGHORNED TICK, HAEMAPHYSALIS LONGICORNIS, FOR HEARTLAND VIRUS UNDER LABORATORY CONDITIONS

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The Asian Longhorned tick, *Haemaphysalis longicornis*, is native to eastern Asia but recently established populations in the United States where it continues to expand its geographic range. In its native range, *H. longicornis* is the main vector of the bandavirus, Severe Fever with Thrombocytopenia Syndrome virus (SFTSV). SFTSV is genetically closely related to Heartland virus (HRTV), an emerging North American tick-borne bandavirus that is increasing in human disease cases in the midwestern and southern United States since first reported in 2012. As the current geographic range and the predicted range expansion of invasive *H. longicornis* overlap considerably with human cases of HRTV, the objective for this study was to assess the ability of *H. longicornis* to maintain and transmit HRTV. In this study, adult female *H. longicornis* were microinjected in the anal pore with HRTV or media. Ticks were dissected at 14, 21, and 28 days post-injection (d.p.i.). At each time point, HRTV RNA was detected via qRT-PCR in the salivary glands and midguts of HRTV-injected ticks, and infectious virions were also detected in each tick. At 40 d.p.i., BALB/c mice were infested with 8 HRTV-injected *H. longicornis* females (1 tick per mouse), of which 5 ticks fed to repletion. Mice were monitored through 28 days post-tick attachment but did not display any clinical signs of disease. No HRTV RNA was detected in blood or terminal organs of mice exposed to HRTV-injected ticks; however, 4 of the 5 mice fed on by HRTV-injected ticks seroconverted, demonstrating host exposure to HRTV during tick feeding. The engorged female *H. longicornis* were maintained for oviposition. Transovarial and transstadial transmission of HRTV was demonstrated by detection of HRTV RNA in 100% of egg pools and larval pools, respectively. Together, these findings show that *H. longicornis* is a competent vector of HRTV. On-going studies are investigating whether non-viremic transmission of HRTV occurs between co-feeding invasive *H. longicornis* and native *Amblyomma americanum* ticks, as this could amplify transmission of HRTV in natural foci.

0867

A TRANSCRIPT CORRESPONDING TO A PREDICTED SECRETED PROTEIN AFFECTS FLAVIVIRUS INFECTION OF IXODES SCAPULARIS SALIVARY GLAND CULTURES

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Ixodes scapularis transmits a number of pathogens, including the agent of Lyme disease and tick-borne flaviviruses (TBFVs). TBFV infections cause thousands of human encephalitis cases worldwide each year. In the US, confirmed human infections with TBFV Powassan virus (POWV) are increasing and have a fatality rate of 10-15%. In addition, Langat virus (LGTV) is often used as an experimental model TBFV of low neurovirulence. Elucidation of tick-virus interactions may inform countermeasures to reduce TBFV transmission. Understanding the functional effect of tick salivary gland transcripts in TBFV infection within tick organs is limited. Organ cultures are an excellent system for studying virus infection in individual tick organs, and RNAi methodology in tick organ cultures can identify selected tick transcripts as possible countermeasure targets. Through dsRNA-mediated RNAi transcript knockdown studies, a transcript corresponding to a predicted *I. scapularis* secreted protein (SecP) limited LGTV and POWV infection in salivary gland (SG) cultures from unfed female ticks. Because transfection of dsRNA corresponding to a SecP transcript reduced infectious LGTV and POWV replication, SecP is considered to be proviral. Additionally, *in situ* hybridization-localized SecP transcripts were observed in SG granular acini of unfed female ticks. Further investigation into the role of SecP in TBFV infection is currently underway. Complementary studies with TBFV-infected *I. scapularis* are being planned. This research approach defines a translational pipeline by which tick transcripts/proteins can be assayed as targets for TBFV countermeasures. This research was supported by the Intramural Research Program of the NIH, NIAID.

0868

SHREW RESERVOIR HOST FOR DEER TICK VIRUS

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Deer tick virus (DTV) is a Powassan virus subtype found in eastern North America. It causes a severe severe meningoencephalitis and cases are increasingly reported where Lyme disease is endemic. Despite being described over 20 years ago, the enzootic cycle remains poorly studied. About 0.5%-5% of host seeking deer ticks, *Ixodes dammini*, are infected throughout their range. Because white-footed mice have long been considered to be the main host for subadult *I. dammini* as well as the reservoir for the agents of Lyme disease and babesiosis, these mice have been assumed to be the reservoir host for DTV. Serosurveys demonstrate that mice are exposed in nature, but definitive evidence (xenodiagnosis, virus isolation or detection by RT-PCR) has not been reported. We sought to identify the reservoir host of DTV using bloodmeal remnant analysis to directly detect the host upon which ticks had been infected. Infected host-seeking nymphal ticks likely acquired their infection while feeding on a viremic host as a larva. Accordingly, we screened host-seeking nymphs collected from 12 different sites in Massachusetts and Rhode Island from 2018-2020 and found 20 that were infected with DTV. Bloodmeal hosts were identified using our newly developed qPCR assay that targets mammalian retrotransposons of mouse, vole, bird, rabbit, shrew, squirrel/chipmunk or deer. The bloodmeal host was identified for 16 (80%) of the infected ticks; mice were not detected at all. Instead, shrews were identified from 65% of them. Furthermore, the general proportion of shrew-fed nymphs at a site was positively associated with the prevalence of DTV in ticks in a site (p=0.006). We conclude that shrews are likely reservoir hosts for DTV.

0869

POPULATION-WIDE FALL IN SERIOUS BACTERIAL COMPLICATIONS FOLLOWING IVERMECTIN-BASED MASS DRUG ADMINISTRATION FOR SCABIES

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Scabies, with a global prevalence of 200 million people, is the predominant precipitant of impetigo in endemic settings. Impetigo can progress to serious bacterial complications including skin and soft tissue infections, invasive infections and post-infectious sequelae. Ivermectin-based mass drug administration (MDA) can significantly reduce scabies and impetigo prevalence in endemic settings. We aimed to ascertain the impact of this intervention on the serious bacterial complications of scabies. We conducted a before-after trial, administering MDA in Fiji's Northern Division (population: 131,914). We measured the incidence of hospital admissions with skin and soft tissue infections, invasive infections and post streptococcal sequelae over 12 months prior the intervention and compared this with the incidence over the 12 months post. We also compared the incidence of primary healthcare presentations with scabies and skin infections, and the prevalence of scabies and impetigo in the community before and after the intervention. The coverage of MDA achieved was 97% and 87% for the first and second doses respectively. The incidence of hospitalizations with skin and soft tissue infections fell by 17% in the year following the intervention (467.3 to 388.7 per 100,000 person-years, incidence rate ratio 0.83, 95% CI, 0.74 to 0.94; P=0.002). There was a 21% decrease in presentations to primary healthcare with scabies and skin infections (108.3 to 89.2 per 1,000 person-years, IRR 0.79, 95% CI, 0.78 to 0.82). The prevalence of scabies fell from 14.2% to 7.7% (cluster-adjusted prevalence ratio 0.71; 95% CI, 0.28 to 1.17) and impetigo prevalence reduced from 15.3% to 6.1% (cluster-adjusted prevalence ratio 0.4; 95% CI, 0.18 to 0.86). The results of this study demonstrate the extended clinical and public health benefits of reducing scabies prevalence with ivermectin-based MDA through substantial reductions in serious bacterial complications and healthcare burden. Our findings support endemic settings and international health agencies towards prioritising scabies control.

0870

GENERATION OF TRANSFECTED BABESIA BOVIS EXPRESSING AN ANTI-TICK TOXIN TO CONTROL TICKS

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Ticks cause significant economic losses to livestock directly and through transmission of pathogens. Vaccines to control ticks or tick-borne pathogens are urgently needed. Bio-insecticides such as protein spider toxins have potential to reduce tick burdens if they can be efficiently delivered. We propose a novel approach for delivery of protein toxins based on expression by transfected *B. bovis* exclusively during tick specific stages of the parasite. In addition to killing ticks this would also confer protection against disease. Expression of the toxin gene at the appropriate time can be achieved if it is under the transcriptional control of a *B. bovis* tick-stage specific promoter. We first tested this model by generating stably transfected *B. bovis* parasites containing a constitutively expressed promoter and a previously identified *B. bovis* kinete specific promoter controlling the expression of green and red florescent proteins,

respectively. Successful expression of eGFP and RFP by the transfected parasites was confirmed by genotypic and phenotypic characterization. Comparison of the levels of expression of eGFP and RFP in blood stage *B. bovis* parasites developed in *in vitro* cultures showed significantly reduced activity of the kinete-specific promoter compared to the constitutive promoter. Future experiments using this transfected parasite line will be aimed at confirming the activity of the promoter in ticks. These studies suggest that this promoter is a viable candidate for developing vaccines based on transfected *B. bovis* strains with tick-stage specific expression, which could become a valuable tool in our arsenal for fighting ticks and tick-borne diseases.

0871

ISOLATION OF HEARTLAND VIRUS IN FIELD-COLLECTED TICKS, GEORGIA

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Heartland Virus (HRTV) is an emerging tick-borne arbovirus transmitted by *Amblyomma americanum* ticks, recently discovered, linked to more than 40 cases of moderately severe to fatal human disease across 9 US States. In Georgia, evidence of HRTV circulation dates to at least 2001, based on seroprevalence studies of white-tailed deer. A fatal human case from 2005 was identified retrospectively by the CDC. Here, we report the finding of HRTV infection in *A. americanum* ticks from Georgia. The study area was a rural landscape comprising 64 km2 in central Georgia, where historical data indicated HRTV exposure in white-tailed deer. Questing adult and nymphal stages were collected by flagging. Ticks were identified to species morphologically and vialed in pools. RNA was extracted from pooled homogenates and screened using real-time PCR specific for HRTV. Isolation of the virus was attempted in Vero E6 cells using homogenates positive by PCR. A total of 6,398 ticks were collected from the study area in 2019 and grouped in 677 pools. Three pools of *A. americanum* (two adult pools and one nymph pool) were positive for HRTV by real-time PCR (0.4%).). No other tick species (*Amblyomma maculatum, Dermacentor variabilis, Ixodes scapularis*) were found infected with HRTV. HTRV was successfully isolated in Vero E6 cells from 2 of the positive pools collected from 2 separate sites. The identity of each HRTV isolate was confirmed by whole-genome sequencing. The isolates were much more similar to one another than to the three other available HRTV genomes. It is common for tick-borne arboviruses, especially when their emergence or diagnosis is recent, that the occurrence of human disease is sporadic and often underdiagnosed. Unfortunately, little is known about HRTV eco-epidemiology, including their main reservoirs, seasonality of transmission, and drivers of population persistence. Our findings confirm the presence and persistence of HRTV in Georgia and represent the first isolate of HRTV in the southeastern US. Further work is needed to better assess the risk for human disease in the region.

0872

OUTDOOR ACTIVITIES IN THE TIME OF COVID-19 AND ITS IMPLICATIONS FOR EXPOSURE TO TICK-BORNE DISEASES IN THE UNITED STATES

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During the COVID-19 pandemic, governmental and individual responses to COVID-19 risk were focused on behavioral changes to reduce transmission, but inadvertent consequences of such behavioral changes are yet to be fully realized. Herein, we evaluated changes in people's outdoor activity patterns during the spring and summer of 2020 in the Northeast and Midwest United States by comparison with the same period in 2019. Because changes in outdoor activity patterns can lead to changes in vector-borne disease risk, we simultaneously assess changes in exposure to tick vectors, and its association with outdoor activities. We used longitudinal self-reported data collected from daily surveys available in The Tick App, a smartphone application designed to assess outdoor activity patterns in relation to tick exposures. The daily proportion of users reporting any type of outdoor activity, including peridomestic activities, increased by two-fold in 2020 vs. 2019, while recreational activities in green spaces decreased by 70%. Meanwhile, self-reported tick encounters significantly increased by 30% in 2020 compared to 2019, and were mainly associated with outdoor activities and living in a rural county. Changes in daily activity patterns were associated with statewide stay-at-home orders in 2020 and persisted despite increased out-of-home mobility measured at the county-level (i.e., Shelter-in-Place Index). By contrast, individual self-reported impacts on outdoor activities (any impact vs. no impact) were positively associated with the Shelter-in-Place Index and if the participant lived in an urban area. Obtaining granular information on activity patterns proved useful in gaining insights about potential changes in the risk of tick-borne disease exposure during 2020, associated with behavioral changes driven by COVID-19 pandemic. Our findings suggest that public health response should address competing hazards from COVID-19 related outdoor exposure and the increasing risk of tick- and other vector-borne diseases associated with increased outdoor activity.

0873

LOIASIS-MEDIATED FILARIASIS TEST STRIP POSITIVITY IS STABLE OVER ONE YEAR

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The Filariasis Test Strip (FTS) is an essential tool to monitor lymphatic filariasis (LF) elimination efforts. FTS cross-reactivity mediated by *Loa loa* circulating filarial antigens (*Loa*-CFAs) is an obstacle to LF elimination in coendemic regions. Cross-reactive FTS results correlate with increasing *L. loa* microfilaria (Mf) loads, and the protein profile of *Loa*-CFAs suggests FTS-positivity stems from intermittent release of *Loa*-CFAs from dying worms. To further characterize the nature of FTS cross-reactivity, we collected serial plasma samples from a cohort of individuals with high *L. loa* Mf loads (>20,000/mL) in the Okola health district in Cameroon, a region endemic for loiasis but not LF. Seventy (52%) of 135 individuals screened tested positive by FTS. Mf counts were higher among FTS-positive participants compared to the FTS-negative group (median 34,450 versus 10,180, p-value <0.0001 by unpaired t-test). All FTS-positive and 13 FTS-negative participants were followed quarterly for one year to better understand the temporal dynamics of antigenemia. The FTS status registered at baseline remained stable for 80% of the sixty-eight participants who attended all study visits. *Loa*-CFA levels were quantified by an ELISA using the FTS antibody AD12, or the Og4C3 antibody which recognizes the same AD12 core epitope. At baseline, 66 (90%) of the FTS-positive group and 12 (92%) of the FTS-negative group were AD12 ELISA positive, while 25% and 31% of the FTS-positive and FTS-negative groups, respectively, were Og4C3 ELISA positive. Neither baseline Mf counts, nor ELISA results were predictive of FTS status changes. The best predictor was the baseline FTS score, a semiquantitative measure of the amount of circulating antigen. Subjects who converted from FTS-positive

to negative, scored lower (score 1) than those who remained FTS-positive (score 2-3). These findings suggest that FTS cross-reactivity in loiasis patients with high Mf counts is relatively stable over time and stress the importance of developing more specific diagnostic assays to aid public health interventions for LF and loiasis.

0874

OPTIMIZED QPCR STRATEGY FOR THE MOLECULAR DETECTION OF ONCHOCERCA VOLVULUS IN BLACKFLIES

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Global elimination of onchocerciasis remains a lofty but realistic goal, yet there are obstacles that need to be addressed if the ambitions of the global health community are to be met. In this study, we address one such obstacle: the need for diagnostic tools of increased sensitivity and species-specificity that are both optimized and standardized. Current guidelines governing when to stop mass drug administration, and post-treatment surveillance strategies, include screening pools of the blackfly vector for the presence of infectious *Onchocerca volvulus* larvae. Molecular screening is non-invasive and cost effective, since large numbers of insects can be tested at once. However, as the prevalence of disease decreases, the sensitivity of the assay becomes more critical, as infrequent pathogen signal can be missed by sub-optimal detection strategies. A potential confounding factor, the bovine parasite *Onchocerca ochengi,* which is genetically similar to *O. volvulus,* can also be found in blackflies. We have tested 4 qPCR primer/probe combinations in 3 different laboratories and with different reagents to identify a screening approach with optimal sensitivity and specificity. The most sensitive assay targeted the *O. volvulus* O-150 repeat and has a limit of detection of 0.01 pg and consistently amplified as few as one *O. volvulus* L3 in a pool of 100 blackfly heads. However, this assay did cross amplify *O. ochengi* genomic DNA at high concentrations (>1 ng/µL). A qPCR assay with a mitochondrial target (ND5) had lower sensitivity but did not amplify *O. ochengi* DNA, even at concentrations up to 25 ng/µL. Thus, we have determined that using qPCR targeting O-150 for primary testing, followed with a confirmatory ND5 qPCR assay, will provide an optimal diagnostic for *O. volvulus* screening in areas of low endemicity. SOPs for DNA extraction and qPCR have been developed and optimized. Aiming to export this diagnostic tool for use in endemic locations, we have designed the assay to be shelf-stable, centrally produced, and easily shipped, allowing for increased standardization and reagent availability while minimizing logistical costs and challenges.

0875

LYMPHATIC FILARIASIS ELIMINATION IN SAMOA: EVALUATING THE USE OF MOLECULAR XENOMONITORING AS A SURVEILLANCE TOOL

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The World Health Organization recently recommended triple-drug mass drug administration (ivermectin, diethylcarbamazine, albendazole: IDA) in areas with slow progress towards lymphatic filariasis (LF) elimination. Samoa distributed the first round of IDA in 2018. Antigen (Ag) prevalence was 4.0% (2.8-5.6%) in 2018, and 4.1% (2.8-5.9%) post-IDA in 2019. The 2019 'Surveillance and Monitoring to Eliminate Lymphatic Filariasis and Scabies from Samoa' project consisted of human and mosquito surveys to identify the best indicators for post-IDA surveillance. Delays in Ag clearance post-IDA limits its usefulness as an indicator in the immediate post-IDA period, and molecular xenomonitoring (MX) may be more sensitive in this setting. We compared estimated prevalence of mosquitoes containing *Wuchereria bancrofti* DNA pre- and post-IDA and investigated associations between PCR+ mosquitoes and Ag+ humans. Mosquitoes were collected from 35 villages using BG Sentinel traps and sorted into 9 categories: *Aedes polynesiensis* (main vector), *Ae. aegypti, Ae. albopictus, Ae. upolensis, Ae. (Finlaya) spp*., other *Ae. spp, Culex quinquefasciatus* and other *Culex spp*. Pools of ≤25 mosquitoes of each category were tested. Analyses were conducted for each category, 'all *Aedes*' and 'all species' using novel PoolTestR software that adjusts for sample hierarchical clustering. Of 2,638 pools (total 34,299 mosquitoes), 6.5% were PCR+. Pools with highest PCR-positivity were *Ae. polynesiensis* (14.1%) and *Ae. aegypti* (7.0%). Estimated infection prevalence in Samoa in 2019 was 0.8% for *Ae. polynesiensis, 0.6% for Ae. aegypti*, 0.6% for 'all *Aedes*' and 0.4% for 'all species', a reduction from 2018 (1.2%, 1.4% and 0.8%, respectively). 'All species' proved most sensitive for detecting villages with Ag+ humans, with PCR+ pools identified in 78.6% of these villages, compared to 67.9% for 'all *Aedes*' and 64.3% for *Ae. polynesiensis*. PCR+ *Ae. polynesiensis* pools provided the highest positive predictive value (81.8%) of villages with Ag-positive people. Our study provides promising evidence to support the value of MX (even using 'all species') in post-MDA surveillance.

0876

ESTABLISHMENT AND VALIDATION OF A REAL-TIME PCR FOR ABSOLUTE QUANTIFICATION OF WOLBACHIA ENDOSYMBIONTS OF ONCHOCERCA VOLVULUS IN SAMPLES WITH VERY FEW TO A SINGLE MICROFILARIA

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The neglected tropical disease Onchocerciasis (river blindness) affects 21 mio people in Sub-Saharan Africa and a few loci in Latin America. It is caused by the filarial nematode *Onchocerca volvulus* which lives in symbiosis with the Gram-negative bacteria *Wolbachia.* Targeting the endosymbiont *Wolbachia* using antibiotics leads to permanent sterilization of female worms and an adulticidal effect. The depletion of *Wolbachia* precedes the adulticidal effect. Quantifying *Wolbachia* after therapy is critical for clinical trials on antiwolbachial regimens. The gold standard to assess *Wolbachia* depletion is histological examination of adult worms in subcutaneous nodules 20-27 months after treatment. A diagnostic tool for detecting *Wolbachia* depletion at an earlier time point in microfilariae (MF) would accelerate new antiwolbachial drug clinical trials. Successful treatment will result in loss of MF, therefore the assay must work with few MF. We have established a real-time PCR (qPCR) for quantifying *Wolbachia* in *O. volvulus* MF that had migrated from skin biopsies. Reproducibility of the procedure was tested on *Litomosoides sigmodontis* MF in blood of infected mice. After counting MF with an inverse microscope, DNA was extracted using a modified protocol of the QiaAmp DNA Micro Kit protocol (Qiagen). *Wolbachia* were quantified by amplifying the single-copy gene *ftsZ* with specific primers and a Taqman® probe.

Reaction efficiency was determined by a 1:10 dilution series of plasmid containing the target sequence. For absolute quantification linearized plasmid standards and synthetic DNA fragments (gBlocks®) were used. The qPCR enabled reproducible *Wolbachia* quantification in isolated MF. The detection limit of the diagnostic tool was 1 MF. Detection of *ftsZ* in 1 MF was possible in 66.7% in field isolates and was 100% in DNA extracted from >2 MF. By using linearized plasmids the qPCR delivered an absolute quantification of *Wolbachia* endosymbionts per MF. The qPCR is reliable for quantifying *Wolbachia* in isolated MF from skin biopsies. Its establishment provides a tool for early treatment monitoring (≤6 months) of new antiwolbachial treatments.

0877

HIGH-THROUGHPUT MOLECULAR XENOMONITORING USING AN AMPLICON SEQUENCING-BASED APPROACH TO PATHOGEN DISCOVERY

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Molecular xenomonitoring (MX) is the molecular testing of vector insects for the presence of pathogen. In recent years, recognition of the utility of this approach has grown, resulting in its adaptation by various tropical disease control and elimination programs as a means of augmenting traditional monitoring and surveillance methods. However, the full potential of MX remains unrealized, as limitations to throughput of testing, vector capture, and cost of testing represent obstacles for many applications. In an attempt to mitigate these challenges, we have developed an amplicon sequencing-based approach to MX, which allows for high-throughput screening of large numbers of mosquito pools as part of a single next-generation sequencing (NGS) reaction. Employing a conserved 18S ribosomal sequence target and "blocking" primers, which prevent amplification of both mosquito and mammalian host sequences, this method allows for the amplification of all eukaryotic species harbored by the mosquito host. Amplification occurs in a universal fashion, meaning prior knowledge of potential targets is not required. In this manner, surveillance is integrated, providing a high-throughput, cost-minimized approach to the screening of mosquito pools, while enabling the identification of all eukaryotic pathogens present in the pools, whether or not they are vectored by the mosquitoes being tested. To demonstrate proof-of-concept for this approach, we screened 50 DNA extracts isolated from mosquito pools previously collected in Zanzibar as part of an unrelated study. NGS-based amplicon sequencing identified the presence of multiple pathogens, including the filarial pathogen *Wuchereria bancrofti*, the presence of which was confirmed in 24 samples using qPCR. Utilization of this technique should enable entire xenomonitoring studies to occur as part of a single NGS reaction. Testing of as many as 25,000 mosquitoes in a single sequencing run is possible, and at an estimated cost of under $2,000 per study, a per-mosquito cost of $0.08 is realized, with each mosquito screened for all eukaryotic pathogens present in the study's sample population.

0878

MOLECULAR DIAGNOSTICS FOR MONITORING HUMAN LYMPHATIC FILARIASIS USING CIRCULATING CELL-FREE NUCLEIC ACIDS IN BODY FLUIDS

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Detecting parasitic circulating cell free nucleic acids (ccfDNA/ccfRNA) in plasma is a promising approach for sensitive and specific detection of active helminth infection including *Wuchereria bancrofti* (Wb). Ccf nucleic acids, given their short half-lives, may be better biomarkers for assessing infection status than more traditional approaches (e.g, circulating antigen). To identify potential Wb RNA targets, we performed Plasma-RNAseq using plasma from 10 Wb microfilaria-positive (mf-positive) individuals and 10 healthy blood bank individuals and used bioinformatic tools to ensure specificity. Six targets were identified that were specific to *Wb* and/or *Brugia malayi (Bm),* the causative agents of LF, and not found in *Loa loa (Ll)* or *Onchocerca volvulus (Ov),* closely related filarial parasites. These targets were compared to the previously described DNA-based targets WbLDR and WbTR1 using newly optimized qPCR assays. After optimizing primer/probe combinations using genomic DNA or RNA from Wb or Bm, we demonstrated that ccfDNA and ccfRNA can be detected in Wb-infected individuals. Because the DNA-based assays appeared to be more sensitive, we next assessed the utility of the most sensitive ccfDNA qPCR assay as a biomarker of active Wb infection. Thus, we extracted ccfDNA from 250 µl of plasma from individuals with Wb infection from India (n=19), Cook Islands (n=71), Mali (n=13), and Haiti (n=1) and Guyana (n=2). Of all the mf-positive individuals, 71 percent were positive for ccfDNA using WbTR1 qPCR; as expected endemic uninfected controls and those with *Mansonella perstans* and/or *Ll* were negative. To understand the kinetics of Wb-specific ccfDNA following treatment, we assessed ccfDNA longitudinally following definitive treatment. In a small number of patients we demonstrated, in time course analyses, that patients had undetectable levels within a year of definitive treatment. Results from different Wb treatment regimens are underway. Overall, ccfDNA/RNA detection in LF holds promise for assessment of infection and treatment response in Wb and Bm infection.

0879

MOLECULAR EPIDEMIOLOGY OF FILARIAL NEMATODES ON BIOKO IS LAND USING NUCLEIC ACIDS EXTRACTED FROM MALARIA RAPID DIAGNOSTIC TESTS (RDTS)

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Implementation of an effective and targeted mass drug administration program against filarial worms needs an accurate identification and mapping of the distribution of these parasites. Molecular diagnostic methods like polymerase chain reaction (PCR) based techniques have shown high sensitivity to detect and distinguish parasites at low levels and even in people amicrofilaremic by microscopy. However, larger scale implementation over time remains a challenge for low-income countries because of logistical complexity and costs, including collection, transportation and preservation of biological samples and running of the analytical tests. To overcome these concerns, we developed a new approach based on the use of nucleic acids (NA) extracted from dried blood retained on used malaria rapid diagnostic (RDT) tests. People of all age groups were recruited during malaria indicator survey carried out in 2018 on Bioko Island (Equatorial Guinea). This yearly survey is based on a detailed questionnaire and includes malaria testing based on malaria RDT. RDTs were barcoded, stored at room temperature, NA was extracted from the roughly five microliter of venous blood retained followed by a multiplex qPCR assay to detect *Mansonella perstans*, *Loa loa* and a control human gene. We identified NA encoding for filarial worms among 254 (8.5%) of the 3214 RDTs screened. Prevalence of *M. perstans* and *L. loa* were 7.1% and 1.6%, respectively. Co-infections between *Plasmodium* spp and *M. perstans* were found in 35 (4.6 %) of 764 *Plasmodium* spp positive RDTs. Elderly male of the lowest socioeconomic quintile and living in rural areas of Bioko Island were affected most by *M. perstans* infection. Our study shows for that malaria RDTs stored at room temperature for around 6 months can be used as source for NA to detect other blood-dwellings pathogens.

0880

USE OF A NOVEL PLASMODIUM VIVAX CHIMERIC PROTEIN FOR MALARIA SEROSURVEILLANCE IN MULTI-SPECIES ENDEMIC COUNTRIES NEARING ELIMINATION

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Reliable estimates of malaria transmission are needed to achieve elimination targets. Detection of anti-malarial antibodies provide exposure history, but previous studies have relied largely on species-specific antigens. The use of pan-*Plasmodium* antigens in serological surveys could provide data for exposure to all *Plasmodium* species circulating in an area. We have recently described a chimeric protein based on *P. vivax* merozoite surface protein 1, designated *P. vivax* recombinant modular chimera based on MSP1 (PvRMC-MSP1), which includes five broadly recognized T cell epitopes, two T helper epitopes from the PvMSP1 33kD fragment, the PvMSP1 19kD fragment, and a $(NANP)_6$ sequence representing the central repeat of the *P. falciparum* circumsporozoite protein. PvRMC-MSP1 binds IgG from a majority of returning US travelers with PCR-confirmed malaria infection regardless of the infecting *Plasmodium* species. Using a multiplex bead-based assay, we present seroprevalence of anti-PvRMC-MSP1 IgG antibodies in population-based surveys using from Ethiopia (n=8,944) and Costa Rica (n=852) collected from household-based cross-sectional studies. Ethiopia is endemic for all four human malarias, and Costa Rica is historically endemic for *P. vivax* but is nearing elimination. Seroconversion rates were calculated for both datasets and were determined to be 0.023 for PvMSP1 and 0.03 for PvRMC-MSP1 in Costa Rica. In Ethiopia, seroconversion rates were 0.044 for PvMSP1 and 0.106 for PvRMC-MSP1. Seroprevalence in Costa Rica was 41.5% for PvMSP1 and 46.7% for PvRMC-MSP1. In Ethiopia, seroprevalence of PvMSP1 was 20.6%, but was 34.5% for PvRMC-MSP1, and comparison of IgG levels showed higher titers to PvRMC-MSP1 when compared to PvMSP1 in both studies. In Ethiopia, the seroprevalence of PfMSP1-specific IgG antibodies was higher than that of PvRMC-MSP1 and PvMSP1 regardless of age, consistent with the relatively high predominance of *P. falciparum* infections in the region. Our data suggest that PvRMC-MSP1 may be able to detect both prior *P. falciparum* and *P. vivax* infections and display higher sensitivity in detecting past malaria exposure.

0881

FIELD ASSESSMENT OF QUANTITATIVE POINT-OF-CARE G6PD ACTIVITY ASSAY—KAMPONG SPEU, CAMBODIA, 2019-2020

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Primaquine and tafenoquine remain the only effective drugs to eliminate the persistent liver stage of *Plasmodium vivax*, thereby providing radical cure for malaria. Both medications may also cause hemolytic anemia in individuals with glucose-6-phosphate dehydrogenase (GP6D) deficiency, a common genetic disorder in many malaria endemic areas. Most methods for screening of G6PD deficiency require special laboratory equipment and can be costly or time-consuming. However, recent advances in G6PD point-of-care (POC) testing aim to improve access to radical cure by accurately identifying individuals at risk of hemolytic anemia secondary to primaquine or tafenoquine therapy. We conducted a clustered, cross-sectional community survey of 840 adults living in 30 villages in Kampong Speu Province, Cambodia to determine performance characteristics of the quantitative STANDARD™ G6PD (SD Biosensor, Suwon, Republic of Korea) POC assay in this population. G6PD activity and hemoglobin concentration in capillary blood were measured at the time and site of collection using the POC assay. Laboratory references for each participant were measured by spectrophotometry and hematology analysis of a corresponding venous specimen in EDTA within three days of collection. Using the nationally proposed G6PD activity threshold for primaquine administration in Cambodia of 6 Units/gram hemoglobin, field diagnosis of individuals with ≤ 30% median G6PD activity by spectrophotometry was 93% (85–100%) sensitive with a negative predictive value (NPV) of 99% (98—100%). Field diagnosis of individuals with ≤ 70% median G6PD activity was 65% (53—78%) sensitive with an NPV of 88% (81—94%). Areas under the curve by receiver operator curve analyses were 0.96 and 0.84, respectively. Though data collection on assay performance among febrile individuals presenting for care at health facilities is ongoing, these results suggest that this POC assay is capable of accurately identifying those at the highest risk of primaquine-induced hemolytic anemia.

0882

IMPACT OF MALARIA RAPID DIAGNOSTIC TESTS SCALE-UP ON ANTIBIOTIC PRESCRIBING AND MORTALITY IN SUB-SAHARAN AFRICA

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The massive scale-up of malaria rapid diagnostic tests (RDTs) in sub-Saharan Africa over the past 10 years is believed to reduce antimalarial overuse and to improve febrile case management. Yet to date there have been no rigorous analysis on whether malaria RDTs scale-up diverts antimalarial overuse to other antimicrobials when test results are negative, especially antibiotics. To estimate the impact of RDTs on antibiotic and antimalarial use and the resulting health implications, we pair country-specific data from the annual operational reports and procurement records from two major donor agencies, the Presidential Malaria Initiative and the Global Fund, with a sample of 125,000 pediatric fever episodes from 42 national household surveys across 13 sub-Saharan Africa countries from 2009-2019. We exploit geographic variation in total number of RDTs distributed via the two donor agencies as an instrument for RDTs take-up. We find that the number of RDTs distributed from donor agencies predicts both blood test take-up at individual level and RDTs use at country level. We find that receiving RDTs increased antibiotic prescribing for febrile children, while antimalarial prescribing remained unchanged. We also examine the implications of receiving RDTs on all-cause mortality among febrile under-five children and find no statistically significant impact on all-cause mortality. However, our results show a large and statistically significant mortality reduction in lower respiratory tract infections concentrated in areas with low RDTs positivity rates. In contrast, respiratory disease mortality increased in areas with high RDTs positivity rates. These results provide the first large-scale causal evidence on the effect of RDTs scale up on the antibiotic prescribing and the resulting health implications in sub-Saharan Africa.

0883

PLASMODIUM FALCIPARUM HRP2|HRP3 DELETION TYPING BY NOVEL DIGITAL PCR METHOD: AN UPDATE ON THE STATUS AND DISTRIBUTION OF DELETIONS IN AFRICA AND SOUTH AMERICA

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The most used *Plasmodium falciparum* rapid diagnostic tests (RDT) target the Histidine-Rich Protein 2 (HRP2) antigen. However, an increasing number of countries report parasites that carry *hpr2|hrp3* gene deletions, which makes them undetectable by these RDTs. Molecular surveillance of *hrp2|hrp3* deletions is thus crucial but proving the absence of a gene is difficult using conventional PCR techniques. We have developed a novel assay for deletion typing based on digital PCR (dPCR), a highly accurate method to quantify DNA. The assay was optimized on 2 digital PCR systems (BioRad QX200 and Qiagen Qiacuity). The latter allows detection of *hrp2|hrp3* deletions in a single tube. To compare the assay to conventional protocols relying on nested PCR (nPCR) and visualization of the product on gel, 248 samples from asymptomatic infections from western Kenya were screened in triplicate by dPCR and nPCR. For the nPCR, *msp2* was run as control, also in triplicate. Eight percent of samples had a band for *msp2* in all replicates, but no band for *hrp2* in any replicate. Thus, a prevalence of deletion of 8% was observed by nPCR. No deletions were observed by dPCR, indicating false negative results by nPCR. In addition, mixed infections with wild type and *hrp2* deleted parasites presented a challenge for surveillance, as the wild type parasite could mask the presence of the deletion. By dPCR, mixed infections were reliably detected when the proportion of parasites carrying the deletion is >40%. We screened 819 samples from Kenya (248), Tanzania (152), Ghana (98), Ecuador (54), Brazil (209), and southwestern Ethiopia (58) by dPCR. No deletions were observed in samples from Kenya, Ghana, and Tanzania. In Ethiopia 2.1% carried a *hrp2* deletion, and 76.9% *hrp3* deletions (2% *hrp2|hrp3* double deletion). In Brazil, 41% of samples carried *hrp2* deletions, and 53% *hrp3* deletions (39% *hrp2|hrp3* double deletions). In Ecuador, no *hrp2* deletions were observed, but 47% of samples carried *hrp3* deletions. In conclusion, dPCR allows for rapid and highly accurate typing of *hrp2|hrp3* deletions. The assay is currently being set up in Ethiopia for molecular surveillance of *hrp2|hrp3* deletions across Africa.

0884

THE NEAR-INFRARED SPECTROSCOPY TECHNIQUE CAN NON-INVASIVELY DETECT MALARIA PARASITES THROUGH THE SKIN OF MICE

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The near-infrared spectroscopy (NIRS) technique has been applied in various studies to non-invasively predict mosquito age, species identity and to detect infections such as *Wolbachia*, Zika, Dengue and malaria in mosquitoes. Here, using a *Plasmodium berghei*-mouse model, we have demonstrated for the first time that NIRS can also non-invasively, through the skin detect and quantify malaria parasites in mice. The technique simply involves shining a beam of infrared light for approximately 5 seconds on a mouse body part, which partially penetrates the skin, and subsequently collecting a reflected spectral signature. Machine learning techniques were then applied on the spectral signatures to differentiate *P. berghei* infected from uninfected mice and to predict parasitaemia of infected mice. A total of 118 mice were used in this experiment. Half were randomly assigned to a control group (i.e. uninfected) and the remaining half to an infected group. The infected group was intraperitoneally injected with *P. berghei* infected red blood cells (RBC) while the control group were injected with uninfected mice RBC. All mice were scanned non-invasively with a NIR spectrometer at pre-parasite inoculation and again at 24, 48 and 72 h post inoculation. Two spectral signatures were collected from the ears, feet, groin and tail of each mouse at each time point. Models were built to differentiate infected from uninfected mice at each time point and to predict parasitaemia of the infected mice. The positive predictive rate (PPR) and negative predictive rate (NPR) of NIRS was 90% and 100% when parasitaemia levels were ≥2%. However, the PPR ranged from 40-55% and the NPR ranged from 47-75% at lower parasitaemia levels. Surprisingly, NIRS predicted parasitaemia values were consistent with microscopy results with parasitaemia ≥2%. The findings of this study are an important first step towards development of the NIRS technique for diagnosis of malaria in the field. If successful, NIRS will provide a non-invasive, reagent-free and rapid platform for screening hundreds of people per day in malaria endemic areas and guide malaria elimination strategies.

0885

POTENTIAL APPLICATION OF THE HEMATOLOGY ANALYZER XN-31 PROTOTYPE IN A FIELD MALARIA SURVEILLANCE

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Early and accurate diagnosis is a key component in malaria control program. Microscopy is the current gold standard; however, it requires training of the examiner, and the results largely rely on the skill of the microscopists. Rapid diagnostic test (RDT) can be performed without training and much faster, but not quantitative. Moreover, recently false negative due to HPR2 deleted parasites are reported. XN-31 prototype (XN-31p), developed by Sysmex cooperation, is an automated hematology analyzer which enables assistance in malaria diagnosis. It can also provide the information of species differentiation and stage specific parasite counts. The machine has a system to analyze the whole blood sample in a minute without any sample preparation. Previous report evaluated this with venous blood samples. Here we test the applicability of this to capillary blood samples and evaluate the effect of sample storage time and temperature on the stability of results. 168 clinically malaria suspected

outpatients in Homa Bay County Referral hospital, Kenya were enrolled in the study. Capillary blood sample was collected from each participant together with venous blood sample. Both samples were tested for malaria with XN-31p, microscopy, RDT, and PCR. The measurement in XN-31p for capillary blood samples was repeated after 6 and 24 hours by storing the samples in room temperature or 4 degrees Celsius. The sensitivity and specificity of XN-31p with capillary blood sample against PCR were 85.7% and 100% respectively and were comparable to that of microscopy and RDT. There was no discordant result between venous blood sample and capillary blood sample in XN-31p. Parasitemia together with major complete blood count are stable under both room temperature and 4 degree even after 24 hours. The results showing the capillary blood samples can be used for this analyzer makes the diagnosis with XN-31p more practical. Furthermore, the stability of the results even under room temperature up to 24 hours gives us the option of transporting and measuring samples collected in a remote area and broaden the applicability of XN-31p not only in the hospital but also in the field survey.

0886

IDENTIFYING FUTURE PLASMODIUM VIVAX RELAPSES WITH SEROLOGICAL MARKERS OF EXPOSURE IN A RETURNING INDONESIAN SOLDIER COHORT

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Distinguishing *Plasmodium vivax* primary infections from mosquito bites and relapse infections caused by re-activation of dormant hypnozoite parasites from a previous primary infection is not possible with current diagnostic tools. Serological markers of exposure to *P. vivax* infection and hypnozoite carriage have been previously identified using longitudinal data and developed into a diagnostic tool. This study aims to further validate this diagnostic tool follow-up of returning Indonesian soldiers from the malaria endemic area of southern Papua to the non-endemic area in East Java, Indonesia. Soldiers who were microscopically diagnosed as free of acute *P. vivax* infection voluntarily participated in the study. They were recruited a few weeks after returning to East Java and followed for 24 weeks. Blood samples were collected on the day of recruitment, during regular follow-up time points and at times of symptomatic relapses. Microscopy diagnosis was conducted on all samples. Serology with a Luminex assay for 19 markers was conducted in all enrollment samples and in a sub-sample of soldiers also in all follow-up samples. The diagnostic tool based on a machine learning Random Forest classification algorithm was used to classify the sero-status of samples during the previous 9 months. A total of 294 soldiers were enrolled in the study in December 2018, of which 24 relapsed during follow-up. Another 45 non-relapsing soldiers were detected by PCR and serology in Eijkman Institute for Molecular Biology, Jakarta. Soldiers who relapsed had higher median antibody titers for several of the biomarkers compared to those who did not relapse. The 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. This study demonstrates that serological markers can identify people at risk of *P. vivax* relapses.

0887

PURIFIED INACTIVATED ZIKA VIRUS VACCINATION IN VOLUNTEERS PREVIOUSLY IMMUNIZED WITH JAPANESE ENCEPHALITIS VACCINE OR YELLOW FEVER VACCINE IN A DOUBLE-BLIND, RANDOMIZED, PLACEBO CONTROLLED CLINICAL TRIAL

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Zika virus (ZIKV) infection is strongly associated with major birth defects and serious neurologic complications. A ZIKV vaccine is therefore an important global health priority. To this end, we developed a Zika purified inactivated vaccine (ZPIV) formulation containing 5µg of formalin inactivated Zika virus with 500µg aluminum hydroxide gel adjuvant. We recruited 75 subjects into three groups of 25 including a flavivirus naïve group, a Japanese encephalitis virus (JEV) vaccine (Ixiaro[tm]) primed group and a yellow fever virus (YFV) vaccine (Sanofi 17D) primed group. Five subjects per group were randomly assigned to receive placebo. In the primed groups, subjects were given either JEV vaccine or YFV vaccine 72-96 days prior to receipt of experimental purified inactivated ZIKV vaccine. Subjects were given ZPIV in either two or three doses at days 0, 28 and 196-234. Vaccinations were well tolerated in all groups with only pain at the injection site occurring more frequently in vaccinated subjects than placebo recipients (65% vs 21%). An 88% seroconversion rate and geometric mean neutralizing antibody titer (GMT) of 100 was observed in the flavivirus naïve group 28 days following 2 doses of ZPIV. In the JEV and YFV primed groups the seroconversion rates (31% and 25%, respectively) and GMTs (11 and 5, respectively) were significantly lower at the same time point. All groups demonstrated a substantial boost following a third dose, with seroconversion rates of 100%, 90% and 60% and GMTs of 511, 174, and 79 for the flavivirus naïve, JEV primed, and YFV primed groups, respectively. This differential immune response is hypothesized to be secondary to biasing of the immune response towards shared non-neutralizing epitopes between ZIKV and JEV and YFV respectively, and is being further evaluated. Overall, ZPIV is a safe and immunogenic tool that represents a potentially effective countermeasure for ZIKV infection. However a third dose is likely necessary to achieve an adequately robust and durable immune response and significant immune interference may occur between related YFV and JEV vaccines.

0888

UNIQUE MICROBIAL SIGNATURES ASSOCIATED WITH ZIKA VIRUS INFECTION AND TEMPERATURE IN AEDES ALBOPICTUS AND IDENTIFICATION OF ANTIVIRAL PROPERTIES OF ELIZABETHKINGIA

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Vector-borne pathogens must survive and replicate in the hostile environment in the insect midgut before successful dissemination and transmission. The insect midgut bacteria can have a modulatory effect on their vectorial capacity. The relationship between infection, temperature and the mosquito gut microbiome are not well characterized. The goal of this study was to delineate the effect of Zika virus (ZIKV) infection and temperature variation on the microbial profile of *Aedes albopictus* mosquitoes. *Aedes. albopictus* were reared at diurnal temperature of

day 28°C/ night 24°C and day 30°C/ night 26°C. Mosquitoes were given infectious blood meals with 8.3 $\log_{10}$ PFU/ml ZIKV and 16S *rRNA* sequencing was performed on midguts at 7 days post infectious bloodmeal exposure. Our results demonstrate that blood feeding, infection and temperature broadly impact midgut microbial communities of *Ae. albopictus*. Further, unique microbes were associated with variable infection outcomes. Most notably, *Elizabethkingia*, a flavobacterium, was found to be associated with both blood digestion and ZIKV infection, particularly at lower temperatures. Subsequent studies demonstrated a negative correlation between ZIKV and *Elizabethkingia* in the midguts of *Ae. albopictus*. Further, *Elizabethkingia* was found to have broad-spectrum antiviral and antibacterial activity inhibiting the replication of *Zika virus* (ZIKV), *Dengue virus* (DENV) and *Chikungunya virus* (CHIKV) and *Escherichia coli in vitro* while significantly reducing the infection rates of *Ae. albopictus* for ZIKV *in vivo*. ZIKV and other mosquito- borne viruses remain major public health threats and lack FDA-approved medication or vaccine to treat or prevent them hence the need for alternative approaches that limit the transmission of the pathogens by the mosquito vector. Identification of a midgut microbe with a broad-spectrum antiviral activity could inform novel transmission barrier tools.

0889

CHIMERIC ZIKA VIRUSES CONTAINING STRUCTURAL PROTEIN GENES OF INSECT-SPECIFIC FLAVIVIRUSES CANNOT REPLICATE IN VERTEBRATE CELLS DUE TO ENTRY AND POST-TRANSLATIONAL RESTRICTIONS

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Long Pine Key virus (LPKV) and Lammi virus are insect-specific flaviviruses that phylogenetically affiliate with dual-host flaviviruses. The goal of this study was to provide insight into the genetic determinants that condition this host range restriction. Chimeras were initially created by replacing select regions of the Zika virus genome, including the premembrane and envelope protein (prM-E) genes, with the corresponding regions of the LPKV genome. Of the four chimeras produced, one (the prM-E swap) yielded virus that replicated in mosquito cells. Another chimeric virus with a mosquito replication-competent phenotype was created by inserting the prM-E genes of Lammi virus into a Zika virus genetic background. Vertebrate cells did not support the replication of either chimeric virus although trace to modest amounts of viral antigen were produced, consistent with suboptimal viral entry. These data suggest that dual-host affiliated insect-specific flaviviruses cannot replicate in vertebrate cells due to entry and post-translational restrictions.

0890

IMMUNE CORRELATES OF PROLONGED ZIKA VIRUS SHEDDING IN HUMAN SEMEN

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Zika virus (ZIKV) can cause significant birth defects following maternal infection and is the only flavivirus known to be sexually transmitted in humans. In a human cohort of ZIKV-infected symptomatic men that was established during the ZIKV pandemic, approximately 50% of the men shed viral RNA in their semen, with a prolonged duration of viral shedding in some individuals. Men shedding ZIKV RNA for over 3 months were classified as long-term shedders, and men with undetectable ZIKV RNA shedding were classified as short-term shedders. The mechanism behind prolonged shedding of ZIKV in semen is unknown; however, semen contains various cell populations and immune mediators which may provide insights into the mechanism(s) of prolonged ZIKV shedding. Case reports from other groups suggest that ZIKV infection increases leukocyte populations and inflammatory cytokine concentrations in semen compared to healthy individuals. Here, we sought to identify changes in semen composition associated with prolonged seminal ZIKV shedding using semen samples from the ZIKV-infected cohort. We hypothesized that long-term shedders would have higher leukocyte counts and inflammatory cytokine concentrations in their semen than short-term shedders. In the first sample collected post-disease onset, leukocyte counts were significantly higher in the long-term shedders compared to the short-term shedders as assessed by flow cytometric analyses. Using Luminex assays, we observed a trend toward higher concentrations of inflammatory cytokines, specifically IL-6 and RANTES, in the semen of long-term shedders compared to the short-term shedders. Increased immune correlates in the semen of long-term shedders may indicate a persistent infection of the male reproductive tract. Future studies will investigate whether these changes in seminal composition are a response to prolonged viral infection in the male reproductive tract. Understanding the mechanisms behind sexual transmission of ZIKV will be crucial to limit ZIKV spread and prevent ZIKV-associated birth defects.

0891

MULTIPLE INTRODUCTIONS OF ZIKA VIRUS IN THE AMERICAS

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Discovered in 1947 in the Zika forest, Uganda, Zika virus (ZIKV) remained mostly unnoticed before the epidemic in the Americas. Locally-transmitted infections were reported in French Polynesia and Easter Island in 2014. The first autochthonous transmissions in the American continent were reported in Brazil in May 2015. Epidemiological observations and phylogenetic analyses have led to the hypothesis that the 2015-2016 ZIKV epidemic in the Americas was the result of a founder event from Southeast Asia to Brazil, followed by spread into South and Central American countries, as well as the USA. Surprisingly, ZIKV was also isolated from the Caribbean in 2014-2015, despite only being reported two years later. The Caribbean isolates originated from patients living in rural areas of Haiti, west of Port-au-Prince, as early as May of 2014. This timeline overlaps with the one of the French Polynesian outbreak. Since the early estimates, new genomes have been sequenced. In light of this data, suggesting an early circulation of ZIKV in the Caribbean, we re-evaluated the timing and the route of introduction of ZIKV into the Americas using 613 ZIKV full genome sequences, and maximum likelihood and Bayesian phylogeography frameworks. Our results highlight a transmission process resulting from multiple introductions, unlike what was previously thought. While confirming the expected role of Brazil as a dissemination hub, we show the near simultaneous and independent emergence of different viral lineages in Brazil and Haiti as early as three years prior the Americas epidemic. Taken together, our findings illustrate the power of timely monitoring of sub-epidemic strains that may represent early indicators of the emergence, or re-emergence, of arboviruses such ZIKV both at the regional and global level.

0892

A NOVEL INSECT-SPECIFIC FLAVIVIRUS OFFERS A PROMISING PLATFORM FOR FLAVIVIRUS VACCINES THAT EMPHASIZE HIGH DEGREES OF SAFETY WITHOUT SACRIFICING IMMUNOGENICITY

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Vaccination remains critical for viral disease outbreak prevention and control, but conventional vaccine development typically involves trade-offs between safety and immunogenicity. To overcome this, we used a novel insect-specific flavivirus (named Aripo virus, ARPV) as a vector to create highly safe, single-dose flavivirus vaccine candidates. ARPV was originally isolated from *Ps. albipes* mosquitoes in Trinidad and, unlike the classical insect-specific flaviviruses, phylogenetically clusters with vertebrate pathogenic flaviviruses. ARPV is also uniquely capable of vertebrate cell entry and induction of an immune response, even in the absence of viral replication. To evaluate this platform, we created a pseudo-inactivated chimeric Zika virus (ZIKV) vaccine candidate, designated ARPV/ZIKV, which expresses genes for the key antigenic ZIKV precursor membrane and envelope (prM-E) proteins. Immunization with ARPV/ZIKV showed exceptional safety due to retention of the ARPV backbone's natural host-restriction in vertebrate cells, as demonstrated by its inability to replicate its genome or translate viral proteins in vertebrate cells *in vitro*. ARPV/ZIKV showed a lack of pathogenicity in multiple murine models, including intracranial inoculation of suckling mice. ARPV/ZIKV was also shown to be highly immunogenic. A single dose of ARPV/ZIKV vaccine offered complete protection against ZIKV-induced morbidity and mortality in various murine models, and against *in utero* transmission of ZIKV after infection of gravid $IFNAR^{-/-}$ mice. T-cell depletion studies in $IFNAR^{-/-}$ mice, and vaccine efficacy studies in $Rag1^{-/-}$, $Tcra^{-/-}$, and $muMt^{-/-}$ mice, showed that neutralizing antibodies are the main contributor to ARPV/ZIKV-induced protection. Murine macrophages exposed to ARPV/ZIKV indicated robust PRR, B-cell receptor, Th1 and Th2 polarization, and antigen presentation signaling. Altogether, success with ARPV/ZIKV shows that chimeric insect-specific flaviviruses are a promising new platform to restrict flavivirus emergence via vaccine development.

0893

CORRELATION BETWEEN FLAVIVIRUS ANTIBODIES IN SERUM AND ZIKA RNA SHEDDING IN BLOOD, SALIVA, URINE, VAGINAL FLUID AND SEMEN OVER TIME

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During acute infection, Zika virus (ZIKV) can be found in several body fluids, and persist for weeks to months, raising questions about whether early ZIKV-specific antibodies are effective in viral clearance. This study explores the correlation between long term shedding of ZIKV RNA in different body fluids and presence of ZIKV and dengue (DENV) IgG in acute and convalescent serum. Furthermore, IgG specific to ZIKV EDIII (Z-EDIII) were examined by ELISA at 7, 14, 21, 28, 60, 90 and 180 days post symptom onset (PSO). Serological analysis was available from 113 of 290 suspected Zika cases from the ZIKA-TS study performed from January 2016 to February 2018 in León, Nicaragua. Of these, 39 participants with confirmed acute ZIKV infection by triplicate RT-qPCR provided blood, saliva and urine samples following the sampling protocol described above; patients were considered negative if RT-qPCR was negative in all fluids at enrollment. Of the 39 patients, long term shedding (≥14 days PSO) was observed in 26% (n=10) in blood, 46% (n=18) in saliva and 67% (n=26) in urine, also in 4 of 6 women that provided vaginal fluid and in 2 of 2 men that provided semen. On acute serum (≤4 days PSO), the mean OD of ZIKV IgG was higher in Zika-Negative than in Zika-Positive (0.920 vs 1.200, $p=0.037$, n = 50 vs 63) patients. Long term shedders in blood had lower OD of ZIKV IgG than those clearing the virus earlier (0.220 vs 0.650, $p=0.047$); in contrast, these subjects had higher OD of DENV IgG on acute serum than those clearing the virus earlier (1.830 vs 0.810, $p=0.01$); this trend was not observed for RNA shedding in other fluids. In long term shedders, the highest OD (2.259) of Z-EDIII IgG was observed at 14 days PSO, decreased to 1.281 at 30 days PSO, remained between 0.529 and 0.548 at 60 and 90 days PSO and reached 0.772 at 180 DPSO. This study provides novel observations on Z-EDIII antibody response for 6 months after natural infection. Given that long term ZIKA shedders exhibited higher DENV IgG OD in acute serum, further investigation is warranted to determine whether pre-existing cross-reactive immunity due to DENV infection promotes persistent ZIKV infection or RNA shedding.

0894

A MULTI-MODAL INTERVENTION TO IMPROVE HAND HYGIENE DURING THE PERINATAL PERIOD: A FEASIBILITY STUDY IN KAMPONG CHHNANG, CAMBODIA

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Globally, infections acquired during birth and the first critical days of postnatal care account for an estimated 15% and 10% of all neonatal and maternal deaths. Risk factors for these infections are predominantly poor hygiene practices in health care facilities and the home environment. Behavioural-science informed interventions targeting a wide range of caregivers and environments are lacking. We assessed the feasibility and limited efficacy of a novel multimodal behaviour change intervention delivered at the facility-level to improve the hand hygiene practices among midwives and caregivers during childbirth through the return to the home environment. The intervention engaged midwives in redesigning the delivery room and incorporating visual cues, leveraged the social environment to promote new norms, and delivered interactive training. For caregivers, the intervention visually demarcated "clean hand zones" in the facility, improved access to hand hygiene infrastructure, and incorporated nurture-related cues to guide, remind and reinforce practice. The hand hygiene practices of all caregivers present during childbirth and the postnatal care period of 99 women and newborns were directly observed across 8 health care facilities - half of which received the 6-month intervention. Multilevel logistic regression models, adjusted for baseline measures, assessed differences in hand hygiene practices between intervention and control facilities. The intervention was associated with increased odds of improved practice prior to labour, delivery or immediate childbirth in the delivery room (Adjusted odds ratio [AOR] = 4.7; 95% confidence interval [CI] = 2.7, 7.7), and prior to newborn care in the post-natal care facility ward (AOR = 9.2; CI = 1.3, 66.2); however, the absolute magnitude of improvements was limited. The intervention was not associated with differences in hygiene practices prior to newborn care in the home environment. Our results suggest the potential of a multimodal behaviour change intervention to improve hand hygiene practices that are critical to maternal and neonatal infection in the facility.

0895

IMPACT OF A DRINKING WATER, SANITATION, HANDWASHING (WASH) IN SCHOOL PROGRAM ON MENSTRUAL HYGIENE KNOWLEDGE, PRACTICES AND MENSTRUAL RELATED ABSENTEEISM AMONG SECONDARY SCHOOL STUDENTS IN BANGLADESH

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We evaluated a menstrual hygiene management (MHM) and behavior change intervention implemented between 2017 and 2019 by WaterAid Bangladesh among 300 schools in three geographic locations in Bangladesh. The intervention provided washing and drying facilities for menstrual cloth, disposal facilities for used pads, MHM corners providing pads inside girls' toilets plus behavior change messages. MHM was promoted by female teachers who educate school girls about MHM. This study aimed to examine whether school interventions influenced menstrual hygiene knowledge and practices among school girls. No baseline data were collected so we enrolled pupils from intervention schools and propensity score matched nonintervention (control) schools. Matching scores were derived using school enrollment, geographical locations, and school infrastructure. From randomly selected intervention schools (n=26) and 26 matched controls a total of 1,040 school girls were enrolled between January and March 2020. Data were collected during student interview on reported knowledge, menstrual hygiene practice and absenteeism attributed to menstruation. Odds ratios (OR) and 95% confidence intervals (95% CIs) were determined using generalized linear models, controlling for cluster level effects and adjusting for individual and school level factors. Students who received the intervention attained significantly higher menstruation knowledge scores. The odds that girls managed menstrual hygiene as recommended in the intervention were significantly higher in the intervention schools than in the control schools (Adjusted OR= 1.32, 95% CI: 1.02, 1.70). Girls from intervention schools missed an average of 1.5 days per menstrual cycle compared to 2.5 days for control schools (adjusted OR: 0.92, 95% CI: 0.87, 0.98). Using a control-matched post intervention study design, courtesy bias among intervention school students and selection bias when recruiting control schools cannot be ruled out. Nevertheless, the evaluation suggests that the intervention was successful in improving menstrual hygiene practices and reduced absenteeism during menstruation.

0896

EFFECTS OF DRINKING WATER, SANITATION, HANDWASHING, AND NUTRITIONAL INTERVENTIONS ON IMMUNE STATUS IN YOUNG CHILDREN: A CLUSTER-RANDOMIZED CONTROLLED TRIAL IN RURAL BANGLADESH

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We hypothesized that a combined drinking water, sanitation, handwashing, and nutritional intervention (N+WSH) would reduce systemic inflammation. Within a trial in rural Bangladesh, we cluster-randomized pregnant women into control and N+WSH arms (ClinicalTrials.gov NCT01590095). Among the birth cohort, we quantified IL-1β, IL-6, TNF-α, IL-2, IL-12p70, IFN-γ, IL-4, IL-5, IL-13, IL-17A, IL-21, IL-10, and GM-CSF at ages 14 and 28 months. Cytokine ratios were included as prespecified outcomes to examine the net inflammatory environment. Analysis was intention-to-treat. We assessed 704 children. After 1 year, TNF-α/IL-10, IL-12/IL-10, IFN-γ/IL-10, and IL-17A/IL-10 ratios were lower in the intervention group compared to the control group (mean difference: -0.11 to -0.19, $p<0.05$), due to a non-significant elevation of IL-10 in the intervention group, indicating the intervention promoted IL-10 driven immuno-regulation. Similar reductions in ratios of pro-inflammatory cytokines to IL-10 were sustained in the intervention group after 2 years. After 1 year, IL-12/IL-4, IL-12/IL-5, IFN-γ/IL-5, and IL-12/IL-13 ratios were lower in the intervention group (mean difference: -0.18 to -0.27, $p<0.05$), suggesting a shift towards a Th2 cytokine response. Subsequently, children in the intervention group experienced a greater decrease in their Th2 response between years 1 and 2. By year 2, there was no difference in Th1 or Th2 response between the groups. These findings indicate that during the first year of life, the N+WSH intervention enhanced IL-10 driven immuno-regulation which continued through year 2. Th2 cytokine driven immune responses, which are important for elimination of extracellular pathogens were also enhanced in year 1, but not in year 2, perhaps because successful reduction of extracellular pathogens could facilitate a rebalancing of the Th1 and Th2 cytokine systems. Our results suggest that the N+WSH intervention enhanced the immuno-protective and immuno-regulatory responses, and suppressed/counteracted the inflammatory/immuno-pathological response, of the immune triad.

0897

LONGITUDINAL EFFECTS OF A SANITATION INTERVENTION ON ENVIRONMENTAL FECAL CONTAMINATION IN A CLUSTER-RANDOMIZED CONTROLLED TRIAL IN RURAL BANGLADESH

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Household latrine access generally is not associated with reduced fecal contamination in the environment, but its long-term effectiveness has not been measured. We conducted an environmental assessment nested within the WASH Benefits Bangladesh randomized controlled trial (NCT01590095) to measure the effectiveness of a sanitation intervention on environmental fecal contamination over time. The intervention included latrines, child feces management tools, and behavioral promotion, and intervention uptake was high. We quantified *E. coli* and fecal coliforms in samples of stored drinking water, child hands, mother hands, soil, and food among a random sample of households (n = 720) from the sanitation and control arms of the trial. A total of 16,732 samples were collected during eight quarterly visits approximately 1–3.5 years after intervention initiation. *E. coli* was detected in 81% of stored water samples, 74% of child hand rinses, 75% of mother hand rinses, 95% of soil samples, and 68% of stored food samples. Overall, there were no substantial differences in environmental fecal contamination between the sanitation and control arms. Statistically significant reductions in mean log10 counts of *E. coli* were found in stored water (Δlog10 = -0.08, 95% CI -0.15, 0.00) and child hands (Δlog10 = -0.08, 95% CI -0.15, 0.00) after pooling across sampling rounds, but the effects were small and not consistent across rounds. In addition, we assessed potential effect modification by follow-up time, season, wealth, community-level latrine density and coverage, population density, and domestic animal ownership. While the intervention

led to statistically significant reductions in *E. coli* counts within some subgroups, there were no consistent patterns of effect modification. Our findings support a growing consensus that on-site latrines are insufficient to prevent fecal contamination in the rural household environment.

0898

PROMOTING IMPROVED BACKYARD POULTRY-RAISING PRACTICES TO REDUCE YOUNG CHILDREN'S EXPOSURE TO POULTRY FECES: A PILOT STUDY IN RURAL BANGLADESH

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Backyard poultry-raising is common in rural households in Bangladesh. Raising poultry contributes to fecal contamination of the domestic environment, increasing children's exposure to enteric pathogens, including *Campylobacter spp.* The objective of this study was to investigate the effectiveness of a behavior change communication and counseling intervention to encourage backyard poultry-raising households to confine poultry outside of the household dwelling in and shed at night and improve poultry feces management. We conducted a two-arm pilot study where household members in both arms participated in a behavior change communication and counseling intervention called Neighborhood-based Environmental Assessment and Planning for four months, and households in one arm received a 23 USD subsidy for the construction of poultry shed for nighttime housing. We administered a household survey and spot-check before and after intervention implementation among 37 subsidy and 42 non-subsidy households. At endline, 58% of all households build a poultry shed (87% of subsidy households and 33% of non-subsidy households). The proportion of households confining all poultry outside the household dwelling the previous night was significantly higher at follow-up (33%) compared to baseline (2.5%) (prevalence difference: 30%, 95% CI: 19 to 41). At follow-up, more households had no visible poultry feces piles inside the dwelling compared to baseline (prevalence difference: 26%, 95% CI: 12 to 41), but there were no significant differences in the number of poultry feces piles in the courtyard or veranda at follow-up compared to baseline. Our intervention effectively encouraged households to confine poultry outside of household dwelling at night and to manage poultry feces better. Households receiving a subsidy may be more likely to build a poultry shed within the study period, but some households may be able to build a shed without monetary support. Future studies should assess if housing all poultry outside the household dwelling reduces children's exposure to poultry feces and related health risks.

0899

$20 SENSOR MEASURED USE CONSISTENCY IN HOUSEHOLD WATER TREATMENT DEVICES IN LIMPOPO, SOUTH AFRICA

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The health benefits from household water treatment and safe storage devices depend on correct and consistent use. Occasional lapses in use can cause dramatically non-linear declines in intervention efficacy. Current measures of intervention use fail to capture this non-linearity, even when sensors are used. We invented and validated a novel sensor embedded in the handle of a water treatment or safe storage device that measures how consistently the device is used. We define consistent use nonlinearly as the proportion of days on which a device was used each day for at least 7 previous days. 232 sensors measured >60,000 water withdrawals on three different types of water treatment or safe storage devices in Limpopo, South Africa. We used our (non-linear) consistent use metric and sensor data to: (1) Demonstrate how households with high, but imperfect use prevalence, are critical targets for behavior change efforts (e.g. increasing use from 89 to 99% of days improves consistent use by 49 percentage points); (2) Emphasize that surveys dramatically overestimate consistent use (by 53 percentage points in our study); and (3) Derive and demonstrate a probabilistic model showing how use prevalence (q) affects contact times in water treatment devices. Mean contact time scales with 1/q and the Nth percentile contact times scales with log(1-N/100)/log(1-q). Only 5% of field-measured contact times exceed our predicted 95th percentile. Relating contact time and use prevalence has implications for household-level disinfection devices reliant on contact times; e.g., those using chlorine, iodine, bromine, silver, and/or solar radiation. Sensors provided useful and objective data on the prevalence of consistent use. The dramatically reduced cost of sensors (10x less than published alternatives) increases how many water treatment sensors can be deployed by an order of magnitude, enabling widespread sensor use for (and potentially beyond) research.

0900

SELECTION AND USE OF A SARS-COV-2 SURROGATE IN SURFACE DISINFECTION EFFICACY STUDIES WITH CHLORINE AND ANTIMICROBIAL SURFACES

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Initial recommendations for surface disinfection to prevent SARS-CoV-2 transmission were developed using previous evidence from potential surrogates. To our knowledge, no appropriate surrogate for SARS-CoV-2 has been identified/confirmed for chlorine and antimicrobial surface disinfection. Furthermore, efficacy of chlorine disinfection against SARS-CoV-2 on surfaces relevant to low-resource contexts is unknown. We completed a study to evaluate the efficacy of two hypothesized antimicrobial surfaces, and four chlorine solutions on non-porous and porous surfaces, against SARS-CoV-2 and three potential SARS-CoV-2 surrogates (coronavirus mouse hepatitis virus (MHV) and bacteriophages Phi6 and MS2), to identify a BSL-1/BSL-2 virus to use in future studies. Additionally, in a follow-on study we used the identified surrogate to test the efficacy of chlorinated disinfection on soiled surfaces in a range of environmental conditions. To mimic soiling conditions, we utilized a combination of BSA, TSA, and bovine mucine as recommended by ASTM test guidelines. Surfaces were maintained in a controlled environmental chamber for one hour, exposed to varying relative humidities (23%, 55%, 85%) and temperatures (4ºC, 25ºC, and 40ºC). We found SARS-CoV-2 can be reduced >4 log10 on porous and non-porous surfaces within 30 seconds exposure to 0.5% NaOCl. Results indicate coronavirus MHV-GFP is inactivated faster than SARS-CoV-2 (0.05% NaOCl k-value 32.59 min-1 vs. 3.07 min-1) and MS2 is inactivated more slowly (across multiple k-value timepoints). Phi6 is inactivated similarly to SARS-CoV-2 (0.5% NaOCl k-value 21.44 min-1 vs. 24.44 min-1) and we propose Phi6 as a slightly conservative surrogate for SARS-CoV-2 chlorine disinfection. Additionally, disinfection of bacteriophages on wood was challenging, and exposure to antimicrobial surfaces had no disinfection efficacy as tested. We recommend using 0.5% chlorine on surfaces to disinfect SARS-CoV-2, and recommend additional research on Phi6 disinfection with other disinfectants relevant to the field, such as simulated sunlight or soapy water.

0901

DIAGNOSTIC YIELD OF ADDING THE BIOFIRE® FILMARRAY® MENINGITIS/ENCEPHALITIS PANEL TO ROUTINELY AVAILABLE DIAGNOSTICS FOR CENTRAL NERVOUS SYSTEM INFECTIONS IN BOTSWANA

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Despite the availability of routine diagnostics for central nervous system (CNS) infections in Botswana many patients do not receive a microbiological diagnosis. Molecular diagnostics may help address this issue. We aimed to determine the additional yield of a multiplex PCR platform used alongside routine diagnostics in a high HIV-prevalence setting. We prospectively evaluated the FilmArray® Meningitis/Encephalitis (ME) panel which detects 14 common pathogens associated with CNS infection. We tested all CSF samples collected at Princess Marina Hospital in Gaborone. Samples also underwent routine testing including microscopy, Gram stain, cell count, culture, India Ink and cryptococcal antigen (CrAg). We calculated the additional diagnostic yield for all patients and, given the widespread availability of highly sensitive diagnostics, excluding those diagnosed with cryptococcal meningitis (CM) on routine testing. Between May 2017 and November 2018, 690 patients underwent ME panel testing. 319 (46.2%) female, 419 (60.7%) adults >15 years, 45 (6.5%) children 2-14 years, 143 (20.7%) infants 1-23 months and 82 (11.9%) neonates <28 days. 317/419 (75.7%) adults were HIV positive. The ME panel increased the number of patients with a microbiological diagnosis from 101 to 146 (relative increase 44.6%; 12.8 tests required per additional diagnosis). The ME panel detected an additional 15 bacterial infections (9 *S. pneumoniae*, 2 *S. agalactiae*, 2 *H. influenzae*, 2 *E. coli*) and 39 viral infections (15 CMV, 13 HHV-6, 5 VZV, 4 enterovirus, 1 HSV-1 and 1 HSV-2). Excluding neonates, among 533 patients without CM, the ME panel increased the number of patients with a microbiological diagnosis from 26 to 71 (relative increase 173.1%; 10.9 tests required per additional diagnosis). 460/690 (66.7%) had normocellular CSF and 82/690 (11.9%) had CSF pleocytosis without a microbiological diagnosis. The ME panel increased the proportion of patients with a microbiological diagnosis. Scale up of enhanced diagnostics, including those for TB which were not widely used during this study, may help to further characterise the epidemiology of CNS infections in Botswana.

0902

DEVELOPMENT AND PERFORMANCE EVALUATION OF A LOW-COST, HIGH THROUGHPUT, MULTIPLEX IMMUNOASSAY OF THIRTEEN FEVER SEVERITY AND ETIOLOGY MARKERS

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Fever is among the most commonly reported symptoms globally. Commercial kits are available that facilitate multiplexed screening of blood markers of diseases, but these may not be tailored to the purpose of investigating fever and/or may be time consuming and costly to perform at scale. We developed a bead-based multiplex immunoassay that estimates the levels of 13 fever-relevant immune response markers in dried blood spot (DBS) specimens. The markers, together or in various combinations, were selected for their known utility, both in the identification of bacterial infection, as well as being prognosticators. The markers were angiopoietins 1 & 2, azurocidin, C-reactive protein, chitinase 3-like 1, interleukins 6, 8 & 10, interferon γ-induced protein 10 kDa, myxovirus resistance protein A, soluble triggering receptor expressed on myeloid cells 1, soluble tumor necrosis factor receptor 1 and tumor necrosis factor-related apoptosis-inducing ligand. All reagents were sourced commercially. Cross-reactivity assessments revealed minimal non-specific interaction between either the antibody pairs, or antibodies and recombinant proteins. The 13 assays worked over a wide range of concentrations including those expected in health and febrile disease of different etiologies. The average within-plate and between-plate coefficients of variation (CVs) were 5.3% and 3.9%, respectively. The fully optimised 13-plex assay cost is around £3.30/$4.60 per sample. The full protocol will be published as an open resource. We tested 720 DBS samples from adult and paediatric inpatient and outpatient fever cases and community controls collected during a prospective study of febrile illness in Lao PDR. Several markers in the panel could differentiate between fever cases and healthy controls, with 4 in particular contributing to this. This assay facilitates large-scale screening of DBS samples from population-based studies to investigate relationships between levels of immune response markers, data from infectious diseases diagnostics and severity of illness in different clinical contexts.

0903

PREDICTING MORTALITY IN FEBRILE ADULT PATIENTS: A PROSPECTIVE VALIDATION OF THE MEWS, QSOFA AND UVA SCORES IN FOUR HEALTH CARE SETTINGS IN AFRICA AND SOUTH-EAST ASIA

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Identifying patients with the highest risk of mortality from easily measurable variables can improve prioritization and thus resource allocation of potentially life-saving interventions. The modified early warning score (MEWS), the quick Sequential (Sepsis-Related) Organ Failure Assessment (qSOFA), and the Universal Vital Assessment (UVA) score were developed as risk-stratification tools but need external validation in new patient groups and settings. We included in the analysis in- and outpatients aged ≥16 years presenting with fever at four sites in Laos, Malawi, Mozambique, and Zimbabwe as part of a prospective study of infectious causes of fever (Febrile Illness Evaluation in a Broad Range of Endemicities - FIEBRE). We determined mortality at a follow-up visit after

at least 26 days. We evaluated predictive capacity based on the area under the receiver operating curve (AUC), as well as sensitivity at a cut-off which gives 90% specificity. We enrolled 4,023 patients; 1,715 (43%) inpatients, 2,336 (58%) female, median (IQR) age 33 (24 - 45) years, and 782 (19%) HIV-infected. Of the total, 1,182 (29%) were in Laos, 807 (20%) in Malawi, 998 (25%) in Mozambique, and 1036 (26%) in Zimbabwe. Complete outcome information was available for 3,432 (85%); 210 (6.1%) died, including 187 (13.2%) inpatients and 23 (1.1%) outpatients. The UVA had an AUC of 0.77 (95% CI 0.74 - 0.81), outperforming both MEWS with AUC 0.65 (95% CI 0.61 - 0.69) and qSOFA with AUC 0.68 (95% CI 0.65 - 0.72). The relative performance of the scores remained the same on sensitivity analysis assuming patients with missing outcome data either all lived, or all died. The observed AUC of UVA was higher than MEWS and qSOFA in each site, varying from 0.88 (95% CI 0.79 - 0.99) in Malawi to 0.75 (95% CI 0.69 - 0.81) in Laos. At a set specificity of 90%, UVA sensitivity (95% CI) was 46.5% (39.9% - 53.0%) compared to 18.6% (10.9% - 27.5%) for qSOFA, and 22.9% (17.5% - 28.8%) for MEWS. Our findings suggest that of the scores assessed, UVA best predicts mortality in febrile adults across a range of health care settings in Africa and south-east Asia, and may be well-suited for clinical use in similar contexts.

0904

DECREASED MUSCLE OXYGENATION AND PH ARE EARLY AND SENSITIVE INDICATORS FOR DENGUE SEVERITY

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Management of dengue is challenging due to the lack of an early indicator for disease severity. We have shown that muscle oxygenation (SmO2) levels determined twice daily correlated with severity in a small number of pediatric dengue cases. In this study, we evaluated the utility of continuous measurement of SmO2 and pH as a monitoring tool for dengue severity in a prospective study of patients hospitalized with suspected dengue. Dengue cases were confirmed by RT-PCR of plasma samples and by paired serology. Dengue cases were classified into dengue fever (DF) and dengue hemorrhagic fever (DHF), and into dengue, dengue with warning signs, and severe dengue. Continuous measurement of SmO2 and pH was carried out with a wearable infrared spectrophotometric device. Forty-four subjects (19 children, 25 adults) completed the study. There were 8 DF, 6 DHF cases in children, and 3 DF and 12 DHF cases in adults. Children with dengue were classified into 7 cases of dengue and 7 cases of dengue with warning signs. There were 8, 5, and 2 cases of dengue, dengue with warning signs, and severe dengue in adults. Different patterns of SmO2 and pH were observed between DF and DHF with declines in SmO2and pH around the time of fever resolution in DHF. A similar trend was found in severe dengue but not in dengue with warning signs. Differences in SmO2 levels between DF and DHF cases were detected starting 12 hours before fever resolution and persisted for 48 hours. SmO2 levels were lower in adults with DHF compared to children six hours before defervescence (75.4 (3)% (mean (SD)) in children and 60.3 (7)% in adults). The nadir of muscle oxygenation levels correlated with minimum systolic and diastolic blood pressures, minimum pulse pressures, and muscle pH. The study demonstrated that tissue hypoxia occurred in dengue hemorrhagic fever as early as twelve hours before defervescence, even in the absence of clinical signs of circulatory compromise. Adults with DHF appeared to be more severely affected than children. Monitoring of muscle oxygenation and pH is a promising tool in clinical management of dengue.

0905

TREATMENT OF COVID-19 WITH REMDESIVIR AND CONVALESCENT PLASMA: A PROSPECTIVE, OBSERVATIONAL STUDY

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Remdesivir and convalescent plasma therapy (CPT) were approved as investigational therapies to treat COVID-19 in Nepal. We conducted a prospective, multicentered, observational study to evaluate the safety and outcomes of remdesivir and CPT to treat COVID-19 in hospitalized patients. Patients over 18 years age, who were admitted to hospitals with a diagnosis of COVID-19 and a positive PCR, and received remdesivir, CPT, or both, were eligible for study enrollment. Other antiviral agents were excluded but immunomodulators including steroids were allowed. Clinical findings, lab results, adverse events, and outcomes were collected in a central electronic database. We enrolled 1315 patients from 31 hospitals across Nepal. Patients were classified as having moderate (24.2%), severe (64%) or life-threatening (11.7%) COVID-19. Of 1083 patients with reported outcomes, 78.4% were discharged (74% in good condition, 4.4% with disability) and 21.6% died. Discharge rate was 84% for remdesivir recipients (N=910), 39% for CPT recipients (N=59), and 54.4% for CPT+REM (N=114) recipients. Most of CPT recipients (98.3%) or CPT+REM recipients (92.1%) had severe to life-threatening infections, and they were admitted to the ICU (CPT 91.8%; CPT+REM 94.6%) compared to REM alone recipients (severe to life threatening 73.3% and ICU admission 57.5%). In a logistic model comparing death vs discharge and adjusted for age, gender, steroid use, and severity, the predicted margin for discharge was higher for remdesivir alone recipients (0.82; 95%CI 0.79-0.84) compared to CPT (0.58; 95%CI 0.47-0.70) or CPT+REM (0.67; 95%CI 0.60-0.74) recipients. Adverse events of Remdesivir and CPT were reported in less than 5% of the patients. In conclusion, most patients who received CPT alone or CPT with remdesivir had severe to life threatening COVID-19 infection and a higher mortality compared to remdesivir alone recipients. Both CPT and remdesivir were tolerated well by the patients in Nepal.

0906

LIMITED CLINICAL DISEASE DESPITE RAPIDLY INCREASING SARS-COV-2 SEROPREVALENCE IN MALI, WEST AFRICA

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The burden of SARS-CoV-2 in West Africa is not well understood. We have previously reported that over half of the population around Bamako may have been exposed. To understand the clinical burden associated with SARS-CoV-2 seroconversion we analyzed clinical information collected during a two visit serosurvey at Sotuba (urban), Bancoumana (rural) and Doneguebougou (rural). In a subset of participants co-enrolled in clinical trials at study sites, adverse events (AEs) occurring between serosurvey visits were also assessed. Study visit 1 was completed just before the malaria season (July/October) and visit 2 was completed just after the malaria season (December 2020/January 2021). The two visit completion rate was 94.8% (2533/2672). At visit 1 symptoms were reported

infrequently. Compared to seronegative cases, several systemic symptoms were more common in seropositive cases including fever (8.5% vs 4.0%), chills (1.7% vs 0.4%), myalgia (3.5% vs 0.7%), and headache (11% vs 3.7%). At visit 2, recent symptoms were reported more frequently in all participants. Only chills (3.7% vs 2.1%) and fatigue (4.3% vs 2.5%) were more common among seropositive cases. At each visit, systemic symptoms (any) approached independent association with serostatus following multiple logistic regression (OR 1.72, 95% CI: 0.96-2.98 and OR 1.22, 95% CI: 0.97-1.52). Respiratory and gastrointestinal symptoms did not clearly differ based on serostatus at either visit. In a subset (n=146) at Bancoumana, AEs possibly related to COVID-19 did not differ based on serostatus. In a subset (n=1037) at Doneguebougou, rhinitis (33.3% vs 25.1%) and headache (18.3% vs 9.4%) were more common among seropositive cases. The rate of malaria infection did not differ with serostatus and was approximately 35%. Six hospitalizations were reported in the study period (3 seropositive and 3 seronegative cases), below the rate predicted using age-stratified US COVID-19 data. COVID-19 attributable symptoms and severe complications are uncommon in the community in Mali despite high seroconversion rates and may fall within background illness rates during the malaria transmission season.

0907

METAGENOMIC NEXT-GENERATION SEQUENCING OF FEBRILE PATIENTS REVEALS DIVERSE VECTOR-BORNE PATHOGEN LANDSCAPE IN CAMBODIA

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Metagenomic investigation of pathogens causing undifferentiated fevers can identify an underappreciated diversity of pathogens, particularly in resource-scarce settings where extensive diagnostics are not available. In peri-urban Cambodia, we enrolled 489 febrile participants from March 2019 to October 2020 via: 1) a cross-sectional hospital-based study of patients 2 to 65 years of age; 2) a community-based longitudinal cohort aged 2-9 years old. Demographics, symptoms, behavioral and risk factor data was collected and stored in a REDCAP® database. Locational data of village locations were referenced using Google Earth. Environmental indices (EI) for surface water and vegetation were extracted from Moderate Resolution Imaging Spectroradiometer products. We isolated RNA from sera and prepared sequencing libraries that were run on an iSeq100 (Illumina) in Phnom Penh, Cambodia. Next, we performed bioinformatic analysis using IDseq, an open-source cloud-based platform, to identify pathogens. We used a multivariate analysis to identify determinants of vector-borne disease. Ultimately, we identified dengue virus (124/487), chikungunya (10/487), *Plasmodium vivax* (6/487), rickettsial-like pathogens (13/487), and rotavirus A (6/487) in addition to a variety of other pathogens of low abundance. Participants 5 to 18 years of age were more likely to contract vector-borne diseases (for 5 to 10 years of age; OR 2.35, 95% CI (1.11–5.06); OR 2.68 for 10 to 18 years of age; 1.4–5.29) compared to those older than 18 years of age. Likelihood of infection with a vector-borne disease was also increased by household car ownership (OR 1.95, 95% 1.19–3.21) and proximity to surface flooding (OR 2.04, 95% CI 1.24–3.49). The pathogen landscape defined by metagenomic sequencing is diverse, but predominantly vector-borne owing to arboviral epidemics during the study.

0908

KINETICS AND FUNCTIONALITY OF NATURALLY ACQUIRED ANTIBODY RESPONSES TO PLASMODIUM FALCIPARUM GAMETOCYTE ANTIGENS IN AN AREA OF INTENSE MALARIA TRANSMISSION IN UGANDA

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Naturally acquired antibody responses to gametocyte antigens can reduce the transmission of *P. falciparum* gametocytes to mosquitoes. Plasma samples were taken every 3 months between 2011 and 2017 from 433 cohort participants who resided in Tororo, Uganda, during which transmission declined due to highly effective malaria control. Parasite carriage was determined by microscopy and a molecular assay. Overall, 2484 plasma samples (4 time-points over 5 years) were analysed by ELISA for reactivity against total gametocyte (NF54) lysate or recombinant full-length Pfs48/45 and Pfs230-CMB, and for surface recognition in a novel gamete binding assay. Functional transmission blocking immunity was assessed by standard membrane feeding assay (SMFA) with NF54 gametocytes. Analyses are ongoing; preliminary results indicate prevalence of antibodies against Pfs230 of 39.5% (163/413) in children under 5, 65.3% (571/875) in children 5-11 years and 84.9% (372/438) in adults (>18 years). For Pfs48/45, antibody prevalences were 13.4% (37/277), 44.6% (263/590) and 76.0% (231/304), respectively. Compared to parasite-free individuals, anti-Pfs48/45 antibody prevalence was higher in those with submicroscopic infections (OR 2.02, 95%CI 1.28-3.17), microscopically-detected infections (OR 1.80, 95%CI 1.11-2.91) or microscopically-detected gametocytes (OR 3.96, 95%CI 1.35-11.7). Among the first 263 samples tested, we identified 11 samples (individuals aged 6.0 – 65 years) that fully blocked transmission in the SMFA. Ongoing work will examine the kinetics of (functional) immunity, with up to 16 time-points of individuals classified as transmission-blockers and selected non-blockers in relation to malaria exposure. In the largest longitudinal study of its kind, we detected high recognition of recombinant gametocyte antigens. Our study addresses a knowledge gap in our understanding of the relevance of anti-gametocyte immunity in relation to (changes in) parasite exposure and may inform the development and deployment of transmission-blocking vaccines.

0909

SEX-BASED IMMUNOLOGICAL DIFFERENCES AMONG MALARIA-EXPOSED UGANDANS INCLUDES ABERRANT HEMATOPOIESIS

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Multiple studies have demonstrated a sex-bias in *Plasmodium falciparum* prevalence in malaria-endemic settings, with greater prevalence in males compared to females. A recent study from our collaboration found that this bias was not explained by sex-based differences in behavior, malaria incidence, or force of infection, but was instead attributed to a slower rate of infection clearance among males, suggesting an immunological basis. We therefore compared immune profiles between 49 males and females living in malaria-endemic Tororo, Uganda. Innate and adaptive cellular phenotypes as well as responses to toll-like receptor and malaria antigenic stimulation were compared in males and females by flow cytometry. Malaria-specific antibodies were compared by a high throughput, Luminex-based magnetic bead-based assay. Our analysis yielded sex-based differences in immune cell population frequencies, with men displaying more plasmacytoid dendritic cells and $CD14^+$ $CD16^-$ classical monocytes but fewer B cells and T follicular $CD4^+$ T cells. Additionally, conventional dendritic cells of women more frequently produced TNFα in response to TLR1/2 stimulation. Curiously, our flow cytometric analyses also identified a heterogeneous population of $CD19^-$, $CD3^-$, $HLA\text{-}DR^+$, $CD11c^+$ myeloid cells expressing CD123, which is not typically expressed at high levels on mature myeloid cells in the peripheral blood of healthy individuals. This $CD123^+$ population was more pronounced among men and appeared to be highly inflammatory, expressing TNFα and IL-6 following TLR1/2 stimulation at approximately twice the frequency of $CD123^-$ myeloid cells. To further characterize this $CD123^+$ population, we are performing a single-cell transcriptomic and epitope analysis (CITE-seq) on peripheral blood mononuclear cells of four age-matched men and women who displayed prominent populations of $CD123^+$ myeloid cells; results will be presented at the conference. We hypothesize that $CD123^+$ myeloid cells present in the peripheral blood of Ugandan patients constitute an immature population of precursor cells that may arise as a consequence of recurrent malaria infection.

0910

DYSREGULATION OF THE IMMUNE RESPONSE IL-5 SIGNALING VIA JAK/STAT PATHWAY IN KENYAN CHILDREN WITH SEVERE MALARIAL ANEMIA

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Despite the global decline in malaria over the past two decades, malaria-related morbidity and mortality remains a significant health burden in sub-Saharan Africa. In holoendemic *Plasmodium falciparum* regions, such as western Kenya, severe malaria in children under five years primarily manifests as severe malaria anemia (SMA, Hb<5.0g/dL and any parasite density). Since previous studies including ours have shown that dysregulation in innate immunity influences the development of SMA, transcriptomic analysis (mRNA-seq) was performed by Next-Generation Sequencing of samples from Kenyan children (3-36 months) with non-SMA (Hb>5.0 g/dL, n=41) and SMA (n=29). Enrichment analyses were performed using MetaCore™ with only false discovery rate (FDR)-adjusted (P<0.05) differentially expressed genes ($\log_2$) in the models (total=6,862 genes/network objects). IL-5 signaling via JAK/STAT emerged as a top-ranked pathway (FDR-$3.87x10^{-10}$). Upon binding of IL-5 to the IL-5 receptor α (IL5RA) and β (CSF2RB) subunits, several signaling pathways are activated, including the JAK/STAT pathway. Although IL-5 is important for cell differentiation, proliferation, survival, B cell maturation, and induction of pro-inflammatory mediators, its role in the pathogenesis of SMA remains undefined. Transcriptomics analyses revealed that IL5RA gene (*IL5RA*) was upregulated (+1.01), while *CSF2RB* was downregulated (-0.99). Moreover, genes for kinase JAK2 and for a group of proteins phosphorylated by JAK2 were downregulated: *JAK2* (-0.45), *STAT1* (-0.33), *STAT3* (-0.56), *STAT5A* (-0.32), and *STAT5B* (-0.41). Downregulation also occurred for *cyclin D3* (-0.48), *BLIMP1* (-0.59), and *CISH* (-0.57). Results presented here illustrate that SMA is characterized by dysregulation of genes in the IL-5 JAK/STAT signaling pathway that can result in decreased class switch recombination upon antigen encounter, perturbations in B cell differentiation, and reduced cell proliferation.

0911

A SYSTEMS SEROLOGY APPROACH TO IDENTIFY DOMAIN TARGETS AND FC FUNCTIONS OF ANTIBODIES AGAINST PFEMP1 THAT ARE ASSOCIATED WITH PROTECTION FROM CEREBRAL PLASMODIUM FALCIPARUM MALARIA

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Plasmodium falciparum erythrocyte membrane protein 1 (*Pf*EMP1), a key malaria virulence factor, mediates sequestration of infected erythrocytes in the vasculature. Antibody responses to several semi-conserved domains of *Pf*EMP1 have been associated with protection from severe malaria (SM), and it is hypothesized that antibodies to a key subset of *Pf*EMP1 domains may confer protection. Previous studies measured IgG antibodies to *Pf*EMP1 but did not consider the quality of the antibody response. Several properties of antibody Fc region influence interactions with the innate immune system to induce functions such as phagocytosis, cell cytotoxicity or complement activation. We used a systems serology approach to profile the PfEMP1 targets and functional Fc features of antibodies that correlate with protection from cerebral malaria (CM). Plasma from Malawian children collected at presentation to hospital with CM (n=46) or uncomplicated malaria (UM; n=46). Antibody responses were measured by bead-based multiplex array for 33 recombinant *P. falciparum* domains that have been associated with SM or UM. We profiled antibody isotypes and subclasses (IgG, IgM, IgG1-4), Fc receptor binding (FcγRIIa, IIb, IIIa, IIIb) and complement fixation (C1q) for each antigen. Univariate comparison and multivariate machine learning were used to select 17 out of 418 antibody features that distinguish between UM and CM, with 87% accuracy (ROC). The target antigens associated with a protective response were predominantly *Pf*EMP1 domains that bind to endothelial cell receptors ICAM1 or EPCR, which are thought to mediate cerebral sequestration and cause CM. Total levels of IgG antibody could not distinguish between CM and UM, but the functional Fc properties of the antigen specific antibodies could. Specifically, antibody engagement with FcγR IIa, IIIb and

c1q were associated with protection, suggesting activation of monocytes, neutrophils and complement may be important protective responses. Characterizing the functional properties of antibodies that relate to protection from SM may advance the development of novel vaccines and therapeutics for malaria.

0912

DETERMINATION OF MALARIA EXPOSURE IN ETHIOPIAN COMMUNITIES AND ITS RELATIONSHIP TO PLASMODIUM VIVAX MALARIAL BURDEN IN DUFFY-NEGATIVE INDIVIDUALS

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Plasmodium vivax is the most common malarial species worldwide, yet it remains rare in Sub-Saharan Africa. During merozoite invasion *P. vivax* must bind to the Duffy antigen receptor expressed on erythrocytes and it has long been understood that Duffy negativity, or the lack of Duffy expression, confers resistance to infection. Duffy negativity is very common in most of Sub-Saharan Africa and generally credited for the lack of *P. vivax* infection on the continent. This antigen system is well studied, yet there has been little exploration on antigenic reactivity of individuals with differing Duffy phenotype. This study seeks to examine how multiple *P. vivax* and *P. falciparum* antigens, as well as mosquito salivary gland antigens, relate to differences in Duffy phenotypes; and to explore if the supposed resistance of Duffy negative individuals to *P. vivax* still yields naturally acquired immunity despite the parasites inability to establish blood stage infection. This is being examined through whole blood and dried blood spots collected during cross sectional survey in southwestern Ethiopia, an area with endemic *P. vivax* transmission and with significant variation in Duffy phenotypes in the human population. Preliminary results indicate that the Duffy phenotype distribution is 54.1% positive, and 48.8% negative. To assess overall seroprevalence patterns antigenic markers were combined and mean fluorescent intensity (MFI) was standardized. Seroprevalence was not significantly different between Duffy negative and Duffy positive peoples for *P. falciparum* antigens ($P>0.05$), yet it exhibited highly significant variation for *P. vivax* antigens ($P<0.0001$). Additionally, preliminary PCA analyses of MFI for all antigens show distinct trends between malarial species; while *P. falciparum* antigens exhibit no apparent clustering related to Duffy expression, *P. vivax* antigens show a clear clustering affect between Duffy positive and Duffy negative phenotypes. Further detailed analyses will likely yield more insight into these trends yet our data are the first to show *P. vivax* antigenic variation across Duffy phenotypes.

0913

IMPACT OF ENDOGENOUS STEROIDS ON HOST IMMUNE RESPONSE DURING BLOOD STAGE PLASMODIUM FALCIPARUM MALARIA

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Host responses in *Plasmodium falciparum* malaria vary between individuals and ethnic groups through poorly understood mechanisms. During blood-stage malaria, host and *P. falciparum* metabolomes become heavily intertwined, highlighting the need to understand how peripheral blood metabolites impact immune response and parasite proliferation. Here we use a longitudinal matched *in vivo* metabolomic and transcriptomic profiling of children of the Gouin and Fulani ethnic groups in Burkina Faso before and during infection to investigate the metabolomic perturbations that impact host immune response. We identify distinct perturbations in steroid biosynthesis as hallmark of *P. falciparum* infection and demonstrate their major role in the host response to infection implicating critical metabolic and immune response pathways. Integrative multi-omic analysis reveals steroid-driven immunosuppression of T-lymphocytes signaling pathways. Analysis of the less malaria-susceptible Fulani ethnic group demonstrates opposing steroid responses during infection supporting the immunosuppressive role of endogenous steroids during blood stage *P. falciparum* malaria.

0914

PROTECTION-ASSOCIATED IMMUNE RESPONSES FOLLOWING VACCINATION WITH RADIATION-ATTENUATED PLASMODIUM FALCIPARUM SPOROZOITES

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Immunization with radiation-attenuated sporozoites (RAS) can confer sterilizing protection, although the mechanism behind this protection is incompletely understood. *In vivo* animal studies have provided some mechanistic insight into how anti-malarial immunity is achieved, although some of these mechanisms are challenging to study in humans.This project aims to understand protective anti-malarial immunity in humans and in this way, aid the development of a malaria vaccine. To this end, we performed immunological analyses of samples from clinical trials in which malaria naïve volunteers were vaccinated with *Plasmodium falciparum* RAS and whose protection from malaria infection was assayed by controlled human malaria infection (CHMI). Blood samples collected at multiple timepoints during the immunization schedule and after CHMI were analyzed to compare immune responses between protected and non-protected volunteers leveraging integrative analysis of whole blood RNAseq, single cell CITEseq and high parameter flow cytometry on PBMCs. These combined datasets revealed differences in early innate immune responses that indicate divergent paths that either lead to protective immunity or the lack thereof. Non-classical monocytes, early type I interferon responses and T-helper 2 T cells correlate with impaired immunity whereas mature CD1c expressing dendritic cells correlate with T-helper 1 skewed responses in protected subjects. In addition, proliferating Vδ2 γδ-T cells after each immunization were associated with protective immunity. Furthermore, preexisting differences in certain cell subsets correlate with protection, suggesting that these baseline immune states can affect immune responses to RAS immunization. Our data indicates that elevated levels of circulating innate lymphoid cells type 2 (ILC2) at baseline are associated with impaired immunity. These findings give insight into the immune responses that confer protection against malaria and may guide further malaria vaccine development.

0915

EXPANDING INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY (IPTP) THROUGH COMMUNITY DISTRIBUTION ALSO INCREASED IPTP UPTAKE THROUGH ANTENATAL CARE VISITS IN THREE DISTRICTS OF MADAGASCAR

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In Madagascar, only 10% of eligible women receive the WHO-recommended 3 doses of intermittent preventive treatment in pregnancy (IPTp) with quality-assured sulfadoxine-pyrimethamine (SP), well below the

National Malaria Strategy Plan target of 80%. To improve IPTp coverage, while promoting antenatal care (ANC) attendance, community-delivery of IPTp (C-IPTp) was introduced in three districts. In August 2018, C-IPTp launched in Mananjary district and expanded to Vohipeno and Toliara-2 districts in November 2019. Community health workers (CHW) were trained to identify pregnant women, refer them to ANC, and administer IPTp. The first dose of SP must be administered during ANC while subsequent doses are administered either by CHWs or during ANC. Data were analyzed from the national health management information system and supplementary reporting by CHWs. In Mananjary IPTp3 increased from 33% in December 2018 to 78% in December 2020. Similarly, between December 2019 and December 2020, IPTp3 increased from 44% to 69% in Vohipeno and 52% to 66% in Toliara-2. Initially these increases were driven by C-IPTp with 70% of doses being distributed by CHWs and 30% during ANC. In 2020, the intervention increased ANC awareness through social behavior change and communications (SBCC), community engagement, strengthened collaboration between CHWs and facilities, and an implemented system to harmonize scheduling of ANC4 visits with timing of IPTp3. Alongside increases in overall IPTp3 coverage across all three districts, there was also a meaningful shift in IPTp3 uptake during ANC – moving from 18.6% in December 2018 to 68% in December 2020. In addition, the percentage of pregnant women attending 4 ANC visits increased from 16% in December 2018 to 55% in December 2020. Introduction of C-IPTp with SBCC and improved scheduling practices has boosted both the number of pregnant women protected against malaria and utilization of ANC services. Through concerted project efforts, improvements in IPTp3 coverage originally gained from community-based distribution are gradually being shifted to facility-based distribution.

0916

CHANGES IN ANTENATAL CARE (ANC) ATTENDANCE AND UPTAKE OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY (IPTP) AFTER INTRODUCTION OF COMMUNITY-BASED DISTRIBUTION OF IPTP IN THREE LOCAL GOVERNMENT AREAS (LGA) IN NIGERIA

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In Nigeria, community health workers (CHWs) from three LGAs were engaged to introduce community delivery of IPTp (C-IPTp) with quality-assured sulfadoxine-pyrimethamine to prevent malaria. This approach, which complements IPTp delivery during ANC visits, was carried out in Ohaukwu, Akure South, and Bosso LGAs. C-IPTp was introduced in September 2018 in Ohaukwu and in December 2019 in Akure South and Bosso. A total of 1,062 CHWs were trained on early identification of pregnant women, referral to ANC, IPTp administration, and use of mobile phones to capture and report data. CHWs conduct household visits, provide malaria health education, refer and encourage pregnant women to attend ANC, and provide IPTp. Routine facility data from 2017 before introduction of C-IPTp were compared with 2020 facility and CHW data to understand the effect of C-IPTp across the LGAs. Before C-IPTp, 43% of the estimated number of pregnant women in these areas attended at least one ANC visit as compared to 57% in 2020 ($p<.05$). Attending at least four ANC (ANC4) visits increased in Ohaukwu by ten percentage points to 29% in 2020 ($p<.05$). In Akure South, ANC4 remained steady Bosso pre-C-IPTp data on ANC4 visits were of too poor quality to conduct a meaningful analysis. Though this project focused on C-IPTp, it also resulted in statistically significant increases in IPTp distribution during ANC visits. From 2017 to 2020, coverage of IPTp doses 1, 2, and 3 all saw increases across the three sites: IPTp1 increased from 54% to 57%; IPTp2 from 36% to 42%; and IPTp3 increased 20 percentage points from 5% to 25%. Of pregnant women receiving all doses of IPTp, 39% did so through ANC with 61% receiving IPTp from CHWs. These data suggest that in addition to contributing to overall increases in IPTp coverage, C-IPTp may also contribute to increases IPTp delivery in ANC and ANC attendance.

0917

MAPPING OUT OF YAWS ENDEMICITY IN GHANA, LESSONS TO STRENGTHEN THE PLANNING AND IMPLEMENTATION OF YAWS ERADICATION

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Yaws caused by Treponema pallidum subsp pertenue is a disease of poverty and affects communities where basic socio-economic amenities are lacking. With results showing that single-dose azithromycin is effective in the treatment of yaws, the World Health Organisation introduced the Morges strategy with the intent to eradicate yaws by 2030. Ghana is one of the countries with the most yaws cases globally, and the National Yaws Eradication Program in Ghana intends to conduct Mass Drug Administration (MDA) of endemic communities in line with the Total Community Treatment plan of the Morges strategy. It is therefore important to map out endemic communities to ensure that MDA is both effective and financially efficient. Children with suspected yaws lesions were actively selected from schools and communities. A full medical history, study site information including GPS coordinates, demographic data including communities of residence and clinical assessment were taken. Each of the clinically diagnosed children were screened using the DPP® Syphilis Screen and Confirm Assay (DPP). Samples for PCR were collected by swabbing ulcerative lesions of participants and tested for Treponema pallidum subsp pertenue and Haemophilus ducreyi DNA. In all, 625 children with a median age of 10 years were recruited into the study. While 401(64.2%) were DPP positive, only 141 (22.6%) of them had Treponema pallidum subsp pertenue DNA (TPE_DNA). Based on the DPP results, yaws was endemic in all 4 study sites with participants from 88 communities in 13 districts in 4 regions in Ghana. There was no statistically significant difference between the various districts in terms of DPP results ($x2=0.9364$, $p= 0.817$) and 154 (24.6%) of those clinically diagnosed as yaws were positive for Haemophilus ducreyi DNA. Our study shows that communities endemic for yaws are also endemic for Haemophilus ducreyi. Most yaws endemic communities were found at the border of other districts and regions. It is recommended that MDA should not only target endemic communities, but it should also target entire endemic districts as well as neighbouring districts in order to be effective.

0918

BASELINE PREVALENCE OF TRACHOMA IN REFUGEE SETTLEMENTS IN UGANDA: RESULTS OF ELEVEN POPULATION-BASED SURVEYS

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The health risks and needs of refugee populations are often distinct from the surrounding permanent inhabitants of the areas in which they live. There are several settlements in the Northern and Western Regions of Uganda serving refugees from South Sudan and Democratic Republic of Congo (DRC), respectively. Trachoma prevalence surveys were conducted in a number of those settlements with the aim of determining whether interventions for trachoma are required. For the purposes of these surveys, an evaluation unit (EU) was defined as all refugee settlements in one district. Internationally standardized population-based trachoma prevalence survey methods were deployed in 11 EUs to assess prevalence of trachomatous inflammation-follicular (TF) in 1-9-year-olds and

trachomatous trichiasis (TT) unknown to the health system in ≥15-year-olds. Household-level water, sanitation and hygiene coverage was also assessed in study populations. A total of 40,892 people were examined across 11 EUs. The prevalence of TF in 1-9-year-olds was <5% in all EUs surveyed. The prevalence of trachomatous trichiasis (TT) unknown to the health system in ≥15-year-olds was <0.2% in 5 out of 11 EUs surveyed. Implementation of the antibiotic, facial cleanliness and environmental improvement components of the SAFE strategy are not needed for the purposes of trachoma's elimination as a public health problem in these refugee settlements; however, intervention with TT surgery is needed in 6 EUs. Since instability continues to drive displacement of people from South Sudan and DRC into Uganda, there is likely to be a high rate of new arrivals to the settlements over the coming years. These populations may therefore have trachoma surveillance needs that are distinct from the surrounding non-refugee communities. These methods and approaches to surveys in refugee settlement are applicable to similar settings or in areas where there are internally displaced populations.

0919

IDENTIFICATION OF SNAILS AND SCHISTOSOMA OF MEDICAL IMPORTANCE VIA CONVOLUTIONAL NEURAL NETWORKS

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In recent decades, computer vision has proven remarkably effective in addressing diverse issues in public health. We investigate the effectiveness of convolutional neural networks (CNNs) in classifying the environmental stages of parasites and respective invertebrate hosts of medical importance, with schistosomiasis as a reference model. Schistosomiasis is a debilitating parasitic disease, affecting more than 200 million people in tropical and subtropical regions, and transmitted to humans via snail intermediate hosts. We trained our CNN, a feed-forward neural network, on a limited dataset of 5,500 images of snails and 5,100 images of cercariae obtained from schistosomiasis transmission sites in the Senegal River Basin, a region in western Africa that is hyper-endemic for the disease. The image set included both images of two snail genera that are relevant to schistosomiasis transmission-that is, *Bulinus* spp. and *Biomphalaria pfeifferi*-as well as snail images that are non-component hosts for human schistosomiasis. Cercariae shed from *Bi. pfeifferi and Bulinus spp.* snails were classified into 11 categories, of which only two, *S. haematobium* and *S. mansoni*, are major etiological agents of human schistosomiasis. The algorithms, trained on 80% of the snail and parasite dataset, achieved 99% and 91% accuracy for snail and parasite classification respectively, when used on the hold-out validation dataset-a performance comparable to that of experienced parasitologists. The promising results of this proof-of-concept study suggests that this CNN model, and potentially similar replicable models, have the potential to support the classification of snails and parasite of medical importance. In remote field settings where machine learning algorithms can be deployed on cost-effective and widely used mobile devices, such as smartphones, these models can be a valuable complement to laboratory identification by trained technicians. Future efforts must be dedicated to increasing dataset sizes for model training and validation, and testing these algorithms in diverse transmission settings and geographies.

0920

USING IMAGE-RECOGNITION SOFTWARE TO STRENGTHEN THE CAPACITY OF COMMUNITY HEALTH WORKERS

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Disease identification is a key component for reducing the burden of neglected tropical diseases. Many diseases require screening throughout the community, which necessitates training community members how to identify the disease in question. This process can be costly, and often does not yield optimal outcomes. Evaluation of trachomatis trichiasis (TT) case finders shows that the accuracy of appropriate case detection can be as low as 15-30%. Thus, in this project, we aimed to develop and evaluate two integrated apps focused on better TT case identification, one for automated detection of TT using photographic images and another for taking TT images in community settings and deploying the algorithm. We utilized 3,000 pre-operative photos from an ongoing clinical trial to train the algorithm. Algorithm development included segmentation, extraction and classification steps. We trained different neural networks to evaluate the classification task. The current accuracy of the algorithm is 87%,with a sensitivity of 88% and a specificity of 82%, and we are continuing to refine it. We have successfully integrated the algorithm app onto smartphones, with current processing time of 13 seconds to return a TT screening result following image capture. The associated image-capture app provides a user-friendly interface. We have also demonstrated that lay community members can easily be trained to take photos using smartphones, with over 98% gradable images. We will also report results of field testing in Mozambique and Ethiopia. This approach could be expanded to additional tropical diseases and other ocular conditions and has the potential to improve efficiency and effectiveness in screening communities for these diseases.

0921

INCORPORATION OF SARS-COV-2 ANTIGENS INTO AN EXISTING MULTIPLEX BEAD ASSAY FOR INTEGRATED SEROLOGICAL SURVEILLANCE OF NEGLECTED TROPICAL AND OTHER DISEASES

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SARS-CoV-2 emerged in December 2019, with confirmed cases reported from most countries. Antibodies to SARS-CoV-2 can estimate the proportion of a population with evidence of previous infection. We validated 4 SARS-CoV-2 antigens (1 spike protein, 2 receptor binding domain proteins and 1 nucleocapsid protein) on an existing multiplex bead assay (MBA) for integrated serosurveillance of vaccine-preventable diseases, malaria, neglected tropical diseases, and waterborne diseases. Receiver operating characteristic curves were created using 87 specimens from confirmed cases positive for SARS-CoV-2 by reverse transcription polymerase chain reaction (RT-PCR) and 118 pre-pandemic (presumed negative) and RT-PCR negative specimens. These were used to determine sensitivity and specificity for each antigen. Positive percent agreement (PPA) was determined using a panel of RT-PCR positive specimens, collected less than (n = 54) or more than (n = 48) 3 weeks post-symptom onset. Negative percent agreement (NPA) was determined using 97 pre-pandemic specimens. A subset of 119 samples (108 RT-PCR positives,

11 pre-pandemic negatives) were tested at both the Centers for Disease Control and Prevention (CDC) laboratory in Atlanta, GA, USA, and the National Reference Laboratory (NRL) in Gaduwa, Nigeria. Inter-laboratory comparisons were done using linear regression analysis of antibody levels. All assays had a sensitivity >94.3% (95% CI: 87.1-98.7%) and specificity >97.4% (95% CI: 92.7-99.5%). PPA for samples collected >3 weeks post-symptom onset ranged from 93.8% to 97.9% for all antigens, which was significantly higher than PPA for samples collected <3 weeks of symptom onset (55.6-81.5%). NPA for all antigens was 99-100%. The MBA signal was significantly higher in samples collected >3 weeks post-symptom onset than <3 weeks of symptom onset. The correlation coefficient between samples run at the CDC laboratory and the NRL was >0.98 for all antigens. The ability to simultaneously test for antibodies to SARS-CoV-2 antigens and other diseases of public health interest will potentially add value to SARS-CoV-2 or other disease-specific serosurveys.

0922

COMPLETE GENETIC ATTENUATION OF PLASMODIUM BERGHEI THROUGH A LIVER STAGE-SPECIFIC CRISPR-RGR GENE DELETION STRATEGY

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The aim of eliminating malaria will, in all likelihood, require an effective vaccine; a goal that currently remains unrealized. Live-attenuated sporozoite vaccine candidates represent the most promising tool for malaria elimination because they can achieve sterile protection. Genetic engineering offers a versatile platform to create Genetically Attenuated Parasites (GAPs). The identification and functional characterization of suitable genes also depends on a flexible and efficient tool for parasite manipulation during liver stage development. To address these twin aims in the rodent *Plasmodium berghei* malaria model for the first time, we adapted a ribozyme-mediated CRISPR gene editing system (CRISPR-RGR) previously established in *P. yoelii* for liver stage-specific gene editing. We generated a single CRISPR-RGR plasmid to express *Streptococcus pyogenes* SpCas9 and a dual ribozyme-guide-ribozyme (RGR) single guide RNA (sgRNA) under the control of liver-stage specific promoters 1 and 2 respectively. Moreover, the construct was designed for site-specific integration at the Silent Intergenic Locus 6 (SIL6). To functionally test this system, we targeted the gene encoding the Schizont Egress Antigen-1 (PbSEA1, PBANKA_0506000), recently described in *P. falciparum* as a gene involved in erythrocytic schizogony, with two sgRNAs targeting the 5' and 3' ends of its coding sequence. Our preliminary results reveal that the CRISPR-RGR plasmid achieves complete *Pbsea1* gene deletion during parasite liver stage development. Extensive cell growth and DNA replication was observed for *pbsea1*$^{-}$ liver stage parasites *in vitro* in human hepatoma cells (Huh7), and yet these parasites failed to establish an asexual blood stage infection in mice. Therefore, the use of liver stage-specific gene editing by CRISPR-RGR to delete blood stage-essential genes such as *sea1* represents a novel strategy to create genetically attenuated parasites for the production of malaria vaccine candidates.

0923

A NOVEL K13 MUTATION MEDIATES PLASMODIUM FALCIPARUM RESISTANCE TO OZ439

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The synthetic ozonide OZ439 is a preclinical antimalarial candidate to replace first-line artemisinin derivatives relied on to treat *Plasmodium falciparum* malaria, including artemisinin-resistant parasites in Southeast Asia. OZ439 shares the same bioactive endoperoxide moiety with artemisinins but benefits from a longer *in vivo* half-life. Here, we performed *in vitro* resistance selections with OZ439 in the isogenic parasite lines Cam3.II and Cam3.II_rev that express, respectively, the R539T mutant or wild-type alleles of the artemisinin resistance determinant *k13* gene. Using a ramping approach wherein step-wise increases in drug concentrations were applied over 34 cycles spanning 18 months, we obtained parasites that could survive and recover from a 96h exposure to 1 μM OZ439 (227x the IC_{50} and the pharmacological C_{max} for a 250mg dose). These OZ439-selected parasites recovered within 7-10 days and 18-22 days for Cam3.II and Cam3.II_rev parasites, respectively. Whole-genome sequencing revealed a novel K13 A212T mutation in the OZ439-selected Cam3.II clones. We used CRISPR/Cas9 editing to introduce A212T into Cam3.II and Cam3.II_rev parasites and to remove this mutation from a OZ439-selected clone. Phenotypic characterization revealed that A212T combined with the R539T mutation confers an accelerated rate of recovery following a 48h exposure to OZ439 at concentrations ranging from 8 nM to 250 nM. These parasites also maintain high levels of early ring-stage survival to 700 nM dihydroartemisinin, with survival rates of 18-24% after a 4h exposure. However, the A212T mutation alone does not alter the rate of parasite recovery after exposure to OZ439 or the levels of ring-stage survival following a 4h exposure to dihydroartemisinin. Further investigations into the mechanism of resistance in A212T mutant Cam3.II lines using global peptidomic profiling and K13 protein expression studies will be presented. Our results raise important implications for the use of OZ439 to treat artemisinin-resistant malaria.

0924

MITOCHONDRIA AND APICOPLAST INHERITANCE PATTERNS IN PLASMODIUM FALCIPARUM GENETIC CROSSES SUGGEST INCOMPATIBILITIES ACROSS THE LIFE CYCLE

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Genetic crosses provide power for linkage analysis when (i) progeny numbers are high, (ii) parental alleles segregate evenly and (iii) numbers of inbred progeny are minimized. Due to meiosis and chromosome crossover events, recombinant parasites inherit nuclear DNA from both parents but inherit their cytoplasmic DNA genomes from only the female gamete. *Plasmodium falciparum* has two cytoplasmic organelles: the mitochondria, with a 6 kb genome, and an organelle of plastid origin, the apicoplast, with a 35 kb genome. To study cytoplasmic genome inheritance and possible incompatibilities, we carried out three unique *P. falciparum* genetic crosses: (1) an allopatric cross between NF54, of African origin with NHP4026, of Southeast Asian origin (2) a sympatric cross between Southeast Asian parasites, MKK2035 and NHP1337 and (3) an allopatric cross between a Cambodian parasite KH004 and a Malawian parasite, Mal31. To date we have recovered 193 recombinant progeny for NF54×NHP4026, 60 recombinant progeny for MK22835 × NHP1337, and 280 recombinant progeny for KH004× Mal31. In the sympatric MK22835×NHP1337 cross, even inheritance of cytoplasmic organellar genomes was observed but in the allopatric crosses (NF54×NHP4026 and KH004×Mal31) 92% and 74% recombinant progeny inherited mitochondria and apicoplast genomes from NHP4026 and KH004 respectively. Since these strains successfully infect mosquitoes, incompetence of male or female gametes is unlikely to explain this inheritance pattern. However, we cannot completely exclude asymmetry in gamete fusion - i.e. an excess of female gametes from NHP4026 and KH004 parents - as an explanation. Using bulk segregant analysis (BSA)

of the progeny from the NF54×NHP4026 cross, we show that there is a significant decrease in NF54 cytoplasmic genome inheritance between the sporozoite and liver stages suggesting a cytoplasmic genome incompatibility during this life cycle transition. Thus, BSA allows us to probe selection across the entire life cycle and suggests incompatibility in cytoplasmic genome inheritance in allopatric genetic crosses.

0925

FINE-SCALE RELATEDNESS ANALYSIS REVEALS THE RECENT EPIDEMIOLOGICAL HISTORY OF PLASMODIUM FALCIPARUM POPULATIONS IN THE PACIFIC COAST REGION OF SOUTH AMERICA

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While global malaria rates have stayed relatively flat in recent years, there is significant geographical heterogeneity within this overall picture. Markedly, there has been a 36% increase in cases reported in the Americas since 2017. In Colombia and Ecuador, 60% and 10% of malaria cases occur in the Pacific coast region, respectively, and are mainly caused by *Plasmodium falciparum*. This region represents a pre-elimination scenario of low transmission that generally permits few outcrossing events. In recent years, however, the frequency of local outbreaks has risen. By increasing the opportunities for recombination, these outbreaks may generate new combinations of drug resistance haplotypes, thus posing a risk for elimination efforts. Here, we analyze 164 whole genome sequences from monoclonal infections sampled in Colombia (126) and Ecuador (38) between 2013 and 2016, to assess whether increased recombination during outbreaks generates genomic novelty. We use identity-by-descent (IBD) to estimate relatedness between parasites and find both high background relatedness and 14 multi-member clonal lineages that account for 98% of samples. Six of these clusters have persisted for at least 10 years, but the population as a whole is not static. By analyzing shared chromosomal segments between pairs of samples, we identify five complete crosses, including a two-generation pedigree. Two of these crosses generated novel combinations of drug-resistance haplotypes. By integrating this with epidemiological data we show that the timing of these crosses likely coincided with periods of heightened transmission when the parental genotypes were at high frequency. Combining our data set with 17 pre-2005 Colombian genomes, we use patterns of intra-chromosomal IBD to identify two hard selective sweeps driven by chloroquine and soft sweeps imposed by sulfadoxine and pyrimethamine (SP). These results show that parasites in low-transmission, low-diversity settings continue to evolve in response to selection pressures via both recombination and *de novo* mutation, making continued surveillance warranted under such conditions anywhere in the world.

0926

EPIDEMIOLOGICAL AND GENETIC MODELING OF MALARIA TRANSMISSION OFFERS AN IN-SILICO APPROACH TO UNDERSTAND GENETIC FEATURES AND TRANSMISSION PROPERTIES FROM EMPIRICAL DATA

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Genetics can serve as a fingerprint for unobserved transmission dynamics to complement routine malaria surveillance data. Features derived from malaria parasite genetic data, such as complexity of infection (COI) and sample relatedness, have been shown to be correlated with measures of transmission intensity. However, the standard algorithms for computing a highly resolved estimates of COI and relatedness have been tested on simulated datasets that represent cross-sectional diversity, largely divorced from the temporal epidemiology that shapes the genetic structure of a population. Here, we leverage a layered mathematical modeling approach where we combine EMOD, an epidemiological model of malaria transmission which accounts for transmission history, GENEPI, a malaria parasite genetic model that simulates genomic evolution on a transmission network accounting for co-transmission and superinfection, and an observational component to recreate sampling and sequencing uncertainty. The observational component simulates sequencing uncertainty by collapsing strains from an infection into a single, representative genome with heterozygous positions. This flexible model enables pressure testing of common algorithms with simulated genomes derived from different transmission intensities, genomic scope by number of variants, and infection diversity by variant heterozygosity. We then compared modeled data estimates to empirical data estimates from a country-wide Senegal population genetic dataset with over 1,000 sequenced samples from geo-located health facilities for a population-specific sensitivity analysis. Our findings suggest that the model can be used to understand variant panel properties and algorithm uncertainty estimates for different transmission scenarios that affect population genetics. Broadly, the comparisons between modeled and empirical data might help define best sample collection and sequencing technology strategies to support programmatic decisions guided by transmission-informative genetic features.

0927

THRIVING STUDENTS, THRIVING COMMUNITIES: THE IMPACT AND COST-EFFECTIVENESS OF SCHOOL-BASED MALARIA CHEMOPROPHYLAXIS

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Across sub-Saharan Africa, the prevalence of *P. falciparum* infection often peaks in school-age children, who suffer from adverse consequences on

health and education. However, children over 5 years old are not included in seasonal chemoprevention (SMC) and are generally less likely to use bednets or receive effective case management, leaving a gap in current malaria control efforts. Using mathematical models of transmission in diverse endemic sub-Saharan settings that differ with regards to transmission intensity, seasonality, and school calendars, we investigate the impact of using primary schools to deliver intermittent preventive treatment to school-aged children (IPTsc). An IPTsc strategy of presumptive treatment of all students with dihydroartemisinin-piperaquine (DP) once per school term reduces burden in school-age children by about 50%, and reduces the overall clinical burden in the community by about 30%, with greatest community benefit found at lowest transmission intensity. IPTsc outperforms increasing ITN coverage from 70% to 90%, which only reduces overall clinical burden by about 10%. IPTsc also outperforms chemoprophylactic strategies which target children under 5, such as SMC, in terms of overall clinical burden reduction. However, in moderate to high transmission intensity settings where the burden of severe disease is highest in children under 5 years old, chemoprevention for under-5s reduces severe disease burden more than IPTsc. IPTsc combined with traditional under-5 SMC is superior in terms of both impact and cost to expanding SMC to older ages. For the health system perspective, the cost to avert a case with IPTsc is typically much less than the cost of treating a case in a health facility, which means that the cost to perform IPTsc is outweighed by the savings generated from having to treat fewer clinical cases overall. In sum, IPTsc is a promising strategy to reduce the burden of malaria both in school-age children and in the overall community. Operational research will be necessary to optimize IPTsc to specific settings based on transmission intensity, seasonality, and local malaria control interventions.

0928

USING MATHEMATICAL MODELING TO EXPLORE THE POTENTIAL EFFECT SIZE OF A SEASONAL MALARIA CHEMOPREVENTION TRIAL IN KARAMOJA SUB-REGION, UGANDA

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As a platform for exploring complex infectious disease dynamics, mathematical modeling can powerfully aid trial planning. We aim to assess the likely effect size of a forth coming trial of seasonal malaria chemoprevention (SMC) when deployed as planned, and if deviations from the plan occur. Using the microsimulation platform, OpenMalaria, we simulated the trial: treating children <5 years with 5 rounds of SMC, from May to September, at a coverage of 80%. Bednet usage of 28% (derived from MIS 2018) and a 25 day efficacy of the SMC drug (SPAQ) were assumed. Alternative scenarios explored how i. doubling net usage, ii. deploying with a month delay, iii. deploying 4 rounds only, iv. halving drug efficacy duration, and v. decreasing coverage by 10% with each round, would likely influence effect size. Scenarios were run under baseline malaria incidence levels ranging 1,200-3,000 annual episodes/1,000 population, to reflect the heterogeneity of study sites. We defined effect size as the percentage incidence reduction compared to the scenario of no SMC deployment. Baseline incidence assumptions shaped all findings. When deployed as planned, the projected effect size of SMC was greatest when baseline incidence was lowest; the incidence in 2022 decreased by 42% at the lowest incidence level and by 25% at the highest. Effect sizes under the alternative scenarios varied less at higher baseline incidence levels, for instance, when net usage was doubled, the effect size increased 1.5 times at the lowest baseline incidence level, but was no different at the highest incidence level. Shorter drug efficacy duration and decreased coverage lowered the effect size most substantially, followed by deploying 4 rounds instead of 5, while delaying deployment had little effect. The effect sizes estimated can be used for calculating the necessary sample size for the trial. However, it is vital to first understand the baseline incidence of each site, bednet usage, drug efficacy, and any likely impediments to maintaining target coverage. This analysis demonstrates how mathematical modelling can be instrumental in disentangling complex considerations in trial planning.

0929

BLOOD FEEDING ON HUMANS VACCINATED WITH AGS-V PLUS, A MOSQUITO SALIVARY PEPTIDE VACCINE, IMPAIRS THE REPRODUCTIVE CAPACITY OF FEMALE AEDES ALBOPICTUS MOSQUITOES

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Vaccinating humans against mosquito salivary proteins has emerged as a novel approach to protect against mosquito-borne pathogens. While this strategy has focused on blocking infection of the host, it is unclear how the mosquito may be impacted by blood feeding on individuals vaccinated against salivary proteins. As part of a phase 1 clinical trial of AGS-v PLUS, a novel mosquito salivary peptide vaccine (NCT04009824), we tested the hypothesis that blood feeding on individuals vaccinated with AGS-v PLUS would impair mosquito survival and reproduction. Participants were randomized into one of five study arms: placebo, two-dose AGS-v PLUS without adjuvant, single-dose AGS-v PLUS with ISA-51, two-dose AGS-v PLUS with ISA-51, or two-dose AGS-v PLUS with Alhydrogel. Three weeks after the last injection, participants were exposed to feeding by uninfected female *Aedes aegypti* and *Aedes albopictus* mosquitoes, which were then monitored for survival, egg laying capacity, and development from egg to adult. For analysis, each intervention arm was compared individually to the placebo arm at a pre-specified significance threshold of $p<0.10$. Thirty-three participants completed both study drug injections and the mosquito feeding. For *A. albopictus*, feeding on participants vaccinated with two doses of AGS-v PLUS/ISA-51 resulted in decreased numbers of eggs laid (average, 5.1 vs. 13.1 eggs per mosquito; $p=0.083$) and fewer adult offspring per mosquito (average, 1.6 vs 8.4; $p=0.012$), with the other three AGS-v PLUS regimens showing similar trends towards lower numbers of both eggs laid and adult offspring. In contrast, none of the vaccine regimens affected oviposition by *A. aegypti*. For both *A. aegypti* and *A. albopictus*, none of the vaccination regimens affected the number of blood fed mosquitoes or mosquito survival. This clinical study provides the first proof-of-principle demonstration that vaccinating humans with mosquito salivary peptides can impair reproduction in female mosquitoes that blood feed on vaccinated individuals. Current work is focused on identifying human immune correlates for these inhibitory effects on mosquito reproduction.

0930

AEDES ALBOPICTUS BLOOD FEEDING BEHAVIOR: FROM LAB TO FIELD

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Aedes albopictus is a competent vector of numerous pathogens, representing diverse transmission cycles involving unique hosts. Previous blood meal analyses demonstrate a wide range of feeding patterns, from primarily anthropophagic to zoophagic - yet little is known about the drivers of these differences. We assessed *Ae. albopictus* feeding behavior in the field and lab, including feeding patterns, potential fitness implications of host species, and preference. We examined the feeding patterns in Long Island, New York, and contextualized blood meal sources with host availability measured by household interviews and camera traps. We identified 90 blood meals, including 29 human, 22 cat, 16 horse, 12 opossum, 5 dog, 2 goat, and 1 rabbit, rat, squirrel and raccoon. Our study is the first to quantitatively assess *Ae. albopictus* feeding patterns in the context of host availability of wild animals in addition to humans and domestic animals. Host feeding indices showed that cats and dogs were over-utilized compared to humans. Forage ratios suggested a tendency to over-utilize cats and opossums and under-utilize raccoons, squirrels, and birds. This feeding pattern was different from a published study from Baltimore, Maryland, where *Ae. albopictus* fed more often on rats than humans. To understand if these differences were due to host availability or mosquito population variation, we compared the fitness of Long Island and Baltimore *Ae. albopictus* after feeding on rat and human blood. In addition, we examined fitness within the Long Island population after feeding on human, rat, cat, horse, and opossum blood. Our results do not show major mosquito fitness differences by blood hosts, suggesting that fitness benefits do not drive Northeastern *Ae. albopictus* feeding patterns. We then assessed the host preference of *Ae. albopictus* populations across a spectrum of feeding patterns from primarily zoophagic to anthropophagic. Together, these findings provide novel insight into a critical behavior of an important vector of human disease.

0931

BLOOD MEAL SOURCES AND NONRANDOM HUMAN SELECTION BY ANOPHELES VECTORS OF HUMAN MALARIA IN MALAWI: IMPLICATIONS FOR MALARIA TRANSMISSION

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Despite widespread availability of long-lasting insecticide treated bednet (LLINs), malaria transmission with high human infection incidence continues in Malawi. We hypothesize that biases in mosquito human feeding could be driving infection transmission. The study was conducted to quantify the extent and diversity of human blood feeding by vector mosquitoes in areas with extensive use of LLINs including Balaka district with standard permethrin and Machinga district with piperonyl butoxide synergist LLINs (PBO). Mosquitoes were sampled indoors during 2019-2020 by pyrethrum spray catch and light traps. Human demographic data and blood spots on filter paper by finger prick were collected from consented participants. Mosquitoes were identified by morphological and molecular methods. Mosquito blood meal source and *Plasmodium falciparum* infection in abdomen or head-thorax of mosquitoes was determined by qPCR methods, using host-specific oligonucleotide probes and parasite-specific probes, respectively. Human blood meals and human blood spots were analyzed by genotyping 24 human microsatellite loci, to generate genetic profiles that were matched using an algorithm executed in R package. Of 635 blood-fed *Anopheles* spp, 44.1% were *An. arabiensis*, 16.2% *An. gambiae s.s,* 33.5% *An. funestus*, 0.3% *An. parensis,* and 5.8% were unidentified *Anopheles* spp. Blood meals were predominantly from humans (80.5%), but also goats (5.2%), dogs (2.0%), human/goat mixed (11.1%), human/dog (0.9%), and dog/goat (0.2%). Samples from Balaka with standard LLIN had a higher human blood index (81.2%) than Machinga (75.3%) with PBO nets, but it was not significantly different ($p = 0.59$). Sporozoite infection in the head-thorax was 16% (99/633) overall, for an estimated annual EIR of 26 infectious bites/person. Genotyping analysis revealed a highly non-random pattern of human host section with a bias toward feeding upon males aged 6-15 years. These results show a high proportion of human blood meals, regardless of LLIN type, and suggest that the school age males may be important contributors to transmission.

0932

IMPACT OF RANDOMIZED WMEL WOLBACHIA DEPLOYMENTS ON NOTIFIED DENGUE CASES AND INSECTICIDE FOGGING FOR VECTOR CONTROL IN YOGYAKARTA CITY

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We conducted a cluster randomised trial of deployments of *w*Mel-infected *Ae. aegypti* for dengue control in Yogyakarta City, Indonesia (Applying *Wolbachia* to Eliminate Dengue (AWED) trial). A predefined secondary endpoint was to determine the impact of *w*Mel deployment on dengue cases notified to the surveillance system, which requires hospitals to report cases diagnosed clinically as Dengue Hemorrhagic Fever (DHF). Mixed-effect negative binomial regression was used to model monthly DHF case count (Jan 2006 - Apr 2020) comparing *w*Mel-treated kelurahans (urban villages) with both the pre-intervention period and untreated kelurahans. The boundaries of the 35 kelurahans in the trial site do not all align with those of the 24 AWED clusters and rules were defined to apply a binary *w*Mel intervention status to each kelurahan. Nineteen kelurahans were classified as 'treated' (*w*Mel frequency >50% AND >50% trap positivity for 2 monthly monitoring events in a 6-month rolling window), 10 as 'untreated', and six were excluded from the analysis using binary *w*Mel exposure classification due to highly variable %wMel. A secondary model used quintiles of monthly kelurahan-level *w*Mel prevalence as the predictor of dengue incidence. DHF incidence was 54% lower in *w*Mel-treated vs untreated kelurahans (IRR 0.46 [95% CI 0.34, 0.62]), and 61% lower in kelurahan-months with 80-100% wMel prevalence compared to kelurahan-months with 0-20% wMel (0.39 [0.25, 0.60]). An effect was also seen with 60-80% (0.61 [0.37, 1.00]) and 40-60% wMel (0.56 [0.38, 0.82]). The impact of *w*Mel deployment on insecticide fogging activities, undertaken by vector control teams around the homes of notified DHF cases, was also analysed using negative binomial regression. Pre-intervention, fogging occurred at similar frequencies in areas later randomised to *w*Mel-treated and untreated arms of the trial. After *w*Mel deployment, fogging occurred significantly less frequently in treated areas (0.13 [0.07, 0.27]). These findings show that *w*Mel deployments reduced the application of perifocal insecticide spraying by 87%, consistent with lower dengue case notifications in *w*Mel-treated areas.

0933

NATURAL WOLBACHIA DETECTED IN WILD ANOPHELES STEPHENSI IN EASTERN ETHIOPIA

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About 66 percent of Ethiopians are at risk of malaria, a disease caused by the parasite *Plasmodium falciparum* and *P. vivax*. *Anopheles stephensi*, a vector typically found in South Asia and the Middle East, was recently found to be distributed across eastern Ethiopia and is capable of transmitting both *P. falciparum* and *P. vivax.* Between the detection of this vector in the Horn of Africa and increasing insecticide resistance, new methods of vector control need to be investigated in order to control the spread of malaria. *Wolbachia,* a naturally occurring endosymbiotic bacterium of mosquitoes, has been identified as a tool for control of malaria transmission. *Wolbachia* could be used to control the mosquito population through replacement and decrease malaria transmission through suppression, however the presence of *Wolbachia* in wild *An. stephensi* in eastern Ethiopia is unknown. This study aimed to identify the presence and diversity of *Wolbachia* in *An. stephensi* across east Ethiopia. DNA was extracted from *An. stephensi* collected from eastern Ethiopia in 2018 and screened for *Wolbachia* using a 16S targeted PCR assay. Haplotype and phylogenetic analysis of the sequenced 16S amplicons were conducted to compare with *Wolbachia* from other regions. Thus far, 22 mosquitoes were positive for *Wolbachia* out of 193 mosquitoes screened. Multiple haplotypes were detected in the Ethiopian sequences. In addition, phylogenetic analysis revealed that the eastern Ethiopian sequences cluster with other *Wolbachia* sequences in Anopheline mosquitoes in Africa and SE Asia. These findings provide the first evidence of natural *Wolbachia* populations in wild *An. stephensi* in the Horn of Africa and identify the need to do further research to confirm the extent that Wolbachia infects *An. stephensi* and investigate its utility in malaria control in the Horn of Africa.

0934

SUCCESSFUL CRYOPRESERVATION OF MOSQUITO (ANOPHELES STEPHENSI) EGGS

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Research on mosquitoes, and in particular on *Anopheles* spp., is constrained by the lack of a method for the long-term preservation of stocks of the large number of laboratory-bred strains, and genetically modified (GM) lines. Cryopreservation would provide a powerful tool for mosquito research, negating the need for continuous maintenance and supporting the implementation of mass release programs of GM mosquitoes for disease control. Several insect species have been successfully cryopreserved including some dipteran species, but to date, despite considerable effort, no method has been developed for mosquitoes - until now. We present a method for the cryopreservation of *Anopheles stephensi* eggs that reproducibly yields a hatch rate of ~25%. The cryopreserved eggs are stable in liquid nitrogen vapor phase below -150 °C for more than 5 years. Hatched larvae develop normally through to adults and the females blood feed, mate, and produce viable second-generation eggs that also develop normally. Vector competency of adult mosquitoes obtained from cryopreserved eggs was demonstrated by infection with the malaria parasite, *Plasmodium falciparum,* and the development of salivary gland sporozoites in numbers similar to those produced in controls. Critical components of the cryopreservation technique are the time of egg harvest after oviposition, the cryoprotectant additive (CPA), the temperature and duration of exposure to the CPA, cooling and thawing rates, and post-thawing dilution. The technique can easily cryopreserve small or large (100,000+) batches of eggs, adequate for banking species and strains, and to date has been used to bank several batches of *A. stephensi* SDA500, the mosquito used to manufacture Sanaria® PfSPZ Vaccine, PfSPZ Challenge, and PfSPZ-GA1, several genetically altered lines of *A. stephensi*, and *A. gambiae*.

0935

MICROBES INCREASE THERMAL SENSITIVITY IN THE MOSQUITO, AEDES AEGYPTI, WITH THE POTENTIAL TO CHANGE DISEASE DISTRIBUTIONS

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Changes in global climate, that include higher temperatures and more frequent extreme thermal events, are expected to cause dramatic shifts in the distributions of infectious diseases. The mosquito *Aedes aegypti* is the primary vector of many disease-causing viruses, worldwide including dengue (DENV), and the geographic range of the mosquito is increasing, partly, due to changing climate. One emerging solution for vector-borne disease control is the release of the insect endosymbiont, *Wolbachia,* whose infection in mosquitoes reduces virus transmission to humans. When temperatures fluctuate, mosquito vectors will be increasingly exposed to temperatures beyond their upper thermal limits, but how they respond may be dependent on genotypic variation in thermotolerance within, and between populations. Here we examine how DENV and *Wolbachia* infection alter *Ae. aegypti* thermotolerance and have investigated whether thermal tolerance is heritable, using a high throughput physiological 'knockdown' assay modeled on studies in *Drosophila*. Such laboratory measures of thermal tolerance have previously been shown to accurately predict insect distributions in the field. We show that DENV and *Wolbachia* infection similarly increase mosquito thermal sensitivity. Surprisingly, in the coinfected state, *Wolbachia* did not provide protection against DENV associated effects on thermal tolerance, nor were the effects of the two infections additive. The latter suggests that the microbes may act by similar means, potentially through activation of shared immune pathways or energetic tradeoffs. Further, by studying thermal tolerance in the framework of a family breeding design, we found heritability of the trait to be low. In total our work demonstrates that future global projections of DENV transmission risk, and of *Wolbachia's* potential efficacy may need to take into account the impact of these microbes on vector survival. Additional studies are needed to further explore the role of history of adaptation and genetic variation in a mosquito's thermotolerance.

0936

COMPARISON OF STATISTICAL METHODS FOR ANALYSIS OF RECURRENT ADVERSE EVENTS IN THE PRESENCE OF NON-PROPORTIONAL HAZARDS AND UNOBSERVED HETEROGENEITY: A SIMULATION STUDY

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In preventive drug trials such as intermittent presumptive treatment for malaria prevention during pregnancy where treatment is repeatedly administered, recurrence of adverse events(AEs) is common. Analysis of the recurrent AEs is challenging due to time-to-AE variations and within-patient correlations, rendering the conventional methods (e.g. Fisher's exact) prone to bias. The correlation comes from individual patient unobserved heterogeneity (i.e. frailty) and the dependence between AEs. Potential AE-dependence can be modelled via time-dependent treatment effects, event-specific baseline and event-specific random effect, while heterogeneity can be modelled via subject-specific random effect. However, the presence of time-dependent effects can lead to biased estimation of the unobserved heterogeneity. Using both simulation study and *Chloroquine for Malaria prevention in Pregnancy* trial data, we investigated whether the lognormal shared frailty models with restricted cubic splines and non-proportional hazards(LSF-NPH) assumption can improve frailty variance estimates compared to the conventional inverse Gaussian shared frailty with proportional hazard (ISF-PH) model, in the presence of time-dependent treatment effects and unobserved patient heterogeneity. We assessed the bias, precision gain and coverage probability of 95% confidence interval of the frailty variance estimates for the models under varying known unobserved heterogeneity and sample sizes in the presence of non-proportional hazards for the drug effects. The ISF-PH model consistently provided better coverage probability and less bias of the frailty variance estimates. The LSF-NPH models yielded better log hazard ratio estimates, precision and model standard errors for frailty variance estimates. The coverage probability of frailty variance estimates for the LSF-NPH improved as the number of restricted cubic splines increased. This study demonstrates the need for improved safety data analysis and preferring LSF-NPH models when there are recurrent AEs and time-dependent treatment effects in malaria chemoprevention in pregnancy trials.

0937

MODELLING THE EFFECT OF PHARMACEUTICAL AND NON-PHARMACEUTICAL INTERVENTIONS IN THE DYNAMICS OF COVID-19 - A COMPARISON BETWEEN SWEDEN AND THE CZECH REPUBLIC

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A year after the onset of the epidemic and multiple epidemic waves later, the Covid19 pandemic remains a global health emergency. The overwhelming pandemic, which increased with the emergence of new variants of the virus, remains somewhat unclear and has raised awareness for an efficient strategy to mitigate the continuous spread of the disease. Herd immunity as a global response to the pandemic could be achieved either by natural infections or through vaccination campaigns. Initially, non-pharmaceutical interventions, e.g., home isolation, social distancing, were the foremost approach by most countries, while other countries like Sweden did not comply with such measures. As shown by the subsequent dynamic of the pandemic, it was erroneously assumed that herd immunity would be achieved faster in Sweden. However, the success of the first implementation of the non-pharmaceutical interventions in the Czech Republic yielded the world's highest death toll during the next wave - "paradox of success". As vaccines have become available, it is crucial to examine the impact of these pharmaceutical interventions, as well as when and how it best fits for the non-pharmaceutical interventions to be relaxed for herd immunity to be reached. Here, we introduce a predictive model to forecast the effect of different non-pharmaceutical (contact reduction measures, social distancing, and isolation) and pharmaceutical (different vaccines efficacy) interventions on the control of the COVID-19 pandemic.

0938

SPATIAL VIDEO, GEONARRATIVES AND MACHINE LEARNING CONTEXTUALIZATION DISEASE LANDSCAPES

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An ongoing data deficiency in many challenging environments is how to acquire granular contextualized spatial data to support on-the-ground epidemiological investigations. When investigating which microenvironments might be predictive of cholera outbreaks, or how risk of malaria varies with micro seasonality, it is important to acquire locations of associated environmental features, such as water points, or drainage channels. Next, we need to include *context*. For example, how do water points vary in type, contamination risk, local use and what is the local perception about them? Do these features change in risk / role / importance across the seasons? And does the surrounding environment have a direct or indirect influence? In this presentation we will focus on Ghana, Haiti, and Kenya to show how a geospatial field technique and an associated software has been used to capture and map the required data for these questions. We will show how spatial video, which is a small robust video camera with an inbuilt global positioning system, has been used to map local mosquito habitats, or where standing water poses a threat to contaminating drinking water (for example). Using bespoke software, we will show spatial video visualization, data improvements such as correcting coordinate paths, and how to map (through digitizing) risk factors. We will then contextualize these environments with geonarratives, which are the addition of informed commentaries collected from local health workers or residents to explain these environments. Using bespoke software these narratives will create an additional map layer of *context*. Finally, we will show how machine learning can be used to automatically extract and map frames from the video to provide a near real time solution to generate and update these types of local area maps. This presentation will provide a detailed explanation of how these techniques work, with results showing granular spatial and temporal variations for each of the three countries for three commonly accepted multi-disease risk environmental variables: trash, standing water and drainage channels.

0939

USING TECHNOLOGY FOR INSECTICIDE-TREATED NET (ITN) DISTRIBUTIONS - IS IT WORTH IT AND WHY?

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In 2018, CRS supported the government of Nigeria with distributing 12 million nets to 22.5 million people. Integrating technology has been key to operating successfully on such a large scale. The project spent just over $2.4 million on technology as a way to improve geographic coverage, efficiency, transparency, and accountability. An internal study was conducted that involved the use of machine learning to understand whether using technology for ITN distributions was worth the cost. To determine the impact of technology, the study compared the cost of paper-based implementations in the two states of Edo and Osun and the cost of digital implementation in Katsina state. The results showed that while the cost of digital implementation was higher, using technology increased the rate of net pick-up, or redemption, significantly - by up to 10.2%. Machine learning algorithms identified the greatest factors to predict redemption. The Random Forest algorithm had the highest predictive accuracy of 77.9%. Using technology to reduce walking and

wait times, sending reminders, tracking household follow-ups, and identifying missed areas enabled the digital implementation to have a higher impact since more people got a net. Other factors that influenced redemption rates were the amount of time the monitor spent at the household, family size, and day of the week the distribution occurred. Redemption rates in the paper states were 88% for Edo and 89.37% for Osun State, but increased to 98.8% in Katsina state with digital implementation. Technology starts to pay for itself when considering the household health costs of contracting malaria. To understand the value of increasing redemption rates, it is important to understand a myriad of factors: the household cost of contracting malaria, the effectiveness of using an ITN, the malaria prevalence rate in the state, and the likelihood of people using the nets. Assuming that treating a malaria case costs about $21.20 and that ITNs reduce the risk of contracting malaria by 56%, we estimate that the %improvement of net redemption in Katsina state led to an estimated $22.5 million in prevented malaria cases.

0940

ESTIMATING THE IMPACT OF COVID-19 ON HOSPITAL ATTENDANCE IN NIGERIA USING ROUTINE NATIONAL HEALTH MANAGEMENT INFORMATION SYSTEM DATA

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After the first COVID-19 case in Nigeria was reported in February 2020, there has been widespread fear with negative impact on society. We assessed the impact of COVID-19 pandemic on health service utilization through a three-year trend analysis of Health Facility (HF) monthly OPD attendance reported through the National Health Management Information System (NHMIS). The HFs assessed were selected through a two-stage random sampling process where six states and sixty high-volume HFs were selected. Level of health care (primary, secondary, tertiary) and ownership (government, private) were also recorded against each HF. Monthly OPD attendance were routinely collected by health workers using standardized registers and entered into the web based NHMIS by local government staff from where data for the analyses were downloaded. Of the 2,160 expected reports, 288 (10%) were missing. Missing data were rebuilt through interpolation using seasonal forecast. Weighted trend series model accounting for yearly seasonality was used to predict OPD attendance trend during the COVID-19 period and this was compared with actual monthly trend. Chi-square test at 95% significance level was also used to compare whether differences 9 month pre-COVID and during COVID-19 periods were significant across ownership, level of healthcare and state. There was a 45% decline between reported and predicted average monthly OPD attendance during COVID-19 period. The decline in government HFs was 51%, compared to 24% in privately owned HFs ($p<0.0001$). Primary level HFs recorded a 40% decline compared to 43% in secondary and 42% in tertiary HFs ($p = 0.107$). By State, the decline was Bauchi 56%, Imo 52%, Lagos 47%, Taraba 32%, Kaduna 20% and Cross River 12% ($p<0.001$). The COVID-19 pandemic negatively impacted health service utilization in Nigeria. This could potentially worsen health outcomes. The significant differences based on ownership of HFs may reflect the general perception of better infection prevention strategies and availability of higher quality services in privately owned HFs. This needs to be studied further.

0941

DEVELOPING A SPATIAL GRID-BASED TECHNIQUE FOR REPRESENTATIVE SAMPLING IN POPULATION-BASED SURVEYS USING MACHINE LEARNING AND GEOGRAPHIC INFORMATION SYSTEM

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Population-based surveys are essential to generate denominators for health statistics. Conventional sampling techniques used in population-based representative surveys pose considerable challenges, including the need to pre-enumerate households and the availability of an up-to-date census with administrative boundaries. Similarly, the sampling frame in a conventional grid-based technique considers grid cells where households might be absent such as in agricultural lands, which may introduce operational redundancies. Therefore, to overcome the challenges of conventional sampling, we developed a hybrid method by integrating conventional grid-based sampling with a satellite-based grid cell elimination process. The study aims to compare this hybrid approach with the conventional grid-based method using data from a health and demographic surveillance system (HDSS), which tracks every individual in the Baliakandi sub-district of Bangladesh and aims to capture every stillbirth and under-five death in the area. We developed a spatial grid with a cell size of 500 x 500 meters and a land cover model using Sentinel-2 satellite images and the random forest machine learning classifier. HDSS census helped determine a sampling size of 12000 households (n=58869). The sampling frame of the grid-based approach considered all grid cells, but for the hybrid method, the land cover model was used to eliminate cells with no households; the sampling frame was then developed from remaining cells (n=605). Results suggest 42% of uninformative cells could be removed using the hybrid approach and save the time and logistics required to deploy survey teams to any of these cells. In 1000 replicated runs, only the hybrid sampling approach could capture the targeted number of households and substantially reduce under- and over-estimations of stillbirths and under-five death rates. The hybrid approach could detect households with a sensitivity and specificity of 86% and 93%, respectively. In conclusion, the hybrid approach offers fast and targeted representative surveys and reduces under-and over-estimations of vital health statistics.

0942

MACHINE LEARNING APPROACHES FOR COVID-19 MISINFORMATION AND VACCINE ACCEPTANCE IN AFRICA

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In order to understand possible barriers to COVID-19 vaccine acceptance in Africa, Novetta's Rapid Narrative Analysis (RNA) process combined human observation, machine learning, and natural language processing techniques to gain insight into levels of trust on vaccine and vaccine trials from African Twitter users from April 2020 - January 2021. The methodology and foundation for this process was based on the Africa Centres for Disease Control and Prevention Rumor Tracking System (est. April 2020), the purpose of which was to monitor rumors and misinformation around COVID-19 in Africa, and to understand public discussion about the pandemic and obtain background information for public health messaging and programming at the continent level. After five months of operation, an initial analysis was conducted of how social media networking on Twitter impacted COVID-19 vaccine acceptance in Africa. Within the scope of analysis, "trust" or "distrust" in vaccines was defined as positive/negative language, sentiment, or disapproval of a

COVID-19 vaccine. Quantified changes in trust or distrust were measured through the volume of tweets with the respective model-predicted sentiment. A follow-up analysis was conducted retrospectively in January, and covered the time frame from August 2020 - January 2021 to monitor any change in narrative from the first monitored period. RNA's machine learning modeling detected more distrust than trust across all tracked African countries in the first 5 months of data monitoring. Nearly 64% of unique users expressed distrust in vaccines, with only 33% of unique users expressing trust in vaccines. Further analysis through time showed an overall decrease in trusting/distrusting sentiment, and a rise in neutral sentiment, due to increased media exposure of other COVID-19 vaccine trials and increased inoculation.

0943

EVERY CHILD COUNTS: REACHING ZERO-DOSE CHILDREN USING GEOSPATIAL DATA & TECHNOLOGY

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Mass vaccination campaigns introduce new vaccines; provide doses to those who may have missed routine doses; and give a second opportunity to those who may not have developed immunity. These campaigns, however, have been known to miss zero-dose children, leaving communities vulnerable to vaccine preventable disease. To address this issue, we utilized a spatial intelligence approach to identify zero-dose children for follow-up after a nation-wide mass vaccination campaign in Zambia for measles and rubella, held during the Covid-19 pandemic in November 2020. To strategically reach zero-dose children, we applied enumeration methods to satellite imagery to establish structure counts for 10 health facility catchment areas in Choma district. We then used Reveal, an open-source platform, which enabled community health workers (CHWs) to navigate and register children at the household-level. An analysis of these data showed this approach allowed zero-dose children to be identified, mapped and vaccinated, using Reveal to navigate and collect data at the household-level during a mop-up activity that followed the campaign. A total of 43,113 structures were enumerated using satellite imagery. Of these structures, 36,964 were found (86%). Only 12,014 structures were eligible for registration, of which 10,928 (91%) were registered. 13,497 children were found and registered within these households. Of these, 11,498 (85%) were fully vaccinated; 1208 (9%) were partially vaccinated; and 791 (6%) were zero-dose. Following the mass vaccination campaign, nurses and CHWs were tasked with visiting the households of these zero-dose children to verify if they were vaccinated during the campaign. If not, a vaccine was provided by the nurse. We found 565 of the 791 zero-dose children. Of these, 363 (64%) were vaccinated during the campaign, while a further 191 (33%) were vaccinated during this targeted mop-up activity. We demonstrate how linking CHWs with a geospatial solution such as Reveal can enable the identification of zero-dose children, as well as the targeted delivery of vaccines, leading to improved coverage rates and community protection.

0944

ADOPTION OF LOCAL GOVERNMENT AREA PEER MENTORING IMPROVES DATA QUALITY MANAGEMENT ACROSS LOCAL GOVERNMENT AREAS IN CROSS RIVER STATE, NIGERIA

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Available evidence suggests that a functional health system needs quality data to make informed decisions. This cannot be achieved if the personnel generating, reporting, and managing data have gaps in their knowledge and skills. The data generated from all the health facilities are uploaded to the National Health Management Information System (NHMIS) by the local government area (LGA) Monitoring & Evaluation (M&E) officers. Thus, the capacity of the LGA M&E officers can determine the quality of data reported for the district. The problem is further heightened by poor oversight due to inadequate human resource. The President's Malaria Initiative for States (PMI-S) Project conducted a baseline analysis of health facilities in Cross River State in 2019. The report highlighted data quality problems with 398 of 1300 health facilities (31%) reporting inconsistent data in the NHMIS. The LGAs contributing most of the inconsistent data were Akpabuyo and Abi who together contributed 12% of the inconsistent data. PMI-S introduced a peer-to-peer mentoring intervention with a high-performing LGA M&E officer paired with low performing LGA staff across 80 health facilities in the identified LGAs. The main responsibility was to support and improve the capacity of M&E officers in these two neighboring LGAs over a four-month period by helping each other to learn, facilitate skills transfers, and improve performance; and in doing so, encourage learning in themselves, inspire exchange, and learn among other LGAs. After four months of implementation, the proportion of health facilities reporting data quality issues (errors) reduced, for Abi LGA 57.6% (29/51) in 2019 to 0% (0/51) in 2020, and from Akpabuyo LGA 49.8% (19/39) in 2019 to 1% (1/39) in 2020. These findings indicate that health care workers at the LGA level may contribute to better results if they are equipped with the right skills. Peer to peer learning removes hierarchy during learning and encourages networking amongst peers to improve performance. The approach is a cost effective and sustainable approach for the state.

0945

THE ROLE OF GEOSPATIAL DATA IN GLOBAL HEALTH

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Health systems in the global north rely on a wealth of geospatial data to deliver routine healthcare, perform disease surveillance, and respond to disease outbreaks. For example, the United States employs recent population data for its distribution and administration of the COVID-19 vaccine, while most American patients in turn can readily access online information about vaccine availability (often using navigation systems for directions to the nearest vaccination site). These systems rely on precise geo-enabled data that is often lacking in low- and middle-income countries (LMICs). To help address this shortfall, GRID3 (Geo-Referenced Infrastructure and Demographic Data for Development) is working with countries in sub-Saharan Africa to generate, validate, and support the use of geospatial data on population, settlements, infrastructure, and boundaries. GRID3 responds to support requests from government and non-government organizations with tailored technical assistance and skill-building, often working closely with national statistical offices and national health ministries. High-resolution geospatial data on population distributions and characteristics, health infrastructure locations, health catchment areas boundaries, and other operational boundaries can be integrated into local and national systems (such as DHIS2) in order to strengthen the effectiveness and equity of health interventions. This presentation will focus on three use cases of geospatial data in the health sector: 1) malaria prevention; 2) immunization (routine and non-routine); and 3)COVID-19 response.

0946

DATA QUALITY IMPROVEMENT IN MALARIA PROGRAM USING SOCIAL MEDIA MESSAGING AS A SUPERVISION TOOL IN CROSS RIVER STATE, NIGERIA

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The COVID-19 pandemic led to restrictions of movement in Cross River State. This affected routine data management activities such as timely data collection and validation, health facility heads of unit meetings, quarterly data quality assurance and supportive supervisory visits to health facilities. There was an increase in positivity rate, higher clinical diagnosis, indiscriminate use of Artemisinin Combination Therapy (ACTs), low uptake of intermittent preventive treatment during pregnancy (IPTp) 1&2 and a decline in National Health Management Information System (NHMIS) reporting rate. We worked with Cross River health officials to leverage WhatsApp technology to improve data collection, processing and supervision. Through the support of the President's Malaria Initiative for States (PMI-S), the WhatsApp group created in May 2020 established an interactive and mentoring platform for malaria stakeholders at the state and local government area (LGA). Data is uploaded by LGA Monitoring and Evaluation (M&E) officers after completion of data validation for the month. Data quality checks were done to see which LGAs did not report or which data was illogical. Facilities with illogical reporting were filtered and displayed on the WhatsApp platform and emails were sent to LGA M&E officers that reported the data with advice for correction. The group facilitated communication among members with reference to topics that were linked to addressing poor management of malaria as seen in the NHMIS as well as in analyzing and presenting data. Adherence to national guidelines on IPTp and case management were consistently discussed on the WhatsApp group. IPTp1 increased from 66% in 2019 to 73% in 2020, and IPTp2 increased from 47% in 2019 to 51% in 2020. The NHMIS reporting rate improved from 81% in 2019 to 83% in 2020 and health facilities reporting no data quality issues with malaria indicators improved from 61% in 2019 to 95% 2020. We will continue to monitor these data indicators for sustained and improved performance while working with senior officials of governments in state and LGA levels to improve sustainability, functionality, and use of the platform.

0947

ASSESSING THE IMPACT OF COVID-19 ON THE UTILIZATION OF PUBLIC HEALTH SERVICES IN 2020 ON BIOKO ISLAND, EQUATORIAL GUINEA

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The impact of the COVID-19 pandemic on health-seeking behavior and its impact on the utilization of health services on Bioko Island were investigated. Using data from the National Health Information System, the monthly average of outpatient consultations and hospitalizations between 2018 and 2019 were calculated and compared to the monthly number of consultations and hospitalizations of the same months in 2020 and 2021. The mean outpatient consultations per month in 2018 and 2019 was 9801. Mean outpatient consultations in 2020 amounted 7409, representing a 24.4% decrease. Regarding hospitalizations per month, the average between 2018 and 2019 was 1062. In 2020, there were 936 hospitalizations, representing a 12% decrease. When disaggregating these results by year, hospitalizations in 2020 decreased 21% and 0.3% compared to 2019 and 2018, respectively. Preliminary analyses show that the difference between the average of outpatient consultations of 2018 and 2019 compared to 2020 was lower but this was not necessarily the case with hospitalizations. The purpose of this study is to investigate the statistically significant impacts of health services utilization across different diseases and to quantify these differences at temporal scales. These analyses would allow a better understanding of how the pandemic could have affected the burden of disease on Bioko Island outside of COVID-19.

0948

SARS-COV-2 MUTATIONS AND COVID-19 JABS - PREDICTING THE EFFECTS OF VACCINATION CAMPAIGNS

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The world is in the midst of vaccination campaigns as a measure to limit the spread of COVID-19. Several vaccine products have been developed and authorized to put an end to the ongoing COVID-19 pandemic that has put the globe in a state of a global health emergency for more than a year, causing a severe global economic crisis. However, the global COVID-19 response is hindered by the emergence and spread of new virus variants and vaccine distribution inequity among countries. We introduce a generalization of the model underlying the pandemic-preparedness-tool CovidSim 2.1 (http://covidsim.eu/) to optimize vaccination strategies, accounting for different age strata, spatial structure, different viral strains, and vaccination strategies assuming different vaccine products. Vaccines are assumed not to immunize perfectly. Some individuals fail to immunize, and others only reach partial immunity. Most importantly, vaccines are assumed to change susceptibility to specific SARS-CoV-2 variants - reflecting that not every vaccine might protect against every SARS-CoV-2 variant. Additionally, the model accounts for unvaccinable individuals (anti-vaxxers, contraindications) and prioritization of specific age groups during vaccination campaigns. Individuals who recover from an infection with a specific strain of the virus are considered to remain susceptible to other variants with mild disease episodes upon infection. The model is exemplified with parameters reflecting the situation in the Federal Republic of Germany to predict disease incidence (and prevalence), assuming general contact reduction interventions (e.g., curfews, social distancing, prohibition of gatherings) and case isolation (in quarantine wards, and home). The predictive model can serve as an advanced decision support tool for COVID-19 management.

0949

MEASURING GROWTH TRAJECTORIES OF CHILDREN UNDER FIVE YEARS OF AGE: A NOVEL METHOD FOR EXTRACTING ANTHROPOMETRIC DATA FROM HEALTH CARDS

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A child's growth trajectory is a reflection of overall health and nutrition. Growth monitoring therefore provides an opportunity for preventive and curative healthcare to reduce childhood malnutrition and mortality. We present a novel method for collecting data on children's growth trajectories using data from standard *Road to Health* (RTH) cards, which are issued to children at birth in many countries. Many Health and Demographic Surveillance Systems (HDSS), which collect population-based data at regular intervals across Sub-Saharan Africa and Asia, take photographs of these cards. For preliminary analysis, we selected 60 cards photographed during the 2019 data collection at an HDSS in Manhiça district,

Mozambique. We explored methods for extracting data on height/length and weight; a nurse enters these data on the RTH card both numerically and in graph form by at each clinic visit. We found that digitizing the data was easiest using the free Graph Grabber graphical digitizing software to extract anthropometric data. For best results, it is important for field staff to be trained to ensure the quality of the photographs taken. Photographs that were crooked or blurry could not be used, with only about half of records yielding data at the first attempt. From these records, we were able to create a database with children's age-and sex-specific height/length and weight. From analyses of these data, we identify patterns of growth faltering and link these to children's risk of death before the age of 5y. We estimate the validity and reliability of extraction method, of digitizing graph and handwritten data, and exporting the extracted data into a dataset. This work demonstrates that information on children's growth patterns can be extracted from RTH cards, which are issued at birth and are carefully maintained even by parents living in very difficult conditions. These methods can be used to characterize growth patterns efficiently, not requiring new longitudinal data collection but relying on anthropometric measures already being taken through children's standard clinic visits.

0950

ASSESSING THE IMPACT OF COVID-19 PANDEMIC IN RURAL BANGLADESH: FINDINGS FROM A CROSS-SECTIONAL SURVEY IN CHAMPS-BANGLADESH STUDY SITE

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Despite significant public health concerns worldwide, the impact of the COVID-19 pandemic in rural areas is poorly understood. Therefore, this study aims to understand how the pandemic and its associated lockdown from 27 March to 30 May, 2020, have impacted the livelihood, food availability, and uptake of pregnancy and healthcare for children under five in the rural Baliakandi sub-district of Bangladesh. A cross-sectional survey was conducted from 7 November to 13 December 2020, and 1302 women were selected using simple (n=299) and stratified (n=1003) random sampling. A structured questionnaire was used to understand household conditions during the pre-COVID-19 (before mid-March, 2020) and peri-COVID-19 (after mid-March, 2020) period. Results suggested that among the respondents who participated in the survey (n=1176), 16% (n=191) reported that they could not obtain sufficient food and among the respondents who could avail sufficient food (n=985), 44% (n=433) could not obtain the food of their choice after mid-March, 2020. While 73% (n=208) of non-farm business owners (n=286) reported business closures, 44% (n=128) of jobholders (n=288) reported job loss and 34% (n=227) of farmers (n=676) reported disruption in farming since the start of the pandemic. For the women who were pregnant (n=284), only 6 women reported they wanted but could not attend antenatal care due to lockdown-related issues. Similarly, for the households with at least one under five child (n=922), only 6 respondents reported they wanted but could not seek care due to lockdown-related issues. This study revealed that the effect of the pandemic on the healthcare uptake in rural Bangladesh was low within the study period. The worst impact could be found on people's livelihood, particularly in non-agricultural sectors. In fact, people with agricultural livelihood were relatively better at coping with the pandemic-related shocks than the laborers. As the pandemic continues, rural households in Bangladesh could experience food insecurities and malnutrition due to reduced food intake.

0951

REASONS FOR HOME BIRTHS IN THE BIRHAN HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM

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Ethiopia is making significant advances to reducing maternal and newborn morbidity and mortality. However, many women still give birth at home without any skilled delivery care which increases the risk for adverse maternal and neonatal health outcomes. In the BIRHAN Health and Demographic Surveillance System based in North Shewa Zone, Ethiopia, we examined the reasons why women give birth at home in order to understand the challenges and better inform the design of solutions. The study design follows an open cohort of pregnant women who were enrolled upon confirmation of pregnancy. For all cohort births, data on location of delivery, reasons for home delivery and other factors were collected. A total of 2039 pregnancies were followed through birth from December 2018 to April 2020, of which 507 (25%) occurred at home or on the way to a facility. Only 19 (4%) of the home births received skilled delivery care at birth. A subset of 414 women who delivered at home were asked where they initially planned to deliver and their reasons for home delivery. Of those interviewed, 287 (70%) reported that they had initially desired to give birth at a health facility. When asked for specific reasons why they delivered at home, 302 women (73%) mentioned that the health facilities were too far away or that no transportation was available. In addition to this, 80 women (20%) mentioned that they chose to deliver at home based on prior evidence of successful home deliveries and 24 respondents (6%) reported that they had no one to accompany them and hence could not go to a health facility. Four women mentioned that they had a previous bad experience at health facilities and decided not to deliver at a health facility. Many women prefer to deliver in health facilities but end up giving unattended births at home. Long distance to health facilities and lack of transportation are important barriers for women to deliver in a health facility. Efforts to improve birth outcomes and skilled delivery attendance need to consider these barriers and explore community-based interventions aimed at improving maternal and neonatal birth outcomes.

0952

SEX WORK, ANTIBIOTIC USE AND THE MANAGEMENT OF SEXUALLY TRANSMITTED INFECTIONS IN HARARE, ZIMBABWE: AN ETHNOGRAPHIC STUDY

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A key global health challenge of our time is the dramatic increase in infections resistant to available antibiotics, including sexually transmitted infections (STIs) such as gonorrhoea and syphilis. Antimicrobial resistance (AMR) is driven by overuse of antibiotics both within and beyond formal healthcare settings, yet little is known about how and why people rely on these substances, particularly in low and middle-income countries (LMICs). An especially under-researched group is sex workers, who are at heightened risk of contracting and spreading STIs, often have poor access to healthcare and have been shown in previous studies to use antibiotics regularly. This paper presents ethnographic research (participant-

observation and in-depth interviews) conducted with 20 sex workers in Harare, Zimbabwe between 2018-2020, which aimed to understand patterns of antibiotic use among sex workers and reasons for this use. We present three key findings: (1) Sex workers were familiar with and regularly used antibiotics like metronidazole, doxycycline, ceftriaxone, and ciprofloxacin, which enabled them to continue working despite regular occupational exposure to STIs. (2) These antibiotics were largely prescribed by a northern-funded NGO clinic devoted to sex workers, towards which they felt considerable belonging in contrast to stigmatising experiences at public clinics. (3) Many women who were not sex workers and thus not eligible to attend the NGO were unable to access antibiotics for STIs (often contracted via partners paying for sex) because of prohibitive costs and stigmatising treatment. We conclude from these findings that attention to individual behaviour risks overlooking the limitations of the ways in which STIs have been managed through vertical programmes for STIs as a way of circumventing integration challenges in health systems. Our research draws attention to issues both within and beyond these programmes that emerge from the perspective of those affected by STIs. In addressing AMR, further effort is needed to reconsider integrated services, recognising the legacies that shape the challenges of integration.

0953

ASSESSING THE IMPACT OF COVID-19 ON MALARIA TRANSMISSION IN NIGERIA AND ZIMBABWE

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As the COVID-19 pandemic struck several countries globally, low-middle-income countries increasingly shifted their focus on identifying and treating COVID-19; however, the emerging question to date is about the impact of the shift to already on ongoing efforts that had realised public health gains in the control of infectious diseases, such as malaria. Several low-middle-income countries reported a spike in malaria cases during the COVID-19 pandemic. For instance, Zimbabwe's Matabeleland South province reported a significant spike in the number of malaria cases during the COVID-19 crisis. However, the literature reviewed has not clarified whether the increase in the number of malaria-infected patients during the COVID-19 pandemic is linked to misdiagnosis or to other factors such as the insufficient resources needed for malaria control. Malaria control has been argued to largely depend on the mass distribution of long-lasting insecticide-treated nets (LLINs), seasonal malaria chemoprevention (SMC) and indoor residual spraying of insecticide (IRS) across communities and households. There is no doubt that understanding the effect of the concentrated campaigns against malaria is vital to inform future control planning during the COVID-19 crisis. Hence, the WHO has stressed that all routine malaria prevention and control activities should not be hampered and be continued to the extent possible as they tackle the COVID-19 pandemic. However, implementing these preventive activities house-to-house has been more arduous during the COVID-19 pandemic crisis. A modelling analysis by the WHO predicted a greater than 20% rise in malaria morbidity and a greater 50% mortality in sub-Saharan Africa during the COVID-19 pandemic due to a 75% reduction in routine malaria control measures, including ITN distribution and shortage of anti-malarial drugs. In Nigeria alone, interrupting malaria control management, such as delaying LLIN campaigns for six months, could result in 81,000 additional deaths. This study seeks to understand the effect of the COVID-19 pandemic on malaria control in Zimbabwe and Nigeria.

0954

GLOBAL HEALTH TRAINING DURING A PANDEMIC: LESSONS AND IMPACT ON THE FUTURE

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The Master of Public Health (MPH) Program at Case Western Reserve University maintains a focus on Global Health training and collaborations. The training program has grown from a historical global health framework of shared training resources across five schools and nine departments. This CWRU Framework for Global Health continues to provide the administrative and training environment for integrated curricula, workshops, a certificate program, and mentored field experiences that link with MPH students, faculty, MPH-dual degree programs and partnerships into the inter- and trans-disciplinary structure. This serves as a core for MPH Global Health Concentration students and also provides opportunities to students across all five MPH concentrations and 11 dual degrees to foster diverse skills, perspectives, and public health impact. The MPH program recently completed a self-study and strategic planning process that incorporated surveys and feedback from students, faculty, internal partners, external partners, and external reviewers. Through this, our Global Health competencies were updated and aligned with curricula, training experiences, outcomes, and partnerships to prepare our students to be effective leaders and partners in global health. The program has designed approaches to overcome the complexities of global health training including program partner and international mentoring, language barriers, cost, time, and safety. COVID-19 travel and course delivery restrictions have fostered further innovations to global health training. Many students took advantage of new online training and local in-person global health experiences during social distancing and travel restrictions associated with the pandemic. Our courses also shifted learning methodologies, objectives, and examples, during a time of remote and dual delivery education. This presentation highlights our approach to establishing and supporting international training partnerships that meet the needs of our students, partners, and communities.

0955

ADDRESSING GENDER EQUITY AND SOCIAL INCLUSION BARRIERS TO IMPROVE ACCESS AND UPTAKE OF SCHOOL MASS DRUG ADMINISTRATION AMONG SCHOOL-AGED CHILDREN IN TANZANIA

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The Neglected Tropical Disease Control Program (NTDCP) in Tanzania, in partnership with USAID's Act to End Neglected Tropical Diseases (NTDs) | East project (Act | East), implemented a pilot intervention in Pangani District Council, applying WI-HER's iDARE (Identify, Design, Assess, Record, Expand) methodology to identify and address gender equity and social inclusion (GESI)-related barriers and solutions around school-aged children who have missed or refused annual school mass drug administration (SMDA) to treat soil-transmitted helminths (STH) and schistosomiasis (SCH). District MDA records (2018-2020) revealed fluctuating coverage, with none reaching the 100% threshold, as the most recent round reached 91.9% of school-aged children. The pilot focused on a select number of zones and schools with especially low coverage, as low as 68.2% in the previous SMDA. The pilot followed co-creation of a GESI-integrated MDA training curriculum, and application through a national, regional, and district-level training cascade, which included training for the Pangani NTD team implementing the pilot. The Tanga Region NTD Officer led the pilot team, which included Pangani NTD Officers for Health and Education and the Council Social Welfare Officer, four zonal coordinators, front-line health workers (FLHWs), and teachers. Using iDARE, the team identified GESI issues affecting treatment uptake among school-aged children, to include parents not allowing children to take, fears of sterility or impotency, fear of side effects and mistrust of medicines. Community influencers were identified to help implement solutions to tackle these barriers and improve uptake. After the start of testing solutions, 97.7% of children and parents in the two zones who had not previously taken S/MDA reported that they intended to take medication during the next S/MDA. SMDA (March 2021) results showed an average of 99.1% of children across four schools that had previously low coverage, within the

two zones, took the medication. Identifying and addressing GESI barriers can better reach school-aged children and change behaviors to increase SMDA coverage.

0956

MULTI-SECTORAL COLLABORATION TO IMPROVE ACCESS TO MEDICINE FOR SICKLE CELL DISEASE IN SUB-SAHARAN AFRICA

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Acknowledging the potential role of public-private partnerships (PPPs) cited in the Sustainable Development Goals to help address global health challenges, we report outcomes from a novel, fit-for-purpose multi-sectoral collaboration designed to improve access-to-medicines for individuals with sickle cell disease (SCD) in sub-Saharan Africa. Novartis, a global medicines company, worked with stakeholders in Africa for three years to understand how it could constructively contribute to improved health outcomes for people with SCD. Access to hydroxyurea (HU), a generic medicine shown to improve outcomes for patients with SCD, emerged as a priority activity. A Memorandum of Understanding for the establishment a PPP was signed among the Ministry of Health (MOH), Ghana Health Service, Sickle Cell Foundation of Ghana (SCFG), and Novartis with the aim of bolstering healthcare system architecture to address SCD. The company increased its manufacturing capacity for HU and registered the medicine for the indication of SCD in Ghana (constituting the first time that Novartis registered HU for SCD and the first time that HU was registered specifically for SCD in Ghana). To help launch the program, the company provided the Ghana MOH with approximately 6 million capsules of HU as bridging stock according to WHO guidelines. This stock seeded the development of a large, organized HU-for-SCD treatment program involving all major SCD Treatment Centres in Ghana and managed by the SCFG. As subsequent steps, and in response to requests from local partners, the company worked with local partners to identify an optimal pricing scheme for HU in Ghana, initiated drug development for a child-friendly dispersible formulation of HU, and registered HU in six additional countries (Uganda, Tanzania, Kenya, Nigeria, DRC and Congo Brazzaville). We will present details of the collaborations and report lessons learned that include following the lead of local stakeholders and leveraging the core competencies of all partners in pursuit of maximizing opportunities for sustainable health solutions.

0957

DEVELOPING GOOD LABORATORY PRACTICE IN INSECTICIDE TRIAL FACILITIES IN AFRICA: THE VITAL ROLE OF LABORATORY TECHNICIANS AND LABORATORY SUPERVISORS

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New vector control products are urgently needed to maintain the enormous gains we have recently seen in the battle against malaria, moving towards eradication. Vital in this is the network of trials facilities in Africa who evaluate these products by conducting studies against local mosquito populations. However, until recently, lack of quality management systems (QMSs) at these trials facilities made it impossible to assess if studies had been conducted correctly, without significant deviations from study protocols. To address this, the Innovative Vector Control Consortium (IVCC) initiated a project in 2016 to support a network of 7 African trials facilities towards Good Laboratory Practice (GLP) certification. With GLP compliant QMSs, trials facilities can generate data that are reliable, repeatable and auditable. The Liverpool School of Tropical Medicine's Centre for Capacity Research conducted semi-structured interviews with 74 staff at these facilities, to investigate project challenges, enablers, and outcomes. The study found that laboratory technicians and supervisors were vital in developing a GLP compliant QMS and, in turn, many experienced career progression. An important challenge for laboratory staff was developing new habits, particularly working to Standard Operating Procedures (SOPs). Enabling activities were early sensitisation to the purpose and benefits - both individual and institutional - of GLP, a collaborative and iterative SOP development process that considered infrastructural limitations, and regular training to consolidate SOP compliance. As a result of the project, several laboratory staff were promoted, including into Quality Assurance and project management roles, or transitioned into academic roles via IVCC funded PhDs. The project also resulted in inter-facility and interdisciplinary learning, with laboratory staff providing peer-to-peer learning to both IVCC supported trial facilities and to African One Health laboratories. This study emphasises that RCS projects should include technical staff, who form the backbone of data generation and who can in turn support regional RCS efforts.

0958

THE DISPARATE BURDEN OF STRESS AND CONCERN ASSOCIATED WITH THE COVID-19 PANDEMIC: FINDINGS FROM AN ONGOING COMMUNITY-BASED CLUSTER STUDY IN CENTRAL NEW YORK

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The COVID-19 pandemic has wrought profound disparities in infection rates across communities. We hypothesized that the psychological toll, both directly associated with personal risk and indirectly associated with COVID-19-related stressors, would similarly display heterogeneity across populations. We examined factors associated with stress and solicited concerns across diverse domains of physical, mental, and financial health. Starting in June 2020, we identified individuals recently infected with SARS-CoV-2 and enrolled these patients and their households. Surveys were conducted on enrollment and associated infections were identified by RT-PCR, IgM, and IgG testing. Mixed effects logistic regression models, with random effects for clustering by household, were used. A total of 70 households (183 participants) were enrolled as of March 2021. Median age was 34 years (range 1–78 years). 55.1% identified as female and 88.1% as white, non-LatinX. 64.0% of household members were found to have infection on enrollment. 28.2% were unemployed, 39.4% were working from home, and 43.9% were essential workers. Those who reported significant stress were not more likely to have COVID but were more likely to be female (OR=3.74), working from home (OR=9.31), to be essential workers (OR=5.43), and to have been enrolled in 2021 versus 2020 (OR=3.34, p=0.07). Among those reporting significant stress, predominant concerns were mental health (OR=7.53 for somewhat concerned or higher), personal health (OR=13.75), and financial stability (OR=2.82). Levels of concern for community public and economic health were statistically improved in 2021 versus 2020. These findings underscore an unfolding mental health crisis in the context of the COVID-19 pandemic, independent of infection status. Optimism about community health is improving, possibly due to the increasing availability of vaccines. However, accumulating stress and indirect burdens of the pandemic are particularly evident among essential workers, those working from home, and women. These findings should prompt focused efforts for mental health and other supportive interventions.

0959

MEASURING DIVERSITY OF SARS-COV-2 VARIANTS

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A year after the onset of the COVID-19 pandemic, different vaccines have been produced and distributed to countries to help control the global pandemic. However, as vaccination campaign strategies are deployed around the globe, major attention goes to the emerging strains of the virus as the effectiveness of the different vaccine products on those variants is unknown. As the effectiveness of the vaccines might differ for each variant, it is crucial for an effective deployment of the vaccination campaigns to understand and study the diversity of these variants in a spatial and temporal context. Here, we use a definition of diversity measures based on a solid theoretical foundation, which gives us a framework that allows studying patterns of diversity among sub-populations. The term sub-populations can be interpreted at different levels of granularity, hence maximizing the potential applications of the method. Our approach allows studying diversity patterns through a straightforward graphical analysis. We apply the method to study: (i) the diversity of SARS-CoV-2 variants between the seven most modernized countries in the world (G7) and sub-Saharan African countries; (ii) the diversity of variants over a time period in some targeted countries, e.g., UK, USA, South Africa; and (iii) the diversity of the variants between sub-Saharan African countries, South Asian countries, and South American countries. The results show a difference in the diversity of SARS-CoV-2 variants between the G7 and the sub-Saharan African countries, but this shows more in low-frequency variants. Over the studied time period, an increase in diversity is observed in some countries, which shows a change in which strain is dominating. We discuss differences in diversity that is observed between countries in sub-Saharan Africa, South Asia, and South America.

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A CROSS-SECTIONAL STUDY TO CHARACTERIZE CHILDHOOD ANEMIA AND INFORM CONTROL STRATEGIES IN RURAL NORTHERN, PERU

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A third of children globally have anemia, with children under 6 years in low to middle-income countries (LMICs) bearing the greatest burden. Children with mild anemia often present few acute symptoms; however there is evidence of long-term effects such as delayed neurodevelopment. Standard treatment in LMICs is iron supplementation, which is ineffective in the presence of other microdeficiencies, chronic disease, parasitosis, and heavy metal exposure. Iron supplementation in Peru significantly increased since 2010; however, changes in anemia prevalence were minimal. We aim to characterize plausible causes of anemia in Tumbes, Peru, and evaluate if chronic methylmercury and arsenic exposure are associated with childhood anemia. We conducted a cross-sectional study in a rural district near a river with heavy metal exposure from decades of upstream gold mining runoff, and a rural district over 50 km away from the river. We surveyed parents for anemia risk factors, and measured children's blood count (CBC). We are currently measuring ferritin, vitamin B12, folate, and C-reactive protein in blood and testing stool for parasitosis. We will process hair and toenail samples for mercury and other metals by fall. We recruited 264 children aged 6-69 months participated and 16.7% (44/264) had anemia (<11 g/dL by WHO guidelines). In communities near river, anemia prevalence was 17.3% (26/150) and significantly higher among males. In communities far from the river, prevalence was 15.8% (18/114) and significantly higher among children without latrines or toilets. We have completed micronutrient analysis for 17 children with anemia and 8 were ferritin deficient (>30g/L by WHO guidelines). Iron deficiency anemia is typically more prevalent among girls and in households with lower socioeconomic status. Therefore, significant association with being male and lack of association with latrine suggest other types of anemia are prevalent near the river. Evaluating environmental exposure in addition to other causes may help inform and improve development of childhood anemia prevention strategies in this region.

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THE PANIC PHASE OF THE PANDEMIC: IMPACT OF THE EARLY PHASE OF COVID-19 ON REPRODUCTIVE, MATERNAL, NEWBORN AND CHILD HEALTH SERVICE UTILIZATION AT A TERTIARY HOSPITAL AND CATCHMENT HEALTH CENTERS IN ADDIS ABABA: A MIXED-METHOD STUDY

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The emergence of the COVID-19 pandemic has presented a unique challenge to the already strained health system in developing countries. The fear of exposure to COVID-19 in health facilities and the shift of attention and resources from essential services was expected to decrease the reproductive, maternal, newborn, and child health (RMNCH) services utilization. A mixed quantitative and qualitative study was conducted at Saint Paul's Hospital and 16 catchment health centers to assess the impact of the early phase of the COVID-19 pandemic on RMNCH services utilization. Data on the service uptake covering a period of 25 months (April 2018-April 2020) was collected. The service uptake after the COVID-19 first case was detected in-country (March 13, 2020) was compared with the preceding months and the same time frame in the previous years. All most RMNCH service uptake has decreased significantly. The highest impacted services have seen a decrement of 42.8% and 29.4% for family planning, 38.1% and 24.8% for safe abortion, and 28.1% and 31.2% for antenatal care uptake in the two months after the first report of COVID-19 compared to the preceding two months and same time frame in the previous year respectively. Deliveries were the least affected with a decrement of 7.4% compared to the preceding two months. The stillbirth rate has increased from 22.0 to 27.2 per thousand births after COVID-19 compared preceding two months. In the qualitative study, participants have corroborated that there was a significant decline in client flow. The reasons attributed to the decline were the restriction of public transport, change in working hours, and fear of exposure to COVID-19 at health facilities. These findings indicate that such essential health services are highly vulnerable at times of disasters like the current pandemic. Emergency preparedness and resilience measures should be put in place to avoid similar failures in the future. Close monitoring of the RMNCH service uptake should be done and continued till we come out of the COVID-19 pandemic.

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COVID-19 IPC OUTCOME ASSESSMENT IN USAID MTAPS-SUPPORTED HEALTH FACILITIES

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As part of the global COVID-19 pandemic response, USAID tasked its Medicines, Technologies and Pharmaceutical Services (MTaPS) Program with providing technical support to countries in infection prevention and control (IPC). IPC areas of critical importance to health care workers and patient safety included: application of COVID-19 precautions, triage for (severe) acute respiratory infections, and waste management. During

the program's initial COVID-19 response (March to October, 2020) in Cameroon, Côte d'Ivoire, and Mali, MTaPS supported IPC implementation and trained 2390 health workers in 128 health facilities in COVID-19-related IPC topics. To improve facility-level compliance to IPC standards, MTaPS adapted WHO AFRO IPC Scorecards to rapidly identify priority gaps prior to conducting training. The program then reassessed facilities to monitor compliance with local and global guidelines over the course of IPC implementation. Beyond training, our interventions included: remote and face-to-face mentoring and coaching; monitoring visits to health facilities at frequencies prescribed by the Scorecard; provision of needed tools and job aids, and advocacy towards facility managers. Data from 44 facilities across the three countries shows that 91% of supported facilities increased their IPC compliance scores with 41% of facilities improving by at least 25% over the course of the interventions. The percentage of facilities with high IPC compliance (scoring 80-100%) increased from 16% to 55%. The use of the scorecard provided an opportunity to engage facility management - eager to improve their score - to develop strategies to strengthen adherence to IPC guidelines as part of a continuous quality improvement process. This session will present lessons emerging from the experience on how best to rapidly improve IPC readiness for COVID-19 in health facilities. We will also discuss the impact of the program's efforts on countries' COVID-19 response. MTaPS will continue the work to institutionalize these interventions in supported countries to ensure sustainable results.

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UTILIZATION OF FACILITY-BASED ESSENTIAL MATERNAL AND CHILD HEALTH SERVICES DURING COVID-19 PANDEMIC IN NORTH SHEWA ZONE, ETHIOPIA

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Ethiopia reported the first case of COVID-19 on March 13, 2020, and various measures have been taken since then to prevent the spread and transmission of the virus. As a result of the ongoing preventive measures and fear of risk of exposure by the community, it was anticipated that utilization of maternal, newborn and child health (MNCH) services at health facilities would decrease. The aim of the study was to assess the trend of MNCH service utilization during the first six months of the COVID-19 pandemic. The study was conducted at eight health facilities in Ethiopia as part of the BIRHAN Health and Demographic Surveillance System. A mixed quantitative and qualitative study design was used. The trend of service utilization during the first six months of COVID-19 (March to August 2020) was compared to corresponding time and data points of the preceding year (March to August 2019). The utilization of antenatal, postnatal, family planning, facility delivery and child immunization services at health facilities did not change during the initial six months of the pandemic. However, facility attendance for sick visits for children under 5 years old decreased by 42% as compared to the preceding year. This could be due to reduction in the prevalence of childhood illnesses as a result of COVID-19 prevention measures like hand hygiene and mask wearing. Provision and utilization of essential MNCH services are crucial to ascertain favorable maternal and child health outcomes. In the setting of developing countries like Ethiopia, health systems are too fragile to withstand the shock due to effects of the pandemic. Our study presents early findings on the utilization of MNCH services that remained preserved as well as some that were negatively affected. Government leaders, policy makers, and clinicians who want to improve the resilience of their health system will need to continuously monitor service utilization, while engaging with clients to understand and address their evolving concerns about MNCH service uptake, during the pandemic to prevent increases in maternal and child morbidity and mortality.

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DYNAMICS IN THE OPINION OF CAMEROONIAN POPULATION REGARDING ACCEPTABILITY OF COVID-19 VACCINES: A NATIONWIDE ONE-YEAR LONGITUDINAL SURVEY FOR POLICY MAKING

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The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) disease, commonly called COVID-19, is an infectious disease that has imposed an important burden on all life aspects of populations worldwide. A race against time has made possible the development of vaccines with proven efficacy in an extremely short timeframe. However, the success of immunization campaigns and therefore the attainment of herd immunity - a prerequisite to return to normal life - rely on the acceptance of vaccines by populations. In fact, vaccine hesitancy has been reported among several populations worldwide, mostly because of the celerity with which these vaccines have been developed, the potential adverse events arising with the early immunization campaigns, as well as all the fake news surrounding this pandemic. In Cameroon, the decision is yet to be made on the choice of the vaccine that will be used as well as when and in which conditions immunization campaigns will be launched. In this context, it appears urgent to investigate the willingness of the populations to get vaccinated, as well as the dynamics and reasons for changes in their opinions over a year. To this end, we will carry out an online nationwide longitudinal survey with quarterly follow-up among Cameroonians to investigate their willingness to get vaccinated, as well as the dynamics and reasons of changes in their opinions over one year. We will use the health belief model (HBM) to understand the participants' compliance with vaccines and the reason why their initial opinion can change. This study will be soon launched, and the findings generated will be translated as a policy brief and shared with the Ministry of Public Health and stakeholders engaged in the fight against COVID-19 to guide decision making and policy change or adaptation. This will be helpful to design adapted communication messages to convince as many as possible people to get vaccinated and therefore rapidly reach herd immunity.

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GLOBAL SURVEY OF HEALTH WORKER PERCEPTIONS OF LOCAL INFECTION PREVENTION AND CONTROL FOR COVID-19: IMPACTS AND REFLECTIONS

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Health workers face stigma, occupational hazards, and increased risk of exposure to severe acute respiratory syndrome-2 virus (SARS CoV-2) in their role providing treatment and care to patients in health facilities. Protection of health worker physical and mental health was a research priority identified in the 2020 World Health Organization (WHO) COVID-19 Research Roadmap. To aid rapid deployment of research, the Social Science Working Group, the Infection Prevention and Control (IPC) Working Group, and the Global Outbreak Alert and Response Network

(GOARN-Research) developed research guidance - *Global Survey of Healthcare Worker Perceptions of Local Infection Prevention and Control for COVID-19*. The guidance protocol aimed to generate rapid evidence for optimizing COVID-19 IPC by understanding health workers' views of local IPC procedures. A free, pre-packaged, customizable research guidance protocol was rapidly developed and published on the WHO website. This presentation will describe findings from our process evaluation which assessed how the protocol was developed, utilized, and modified to meet the dynamic needs of the COVID-19 pandemic. Data from the process evaluation were collected via observations of fortnightly meetings with the research teams implementing the protocol; surveys across 16 study sites assessing researchers' use of the protocol and impact of their findings; and follow-up key informant interviews with the primary investigators across study sites. We will present our findings of protocol use from 52 countries, that included over 10,000 health workers. Furthermore, key findings from each study site will be discussed. Findings from the process evaluation demonstrate that universal protocols are essential in an outbreak, epidemic, or pandemic setting as resources, time, money, tools, protocols, and personnel are often limited to execute research. In such emergency settings, it is critical that research be rolled out quickly, effectively, and with rigor to aid on the ground response.

0966

IMPLEMENTATION OF ACUTE ILLNESS SURVEILLANCE THROUGH TEXT MESSAGING AMONG A COMMUNITY-BASED COHORT IN PONCE, PUERTO RICO

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Dengue virus (DENV) is a common cause of acute illness in endemic areas, but reported cases represent a small proportion of symptomatic infections. We implemented a community-based cohort study in 2018 in Ponce, Puerto Rico to measure the incidence of DENV and other arboviral infections through annual interviews and serum specimen collection. In May 2020, an acute illness surveillance component was initiated in which participants are sent weekly texts asking if they had fever, cough, or other symptoms. Those reporting fever can provide serum samples for DENV molecular testing. As part of expanded activities for COVID-19 investigation, participants reporting COVID-like symptoms can provide nasal swabs for SARS-CoV-2 and seven other respiratory viruses for molecular testing. During May 2020-March 2021, an average of 2,311 participants (57% of the cohort) received weekly texts; average response rate was 37% (n=854). A total of 687 respondents reported symptoms (2% of all responses received); of these, 28 (4%) provided serum and nasal swabs and 60 (9%) provided nasal swabs only. As of December 2020, of the 88 participants that provided nasal swabs, five (6%) participants tested positive for SARS-CoV-2. To identify strategies to increase the response rate, 102 nonrespondents were surveyed about reasons for not replying to the text messages for 2 consecutive weeks. The most frequently reported reasons were being too busy (23%), confusion about the objective of the text messages (20%), not liking to receive or send text messages (16%), and not having symptoms (13%). To address this, informational videos and flyers were shared with participants via email and social media platforms and text message content underwent additional cognitive testing and revision. Our surveillance findings were consistent with COVID-19 surveillance data trends from Ponce. Text messaging surveillance can be a useful tool to monitor for illness at the community-level to help inform public health response. Activities to increase awareness and highlight the importance of community-based disease surveillance will continue among participants to improve their involvement.

0967

EFFICACY OF MOSQUITO BLOODMEAL- BASED SURVEILLANCE IN RURAL GUATEMALA

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There is an increasing need for surveillance methods that allow for rapid detection of circulating pathogens in low-resource areas. Mosquito blood meals are a convenient sample source that can sensitively detect blood-borne pathogens present in humans (xenosurveillance) and may represent an attractive alternative to traditional surveillance methods. In the present study, we evaluated the feasibility and effectiveness of xenosurveillance in rural Guatemala. Twenty households from two communities were enrolled and followed from August to December 2019. When febrile illness was reported in a household, blood-fed indoor mosquitos as well as blood samples from each member of the household were collected and preserved on FTA cards and sent to Colorado State University for further processing. Shotgun sequencing on a subset of pooled samples was first used to identify relevant circulating viruses. Quantitative PCR (qPCR) was then used for sensitive and targeted detection of identified viruses in all samples. The host source of each mosquito bloodmeal sample was also identified using a tagged cytochrome-oxidase PCR and subsequent consensus-level sequencing (bloodmeal ID). A total of 507 mosquito blood-meals and 175 human blood samples were collected during the course of this study. The majority of blood-fed mosquito species collected were identified as *Aedes aegypti* (43.7%). We have detected human gamma herpesvirus in both mosquito blood-meal and human blood samples and have verified these results using qPCR. Though there was a dengue virus 2/3 outbreak in the area during our study, we did not detect dengue virus (DENV) in any of our samples. This was expected given the prevalence of DENV in the community and limited sample set. Results from bloodmeal ID reveal human bloodmeal sources across all mosquito species, though this rate is highest among *Aedes albopictus* (75%) and Anopheles sp. (72.7%). Our results indicate that xenosurveillance can be used to detect non-mosquito borne pathogens circulating in humans and mosquito host-feeding patterns can be used to hone future mosquito collection.

0968

GLOBALLY PROMOTING COVID 19 VACCINES FOR INTERNATIONAL OIL AND GAS COMPANY

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To globally protect the health and safety of our workforces, in addition to our business operations from the unprecedented impact of COVID-19 pandemic, we implemented preventive and mitigation safeguards, including a global workplace communication approach promoting COVID-19 vaccine awareness, access and use. In order to accomplish the intended results of our global COVID-19 vaccine initiative, we initiated a needs assessment review across our operations to anticipate the vaccine prioritization by local health authorities, their planned distribution by the public and / or private sector and the potential ability to locally obtain vaccines for our workers and dependents. In addition, we regularly reviewed the growing scientific evidence published on the different vaccine types, including their safety and effectiveness while monitoring their approvals by countries with stringent drug regulatory authorities and pre-qualification by the World Health Organization. As vaccines became available across the globe, we continued our scientific surveillance and its coordinated internal sharing with our global Company workforces, clinicians, and senior executives for effective awareness, vaccine use and supportive environment. Main enablers of such initiative relied

on our structured system integrating safety and health with executive Company interfaces built during SARS, MERS, and Ebola with insights from global renowned experts. Key challenges were related to the progressive information availability on the disease, the vaccines safety, effectiveness and ability to prevent transmission, combined with their restricted commercial availability for global access to our workers. As a result, a global workplace framework for COVID 19 vaccine was rolled out with vaccine demand, supply and policy components. It helped affiliates recommend vaccines for our workers, considering individual and community benefits, promote vaccine confidence, and encourage early vaccination through community or workplace sites.

0969

EXPANDING XENOSURVEILLANCE CAPABILITIES USING LONGITUDINAL DATA COLLECTED FROM HOUSEHOLDS IN RURAL GUATEMALA

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Rapid detection of established and emergent human pathogens is necessary for efficient implementation of public health response. This is often inhibited by acquisition of high-quality samples and infrastructure for sample testing and analysis in resource-limited communities. Previously we demonstrated our ability to use blood-fed mosquitoes as tools for human pathogen surveillance (xenosurveillance) in resource limited regions of West Africa. Furthermore, a recent pilot study in Guatemala compared household-matched mosquito and human blood samples to reveal circulating pathogens, including Epstein-Barr virus. Leveraging an expanded cohort in the same rural Guatemalan community, we hypothesize that mosquitoes can be used as a non-invasive, self-supported sampling sources for xenosurveillance. We have enrolled 107 individuals across 40 households in two communities of southwestern Guatemala who will be followed prospectively for one year of weekly syndromic surveillance. Households with individuals reporting symptoms of fever, arthralgia, and/or rash will be activated for a four-week period in which blood will be acquired from enrolled subjects, domestic animals, and replete mosquitoes. Blood samples will be preserved on FTA cards, which will be used for targeted PCR screening of human pathogens, as well as next-generation sequencing. To date, 21 individuals across seven households have reported syndromic illness of fever (n = 9) and arthralgia (n = 6). Replete mosquitoes (n = 520) collected were represented by the genera *Culex* and *Aedes*. Molecular analysis of these samples is ongoing. Data collected throughout this study will allow for comparison between samples (i.e. mosquito versus domestic animal versus mosquito), demonstrating the untapped potential for mosquitoes to be used in pathogen surveillance. This work will also create sustainable infrastructure for disease monitoring in rural and resource-limited communities that are particularly vulnerable to the burden of disease.

0970

ANTIMICROBIAL CONSUMPTION SURVEILLANCE IN A RESOURCE LIMITED SETTING: FINDINGS FROM THIRTEEN HOSPITALS IN UGANDA

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Surveillance of consumption and use of antibiotics is a key strategy to inform antimicrobial stewardship (AMS). This study analyzed public health surveillance data on the use of antibiotics to inform the implementation of AMS programs in 13 hospitals in Uganda. We conducted a cross-sectional study for patients admitted to 13 hospitals in Uganda between September 2019 and September 2020. One hundred patient files from each of the 13 hospitals were collected through interval sampling, for a total of 1,300 files, using an Excel tool to measure and analyze antibiotic use. Most patients, 74.4% (967/1300), had at least one antibiotic prescribed with an average of 2.27 (SD = 0.6) antibiotics prescribed per encounter. Of the administered antibiotics, 86% were injectable. The mean daily defined dose (DDD) per 100 bed days was 0.847 ± 0.86. In total, 18,333.9 DDDs/100 bed days were used across all health facilities. Of these, cephalosporins were the most frequently used class, accounting for 28.6%, followed by penicillins, nitroimidazoles, fluoroquinolones and macrolides at 27%, 26%, 6% and 5% respectively. Based on WHO's antibiotic classification, 1104.2 DDDs/100 bed days were consumed from the "Access" class with 729.8 DDDs/100 bed days from the "Watch" class, p=0.2238. Most (62%) prescriptions were from the "Access" class, with the "Watch" class accounting for 38% of prescriptions. Less than 1% of prescriptions were from the "Reserve" class. Extensive use of antibiotics, a high proportion of which are injectable, should be a focus of AMS interventions. The findings necessitate implementation of periodic antimicrobial use and consumption surveillance in health facilities in order to strengthen AMR control activities.

0971

THE REMOTE EMERGING DISEASE INTELLIGENCE NETWORK (REDI-NET): AN INTRODUCTORY OVERVIEW

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Epidemics of novel emerging infectious diseases are becoming more frequent worldwide and outbreaks will inevitably continue into the foreseeable future. Proactive surveillance efforts are often challenged by a lack of expertise and/or capacity, causing serious delays in sample processing and/or timely data analysis and release. To overcome these obstacles and effectively detect, predict and contain potentially emergent zoonotic diseases of relevance, we need to change our current approach, streamline and improve the accuracy and timeliness of the 'data-to-decision' process, and provide health authorities with timely, comprehensive, actionable data for decision-making on protection and mitigation strategies. The Remote Emerging Disease Intelligence NETwork (REDI-NET) will develop a flexible, scalable and expandable computing substrate—'a system of systems'—upon which a myriad range of global health platforms can be developed and customized to support specific disease modeling, prediction and policy-decisions in support of public health. The REDI-NET consortium will leverage pre-existing and real-time surveillance data to provide a one-stop shop for health decision makers. REDI NET will operate through four specific aims: Aim 1: Establish robust SOPs for rigorous data capture and matched capabilities across field and reference laboratories; Aim 2: Active NGS surveillance across varied ecologies for broad spectrum pathogen detection; Aim 3: Enable remote, in-situ verified, near real-time data acquisition for actionable reporting and Aim 4: Institute a data management pipeline for actionable reporting and threat forecasting. Expected outcomes include more effective surveillance tools and methodologies; an open-access data repository of regional pathogen and infected sample distribution, abundance, ecology; greater numbers of trained laboratory and field personnel with a foundational background in detecting and responding to emerging disease outbreaks; and expert guidance for local jurisdictions and public health agencies on the most appropriate surveillance methods, sample processing and data usage.

0972

COMPREHENSIVE PROFILING OF SOCIAL MIXING PATTERNS IN RESOURCE POOR COUNTRIES

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Social contact patterns are key determinants of the transmission of pathogens that spread directly from person to person. These data are increasingly important in mathematical models investigating the impact of interventions against disease transmission such as physical distancing and targeted vaccination. Unfortunately, there are little data on contact patterns from low-and-middle-income-countries (LMICs) where morbidity and mortality from infectious diseases is high.In this proposed research, we present a framework that aims to characterize epidemiologically relevant household and community social contact patterns in urban and rural sites in Mozambique, Guatemala, India and Pakistan. Through formative work, we will develop standardized social contact diaries sensitive to the social and cultural contexts in each setting. For instance, we will explore the usability of self-report diaries within each setting, as well as the use of "shadows" or third party individuals to record contacts on behalf of children and individuals who cannot read and write. Participants will report sociodemographic characteristics (age, sex, occupation, household structure) and contact attributes defined by the number, frequency, duration and location of contacts over two continuous days. In addition, we will use wearable proximity sensors to automatically detect and record interactions between household members focusing on infants, who experience the highest infectious disease burden. Participants will wear the sensors over 7 days while also keeping a contact and location diary over two days. Primary outcomes will be social mixing matrices stratified by age, gender, urbanity among other key variables of interest. Leveraging the multiple data collection approaches, we will cross-validate the diaries and sensors as tools for measuring age-specific interactions between individuals.Social contact data from these studies will be made freely accessible to the scientific community. These data may be used to parameterize mathematical models of infectious disease spread and control in LMICs.

0973

AN INTEGRATED QUALITATIVE APPROACH TO SURVEILLANCE, MONITORING, AND EVALUATION FOR NATIONAL MALARIA CONTROL PROGRAMS

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Surveillance, Monitoring and Evaluation (SME) is critical for the continued success of National Malaria Control Programs (NMCP) since it provides the information necessary for effective program planning and management. Interventions aimed at improving NMCPs focus on both the target population and the program provider. Qualitative approaches are often used to understand the target population and barriers to intervention success. While there is growing emphasis on qualitative approaches in provider focused SME, metrics of success tend to focus on quantitative measures. The integration of qualitative approaches is one way to identify barriers that impede sustaining the gains made from provider focused capacity building efforts. We analyzed SME tools commonly used by NMCPs including the Performance of Routine Information System Management (PRISM) Tools, and Routine Data Quality and Supervision Tools for current and potential integration of qualitative data collection elements. Data collection tools were imported into NVivo for coding and analysis. Preestablished and emergent codes were applied to assess the extent to which qualitative data was collected and integrated. Findings indicate that qualitative approaches focus on understanding the program implementation and interventions, but the systematic integration of qualitative data is limited. There are greater attempts to integrate qualitative data into planning and supervision. Although several tools examine the capacity of service providers and system performance, there is limited understanding of the barriers that continue to hinder performance. Qualitative data collection efforts can be tailored to facilitate the systematic analysis, interpretation and integration of findings into quantitative metrics or outputs. We propose an approach to systematically collect, analyze and utilize qualitative data to improve SME and highlight how such data is useful for understanding and addressing the barriers that hinder optimal uptake of capacity building efforts and subsequent improvement to NMCPs.

0974

CLINICAL AND LABORATORY CHARACTERISTICS, AND OUTCOMES OF PATIENTS HOSPITALIZED WITH CORONAVIRUS DISEASE 2019: A RETROSPECTIVE ANALYSIS FROM BANGLADESH

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Started on 31 st Dec 2019, from Wuhan city, China, Coronavirus disease 2019 caused a global pandemic by March 11, 2020, and resulted in a total of 3,046,271 (2oth April 2021) global deaths. After one year of its origin, still, different variants are emerging and continuing their havoc worldwide. Bangladesh detected its first case on 8th March`2020, only 66 days later the first cases detected in China. Now also facing its second wave with increasing detection and case fatality rate (out of 732,060 confirmed cases, 10,683 deaths by 20th April 2021). In this retrospective chart analysis, we aimed to describe the epidemiology, clinical features, laboratory and radiological characteristics, treatment, and outcomes of Bangladeshi COVID-19 patients by comparing hypoxemic and non-hypoxemic patients treated in a makeshift COVID-19 unit of icddr,b. Statistical analyses were done using the SPSS software, version 20. By March 2021, among the identified 439 cases, 232 took outpatient care, stayed in home isolation and 207 remained in-patient. 293 (66%), 51 (12%), 95 (22%) were mild, moderate, and severe/critical cases respectively. Nineteen patients (4.3%) died while 10 (2.2%) were referred to different facilities for appropriate care. Out of 207 in-patients, 88 patients required oxygen therapy, which was given by nasal cannula, face mask, non-rebreather mask, HFNC, or mechanical ventilator as appropriate. All hypoxemic patients received steroids, antibiotics, and anticoagulants following standard hospital guidelines on the management of COVID 19 patients. Multivariable logistic regression identified age [1.07 (1.02-1.13)], dyspnea [3.56 (1.06-11.96)], high CRP [(1.13 (1.03-1.25)], and lymphopenia [(6.18 (1.81-21.10)] as the independent predictor for hypoxemia in patients hospitalized for COVID 19 (for all $p<0.05$). These simple clinical and laboratory parameters may help clinicians to identify COVID-19 cases who are at risk of developing fatal hypoxemia and thereby strengthen their follow-up and monitoring to abate mortality and morbidity, especially in resource-limited settings.

0975

MODULATING FACTORS OF HOUSEHOLD TRANSMISSION OF SARS-COV-2 IN A COMMUNITY-BASED STUDY

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Limitations in vaccine availability and uptake in middle and low income countries support an ongoing need to reduce community and household transmission of SARS-CoV-2. To identify factors modulating household transmission, we collected demographic, household composition, infection-control practice, and COVID-19 risk perception data from individuals in the Los Angeles region of Southern California who either recently tested positive or were at risk of infection from a household contact. We monitored respondents daily for new infection using high sensitivity RT-qPCR testing. We compared the combined responses of households where transmission was observed to those without transmission, and we compared the individual responses of participants who became infected during enrollment to those who remained negative. As of mid-April, 141 participants from 41 households (average size of 3.4 persons; ± 1.2 S.D.) have participated, with a broad age distribution (25.5% < 18, 45.4% 18-40, 24.8% 41-65, and 4.3% > 65 years old), 56.0% female, 64.5% reporting Hispanic/Latinx ethnicity, and 53.9% of participants identifying as white, 10.6% African American, and 8.5% Asian by race. Annual household incomes range from less than $5,000 to more than $500,000. After grouping by households with observed transmission (n=17) and those without (n=24), we calculated odds ratios to evaluate if factors like ventilation (e.g. windows, fans, and HEPA filters) or infection control practices (e.g. disinfecting surfaces, isolating sick household members in their own room, and maintaining social distance between all household members) are associated with transmission risk. The efficacy of these factors will be further investigated by analysis of responses from individuals who were initially infected in the household (n=41), became infected (n=35), or did not (n=65), to examine the role of individual behaviors. Study enrollment and data collection is ongoing, and formal results will identify specific interventions (e.g. infrastructure, resources for at-risk households, infection control messaging) to reduce household transmission of SARS-CoV-2 globally.

0976

ASSESSMENT OF KNOWLEDGE AND PERCEPTIONS RELATED TO THE COVID-19 PANDEMIC AMONG HEALTHCARE WORKERS IN NON-GOVERNMENTAL HEALTH FACILITIES IN CAMEROON

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Healthcare workers (HCWs) currently face unprecedented demand and pressure due to the COVID-19 pandemic. In Cameroon, most actions for the pandemic response by the Ministry of Public health (MOPH) is mainly focused on governmental health facilities. However, a recent WHO report showed that the majority of populations in Sub-Saharan African countries prefer to seek care at private clinics/hospitals, Faith-based health centers, and 17.8% sought care from traditional healer. We aimed to assess the knowledge and perceptions of HCWs about COVID-19 in Yaounde and Douala. We did a quantitative descriptive cross-sectional study in Non-Governmental health facilities in Yaoundé and Douala from September to November 2020. A snowball sampling method was then used to include HCWs within selected health facilities. A pre-tested structured questionnaire was administered to HCWs and responses were entered into REDCap database. HCWs from Douala were slightly more knowledgeable on COVID-19 symptoms with 88.3% correct answers compared to 86.6% in Yaoundé. Knowledge on immunity acquisition after recovery did not significantly differ between male and female (70.7% for men and 70.5% for women). In general, 2.9% of survey participants previously had experienced a confirmed COVID-19 infection, medical doctors were the most infected heath profession (6.6%). About one third of healthcare workers did not easily get access to personal protective equipment (PPE) in their workplace. More HCWs in public health facilities (62.9%) felt confident in their institution's ability to manage COVID-19 infected individuals followed by HCWs in non-lucrative health facilities (44%), and denominational ones (35.9%). While 57.9% of respondents found their training on COVID-19 to be insufficient, 90% were concerned about the risk of getting contaminated by COVID-19 with similar proportions (89.3%) amongst both men and women (p=0.013). This study highlights an adequate knowledge on the symptoms and aspects of immunity of COVID-19 by healthcare workers in non-governmental facilities in Cameroon.

0977

COMMUNITY SARS-COV-2 DYNAMICS AND CYCLE THRESHOLD USE FOR ENHANCED PUBLIC HEALTH SURVEILLANCE IN THE DOMINICAN REPUBLIC

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Asymptomatic COVID positive hotspots may be going undetected by public health surveillance systems. This study aimed to understand viral load dynamics of SARS-CoV-2 in the Dominican Republic using the Cycle threshold (Ct) value of RdRP gene amplification as a marker for the public health surveillance of undetected transmission pockets. 3,309 saliva samples were analyzed across 24 hospitals in the Dominican Republic and processed by automatic nucleic acid purification. Levels of viral RNA were determined by RT-qPCR. Results were considered "Detected" when Ct of the RdRP gene amplification was <37 and considered "Undetected" when the Ct was >37 or if no amplification curve was detected. Positivity rates were compared with RT-qPCR Ct values and were used as a proxy for viral load as it is inversely proportional to the sample's viral load. The samples yielded a positivity rate of 18.01%. Mean Ct was 29.3 (r =-0.034, p=0.04) and correlated significantly with community positivity rates. Mean age for positive samples was 39.8 years old (yo) [SD=15] with a distribution from 35-54 yo. There was no significant correlation identified between age groups [F(19, 30)=0.65, p=0.5] or between mean Ct by age groups and community positivity. No difference in viral load was found when comparing symptomatic and asymptomatic COVID-positive patients (t(140)=0.52, p=0.6). Mean Ct in the asymptomatic population was significantly and inversely correlated with the community positivity rate (r=-0.43, R2=0.181, p=0.0002). Significant differences were found in Ct when comparing time elapsed from the date of onset of symptoms to the date of RT-qPCR test [F(3, 319)=6.6, p=0.0002]. Our findings indicate that viral loads are comparable between age groups and between symptomatic and asymptomatic presentations, thus widespread surveillance strategies should be implemented to detect younger and asymptomatic populations that could serve as community transmission pockets. The use of RT-qPCR Ct values to understand community viral load should be considered as a tool for public health surveillance especially in resource-limited countries such as the Dominican Republic.

0978

IMPLEMENTATION OF VACCINATION CAMPAIGNS TO RESPOND TO COVID-19 AND OTHER EPIDEMIC THREATS IN LOW- AND MIDDLE-INCOME COUNTRIES: LEARNING FROM KEY INFORMANTS' EXPERIENCES WITH MENINGITIS A, YELLOW FEVER, AND EBOLA VIRUS DISEASE

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Recent vaccination campaigns to control outbreaks of meningitis A (MenAfriVac), yellow fever (YF-17D), and Ebola virus disease (rVSV-ZEBOV) have faced similar challenges to those expected for the implementation of COVID-19 vaccines in low- and middle-income countries (LMICs). We explored how these three vaccines were implemented to extract transferable lessons for COVID-19 vaccination and inform country preparedness for future epidemics. We conducted 24 semi-structured interviews with purposively selected key informants who had been involved in vaccination campaigns for these three diseases in Africa and the Americas. Participants were asked to reflect on barriers, enablers and lessons across a range of thematic areas relating to vaccine implementation. A number of lessons were developed from key informants' experiences. These include: ensuring the availability of operational funds to support pre-campaign community engagement and social mobilisation activities; recruiting additional health workers from within communities, rather than bringing in external staff; supporting vaccination teams to have open, frank discussions with communities about the disease and response measures; working with communities to 'map' social groups and tailor vaccine delivery strategies; investigating opportunities to integrate vaccine campaigns; investing in active rumour monitoring; strengthening surveillance for case detection and pathogen differentiation; and strengthening national ownership of, access to, and capacity to analyse vaccination campaign data. As COVID-19 vaccines continue to be distributed worldwide, these experiences and perspectives can assist countries in designing implementation strategies that utilise and build on learnings from past campaigns.

0979

CHALLENGES OF OBTAINING NASOPHARYNGEAL AND MINIMALLY INVASIVE TISSUE SAMPLES (MITS) FOR ASSOCIATED RSV MORTALITY AMONG DECEASED INFANTS IN LMIC COMMUNITY SETTING

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The burden of respiratory syncytial virus (RSV) in Pakistan has been established in a community-based surveillance study, where it was found that neonate/infant mortality rate attributed to RSV infection is 2.7% (as high as 4.9% in high season). The process of obtaining 588 nasopharyngeal specimens and 14 lung/thorax tissue specimens using minimally invasive tissue sampling (MITS) from recently deceased infants in low-income communities is currently ongoing and has several challenges. IDIs and FGDs were conducted in the formative phase with community stakeholders before nasopharyngeal/MITS collection regarding community understanding of post-mortem examination methods, perceptions and barriers for household specimen collection and the acceptability of a purpose-built van parked at the household for the MITS procedure. Logistical challenges included design and protocol development of a purpose-built van for MITS procedure outside bereaved family's household. Contextual challenges included working with a religiously conservative population's preconceptions of biopsy and post-mortem examination, particularly among community fears of COVID-19 surveillance. We obtained official rulings from religious scholars and leaders of Islamic, Christian, and Hindu faiths regarding the acceptability and usefulness of MITS as a health tool for ascertaining cause of death or disease in infants. During the sampling phase, regular meetings were conducted with general population and community stakeholders for MITS advocacy and myth surveillance. Staff were trained in counseling, advocacy, communication skills. Receiving timely death alerts and obtaining consent from grieving parents in a critical window of <1 hour before the infant's shrouding, ritual bath & burial was possible through support of key community partners and the presence of study staff: community health workers/mobilizers from the same neighborhood as the parents. In conclusion, advocacy with key community partners including religious scholars and local stakeholders helped in timely death alerts and support for parental consent for MITS in the community.

0980

THE GEOGRAPHICAL AND TEMPORAL SEROPREVALENCE OF BORRELIA BURGDORFERI AND B. MIYAMOTOI IN NEW ENGLAND FROM 2018-2019

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Ixodes ticks are formidable vectors that transmit various bacterial, parasitic, and viral pathogens, including two Borrelia species, *Borrelia burgdorferi*, the causative agent of Lyme disease, and *B. miyamotoi*, the causative agent of tick-borne relapsing fever. Although the relative risk of exposure to these agents has been studied at a few New England sites, no prevalence studies have been carried out for both pathogens over a large geographic region over time. It may be that significant variability in the relative frequency of these pathogens exists over space and time in New England. Accordingly, we collected deidentified residual human sera from 11 clinical laboratories in five New England states (Maine, New Hampshire, Rhode Island, Massachusetts, and Connecticut) between the months of May and August from 2018-2019 to test for exposure to the two pathogens. Antibody detection assays included an FDA cleared ZEUS ELISA (*Borrelia* VlsE1/pepC10 IgG/IgM) for *B. burgdorferi* and a multiplex Luminex assay for *B. miyamotoi*. Residential zip codes were collected at the time of sera acquisition to determine spatial distribution between counties and clinic sites. Of 2778 serum samples, 266 (9.6%) were *B. burgdorferi* seroreactive and 66 (2.4%) were *B. miyamotoi* seroreactive. No significant differences were found for seropositivity to either pathogen in 2018-2019 by performing a two-way ANOVA (p-adj= 0.199). A chi-square test for relative risk pooling seropositivity across all sites found a greater risk of exposure to *B. burgdorferi* compared to *B. miyamotoi* (X^2=126.9, p=<0.016). A total of 12/2778 sera (0.4%) were seropositive for both pathogens. Sufficient sera were available to also test for *Babesia microti* antibody by IFA in 2018. Of 1481 serum samples, 160 (10.8%) were seroreactive to *B. burgdorferi*, 146 (9.9%) to *B. microti*, and 37 (2.5%) to *B. miyamotoi.* Seroprevalence for *B. burgdorferi* and for *B. miyamotoi* were stable across time and space with evidence of variation between geographic locations. The views and opinions expressed here are those of the authors and do not represent the official position of FDA

0981

SOLUBLE FACTORS SECRETED BY BABESIA MICROTI-INFECTED RED BLOOD CELLS INDUCE CYTOKINE PRODUCTION IN MACROPHAGES

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Babesia microti is the primary cause of human babesiosis in North America. Despite recent increases in the disease, the pathogenesis and immune response to *B. microti* infection remain poorly understood. Studies in laboratory mice have shown a critical role for macrophages in eliminating parasites and parasitized red blood cells (RBCs). Notably, the effector parasite molecules that activate macrophages are still unknown. Recent evidence identified a novel protein export mechanism in *B. microti* that features a network of tubes of vesicles that extend from the parasite plasma membrane to the RBC cytoplasm. Vesicles harboring *Babesia* proteins are released from the infected RBC (iRBC) to the extracellular environment. We postulate that these parasite-derived extracellular vesicles (EVs) participate in intercellular communication between iRBCs and neighboring cells. When macrophages function as recipients, changes in the production of cytokines with key roles in the host innate immune response occur. To test this hypothesis, we examined cytokine responses in macrophages exposed to *B. microti*-iRBCs using an *in vitro* co-culture model. Pro-inflammatory cytokines such as IP-10, G-CSF, IL-6, TNF-α, MIP-1α, MIP-2, IL-1rα, and RANTES were markedly increased in the supernatants of macrophages co-incubated with iRBCs, as compared to uninfected RBCs, using antibody arrays. These effects were dependent on parasite growth, as treatment with the antiparasitic drug clindamycin resulted in significant decreases in cytokine secretion by macrophages exposed to iRBCs. These results support the hypothesis that soluble factors released by *B. microti*-iRBCs cause phenotypic changes in macrophages. Identification of secreted parasite antigens found in EVs isolated from iRBC will provide insights into the mechanisms of intercellular communication between *B. microti* and macrophages which contribute to the induction of the innate immune response in the mammalian host.

0982

VARIATION IN COMPOSITIONAL PROFILES OF AMBLYOMMA AMERICANUM MICROBIOTA DRIVEN BY SEX AND RANGE

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Amblyomma americanum ticks have undergone recent range expansion. We examined the microbiota of 187 *A. americanum* ticks from 4 regions spanning their historical and current geographic range . Using massively parallel sequencing targeting the 16S rRNA V4 region, we analyzed the bacterial community composition of each individual tick. Consistently, the most prominent members of the community for each sample were either *Coxiella*, *Rickettsia* or *Rickettsiella*. Alpha diversity was significantly different between males and females at all regions tested; male ticks possess richer but uneven communities. Random forest analysis revealed that sex is a significant predictor of microbiome diversity, and indicator species analysis pinpointed taxa that are most or least commonly associated with each sex (224 for males, 1 for females). Regarding beta diversity, both ordination and PERMANOVA analyses revealed significant differences driven by sex and geographical origin. In addition, a mantel correlogram showed that the dissimilarities were structured by the distance between collection sites. Null model and phylogenetic analyses showed that for females and males, respectively, drift (88.11%; 85.90%), dispersal limitation (11.23%; 12.96%), homogeneous selection (0.61%; 0.10%), and homogeneous dispersal (0.00%; 1.03) were ecological drivers of microbial community dynamics. Our results suggest the microbiome may offer an additional layer of genomic information to study the range expansion of this relevant vector. Diagnosis of pathogenic *Rickettsia* and other pathogens is underway. We aim to identify indicator taxa whose signature profiles allow us to build a model to predict their presence.

0983

INITIAL EVALUATION OF THE INBIOS LYME DETECT™ IGM IGG ELISA FOR DETECTION OF LYME DISEASE

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Tick- borne diseases continue to pose a substantial and growing public health problem in the United States. The most common tickborne disease in the United States is Lyme disease, caused by the spirochete *Borrelia burgdorferi sensu strictu*. With an estimated incidence of 240,000-440,000 new infections annually, Lyme disease is an important and expensive emerging illness in the US. Symptoms of early Lyme disease include fever, fatigue, headaches and weakness. Late Lyme disease symptoms such as arthritis, fatigue, carditis, memory loss and loss of sight are more prevalent than previously estimated. The Johns Hopkins Bloomberg School of Public Health has estimated that Lyme disease costs the US health care system an average of $712m to $1.3b annually. Early detection is important to minimize the debilitating manifestations of late Lyme disease. Diagnosis of Lyme disease currently involves a laborious 2- tier testing algorithm. Due to the complex immune response in Lyme disease, many antigens are needed to achieve necessary sensitivity. InBios developed the Lyme *Detect*™ IgM/IgG ELISA using a recombinant, multi-epitope fusion peptide antigen, VOVO, made by the National Institutes of Health. In initial evaluation, the ELISA had a sensitivity of 100% (14/14) and specificity of 95% (2/40) on commercially obtained Lyme disease and healthy control samples respectively. When the ELISA was tested on the well characterized panel of samples established by the CDC defined by the stage of Lyme disease, good correlation with the 2- tier algorithm was seen. High sensitivity of 100% was observed for Stage 1 convalescent, Stage 2 neurologic Lyme and Stage 3 arthritic Lyme samples. For Stage 1 acute samples which fall within the early "window period" of detection prior to sero- conversion,1/5 samples were IgM positive, better than the 2-tier algorithm which did not detect any of the 5 samples. High specificity was observed on the control samples (92%) in the panel. The InBios Lyme *Detect*™ IgM/IgG ELISA is promising in initial evaluation and upon further development and FDA clearance can be a sensitive, specific, easy to use and interpret 1-tier test for Lyme disease.

0984

IDENTIFICATION OF CODING REGIONS FOR THE INNOVATION OF THE CONFIRMATORY DIAGNOSIS OF CARRION'S DISEASE

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The current gold-standard method for the diagnostic of Carrion's disease is the bacteriological culture which is accurate but time-consuming. Other methods like blood smear inspection are fast but low sensitive, the serological methods are based on the interaction between patient antibodies and all the expressed proteins which limits specificity, molecular methods are based on classical gene regions like gltA, RibC and ialB among other genes that are not unique for Bartonella bacilliformis and therefore subject to cross reactivity. In addition, there are currently two proposed subspecies for Bartonella bacilliformis and no available methods for their identification and distinction. This study aims to find unique and specific coding regions of Bartonella bacilliformis at species and subspecies levels for the development of an accurate and sensitive method for the molecular diagnosis of Carrion's disease. Search was based on sequence similarity for all coding regions identified in Bartonella bacilliformis strain KC584 with 4 databases as follows: against complete genomes of febrile-illness-associated bacterial species from Leptospira, Rickettsia, Anaplasma, Orientia, Neorickettsia, Borrelia and Brucella genera, against

complete available genomes of Bartonella genera species, against 16 B. bacilliformis complete and partial genomes corresponding to both identified subspecies and against Human genome GRCh38.p13. Sequence similarity was assessed with BLASTplus and resulted regions were processed in primer-BLAST for the identification of primers and probes, we finally used Oligoanalyzer for primers quality analysis by measuring GC content, melting temperature, secondary structure and dimer formation. We identified four coding regions, Flagellar hook-length control protein FliK, TOPRIM-domain-containing protein, YdaU family protein and POR-domain-containing protein and one hypothetical protein for the detection of B. bacilliformis and both proposed subspecies. All regions represent good candidates for the implementation of an accurate and fast molecular method by conventional and multiplex real-time PCR.

0985

DERMACENTOR VARIABILIS AND ITS ASSOCIATED RICKETTSIAE IN SOUTHEASTERN VIRGINIA; THE POTENTIAL ROLE OF PATHOGEN SPILLOVER FROM LOCAL SYMPATRIC TICK SPECIES

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Hard-bodied ticks are the most medically important group of arthropods in the United States. *Dermacentor variabilis*, the American dog tick, is a common vector in the United States with its geographic range extending across the eastern portion of the country and parts of the west. This tick is historically the vector of *Rickettsia rickettsii*, the causative agent of Rocky Mountain Spotted Fever; it has also been observed to harbor other spotted fever group rickettsiae. In Virginia, the number of spotted fever group rickettsiosis cases range between 300-400 cases per year, but often the rickettsial agent is not identified to species level. There are a variety of rickettsial species reported in Virginia including *R. rickettsii*, *R. amblyommatis*, *R. montanensis*, and *R. parkeri*. *Rickettsia* can be detected in multiple tick species, but are typically transmitted by a single vector; all of which are sympatric with *D. variabilis*. The purpose of this study was assess current *D. variabilis* populations in Virginia for their associated rickettsiae. From 2012 to 2018 as part of a long-term active surveillance project, adult *D. variabilis* were collected using standard flagging methods while immatures were collected using small mammal trapping. Rickettsiae were detected using real-time PCR and confirmed using Sanger sequencing. *Dermacentor variabilis* in Virginia were observed to harbor *R. montanensis*, *R. parkeri*, and *R. amblyommatis,* with the most common rickettsial species being *R. montanensis*. No samples had detectable *R. rickettsii* infection. The occurrence of pathogen spillover among sympatric tick species appears to be common in *D. variabilis* when other tick species are dominant in the same area.

0986

DETECTION OF AN EXOTIC RICKETTSIA IN AN INVASIVE TICK COLLECTED FROM AN INVASIVE AMPHIBIAN

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Invasive species can transport their associated parasites and pathogens from their native to non-native ranges. In Florida, invasive reptile and amphibian species have established populations with ticks from their native ecosystem. For example, *Amblyomma rotundatum* is a reptile and amphibian feeding tick from South America that can vector tick-borne pathogens. We conducted pathogen surveillance on *A. rotundatum* collected from cane toads (*Rhinella marina)* in Florida to determine if exotic pathogens were detected. We examined 99 cane toads from a population in Homestead, Florida collected from June to October 2019. Individual toads were examined for ticks and collected if present. DNA was extracted from individual ticks. Using PCR, we surveyed for *Rickettsia*, *Anaplasma*, *Ehrlichia* and *Borrelia* bacteria. Positive PCR results were Sanger sequenced to identify pathogen species. Out of the toads examined 18/99 (18.2%) were infested with ticks. We collected 88 ticks and on average toads were infested with 4.89 ticks. PCR results were negative for *Anaplasma*, *Ehrlichia* and *Borrelia*. Of the toads infested with ticks 11/18 were positive for *R. bellii*. *Rickettsia bellii* is a mildly pathogenic Spotted Fever *Rickettsia*. Phylogenetic analysis indicated that the *R. bellii* strain from the toads in Florida matched closely to *R. belii* sequences collected in Central and South America and deposited in Genbank. Our study suggests that exotic ticks can bring their pathogens with them and remain established in the system for 70years. Understanding the pathogens invasive ticks carry can provide valuable insight on the risk of establishment of tick-borne pathogens to native and invasive wildlife. Ongoing research is being conducted to screen ticks for additional parasites and examine more cane toad populations in Florida.

0987

CAUSES OF ACUTE FEBRILE ILLNESS IN SELECTED HOSPITALS OF BANGLADESH

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Understanding region-specific distribution of pathogens causing acute febrile illness (AFI) is important for clinical management in resource-poor settings. We aimed to explore the proportion of AFI caused by specific pathogens in Bangladesh. During May 2019-March 2020, study physicians screened patients aged >2 years presenting to outpatient departments of 4 public hospitals in different regions. We randomly enrolled patients who had measured fever (≥100.4°F) during assessment with onset within the past 14 days but did not take any antibiotics in the past 24 hours. Their blood and urine were tested at icddr,b [rapid diagnostic tests, bacterial culture, polymerase chain reaction (PCR)]. Acute and convalescent samples were sent to Centers for Disease Control and Prevention (USA) for *Rickettsia* and *Orientia* (*R/O*) and *Leptospira* tests. Among 690 AFI patients, 67 (9.7%) had typhoid (*Salmonella* Typhi) detected, 51 (7.4%) *Escherichia coli,* 28 (4.1%) dengue, and 1 hepatitis (HEV). Of the 441 patients tested for *R/O*, 27 (6.1%) had rickettsioses. We found 5 (2%) *Leptospira* cases among the 250 AFI patients tested for it. Eight AFI patients (1%) were hospitalized, but no deaths were reported. Typhoid was more prevalent in Rajshahi (15%, 35/231). Dhaka had most dengue cases (68%, 19/28) with the highest prevalence here than other locations (8%, 19/233). *R/O* affected young adults (IQR 7-23 years) and was more prevalent in the 21-25 years age-group (11%, 8/70). The highest prevalence of rickettsioses was in Rajshahi (10%, 18/182). Though there was no significant difference in gender or urban/rural residency, *R/O* was more likely to be found in patients who had a history of recent animal entry inside their house than not (OR 2.5, 95% CI 1.1-5.5), and in Rajshahi region than in Sylhet (OR 4.7, 1.1-20). Most (67%) *R/O* were from Rajshahi. *S.* Typhi and *E. coli* were the most common bacterial causes of AFI in Bangladeshi hospitals. Dengue was the most common viral infection, predominant in Dhaka. *R/O* was also common based on PCR alone and may be much higher once acute/convalescent antibody results are available. These results can help guide empiric outpatient AFI management.

0988

EVALUATION OF THE ECONOMIC BURDEN OF SCABIES IN FIJI

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We analysed data of the Big Skin Health Intervention: Fiji Trial (BigSHIFT) in order to evaluate the economic burden of scabies in Fiji. The trial included 3643 outpatient and 58 hospital admitted cases of scabies from the Northern Division of Fiji between July 2018 and June 2019. We applied cost of illness approach to estimate the total cost of scabies from the

government perspective. We used micro-costing method to identify items related to direct cost of treating scabies. The cost items were valued by using bottom-up approach. Information on cost was derived from the reports of Fiji Ministry of Health. We also did one way sensitivity analysis to examine the variation in the cost estimates between the report of Fijian Ministry of Health and World Health organisation (WHO). Our study evaluated the direct medical costs associated with scabies in the whole population of Northern Division of Fiji which is highly endemic of scabies and has poor socioeconomic condition. Total economic burden was estimated separately for hospital admitted and outpatient cases. The data indicated that scabies poses a significant burden to public health, health resource utilisation and health budget. The notable contributors for direct medical costs were costs of clinic visits and cost of medicines for outpatient cases. For hospital admitted cases, cost for hospital bed, diagnostic tests and medicines were the main contributors. This study will provide information to make informed decisions about allocation of the limited resources in low-and-middle-income countries like Fiji. The findings will strengthen scabies control program by prioritising scabies alongside other neglected tropical diseases. Because we have evaluated the economic cost from government perspective, the findings of this study will help policy makers and governments to design programs and strategies for scabies control. It will help to identify target groups for scabies control interventions like mass drug administration and provide evidence to inform future cost effectiveness analysis of those interventions.

0989

ALPHA-GAL: AN EMERGING TICK-BORNE DISEASE OF CONCERN IN THE CAROLINAS

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Tick-borne disease (TBD) incidence is increasing globally. The southeastern US, home to multiple ticks which pose public health threats, has been identified as a hotspot of concern regarding TBD. According to the South Carolina Department of Health and Environmental Control, approximately 74% of all reported vector-borne disease cases from the past 5 years have been tick-borne. According to the North Carolina Department of Public Health, approximately 91% of vector-borne disease from the same time frame are tick-borne. One emerging TBD in the southern US and internationally is alpha-gal syndrome, a novel food allergy to the mammalian oligosaccharide galactose-alpha-1,3-galactose (alpha-gal), associated with the bite of a hard tick. The Lone Star tick, *Amblyomma americanum*, is most associated with cases in the US. This disease is not nationally nor state reportable in either of the Carolinas; however recent case reports of alpha-gal have raised concern. Much is still unknown regarding this disease, including definitive clinical manifestations and the induction mechanism for pathogenesis. In response to these recent cases of alpha-gal, environmental sampling began in March 2020 in South Carolina to determine the distribution of the tick vector and understand potential areas of high risk. Ticks were collected through dragging and CO_2-baited traps at state parks and from dogs at local animal shelters. All ticks were identified and processed for pathogen testing. *Am. americanum* was the most collected tick in the state and was found in all regions where alpha-gal human cases have been reported. Testing results for this species revealed multiple pathogens of public health importance. In addition to our tick surveillance data, recent clinical cases of alpha-gal in South Carolina will be described along with their relation to surveillance results. The findings of our two-part state-wide active tick surveillance and passive human surveillance will provide the audience with important information to aid in public health officials' preparedness regarding this emerging TBD threat in the US, South Africa, South America, Europe, Australia and Asia.

0990

FIRST REPORT OF DABIESHAN AND LIHAN TICK VIRUS (BUNYAVIRALES: PHENUIVIRIDAE) IN TICKS IN LAO P.D.R.

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In Laos, definitive diagnosis for vector-borne illnesses is often not available thus causing these infectious diseases to be limitedly defined. In order to identify common and emerging tick-borne pathogens in Laos, NAMRU-2 Singapore (SG) and Institut Pasteur du Laos studied the distribution vector potential of ticks to act as vectors of new or neglected arboviruses. Between 2019-2020, 376 ticks were collected from Xienkhouang Province and screened for tick-borne virus through conducting RT-PCR and Next Generation Sequencing (NGS). The ticks were pooled into 12 large pools with the nucleotide sequences of the L and S segments obtained by NGS. NGS identified 2 viral species, Dabieshan tick virus and Lihan uukuvirus, of the genus *Uukuvirus* (former classified as *Phlebovirus*). Dabieshan tick virus was detected in 2 pools of *Haemaphysali*s ticks collected by dragging, with a 72-88% (L segment) and 53% (S Segment) nucleotide identity with the prototype strain previously identified in Chinese *Haemaphysalis longicornis* ticks. Lihan uukuvirus was detected in 2 pools of *Rhipicephalus microplus* collected from cows, with a 99.6-99.8% (L segment) and 85.3-97.5% (S Segment) nucleotide identity with the prototype strains previously identified in *Rhipicephalus microplus* ticks from various locations. The 4 large positive pools were broken into 18 min pools for RT-PCR pathogen screening. From mini pools, the prevalence rate among these samples was 22% (4 positives out of 18 mini pools (36 ticks)). The results from this study are the first to record Dabieshan and Lihan tick viruses in Lao P.D.R. Future research studies are needed to determine the pathogenicity of these viruses to livestock and humans in Laos.

0991

IDENTIFICATION OF TICK-BORNE RICKETTSIAIN COMMUNAL LANDS, RANCHES, AND PROTECTED AREAS OF ESWATINI

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African tick-bite fever, caused by the bacteria *Rickettsia africae*, is the most commonly reported travel-related tick-borne disease. Furthermore, tick-borne rickettsioses are increasingly implicated in acute febrile illness in rural, pastoral and mixed farming communities across the continent. Living in close association with animals and participating in outdoor activities are key drivers of rickettsial infections. The goal of this study was to identify tick-borne spotted fever group *Rickettsia* from host-seeking ticks in a landscape of wildlife, livestock, and humans, and explore distributional patterns in the ticks and rickettsial species. We collected questing ticks from the vegetation at 26 sites between 2018 and 2019 across conservation areas, ranches, and communal rangelands in Eswatini. We identified ticks using morphology and molecular methods and then tested them for rickettsial DNA using real-time PCR assays, conventional PCR assays, and direct sequencing of multiple loci. We detected rickettsial DNA in three out of seven tick species collected (*Amblyomma hebraeum*, *Haemaphysalis elliptica*, and *Rhipicephalus simus*). Using sequences from three genes, rickettsial samples grouped into four distinct clades: *Ri. africae*, *Ri. conorii*, *Rickettsia massiliae*, and *Rickettisa barbariae*. We found an average prevalence of 55% for *Ri. africae* in *A. hebreaum*, 4.2% for *Ri. conorii* in *H. elliptica*, and 4.4% for *Ri. massiliae* in *Rh. simus*. *Rickettsia africae* was detected in multiple conservation areas, ranches, and communal rangelands, while detection of *Ri. conorii* was limited to a single communal rangeland and single ranch and detection of *Ri. massiliae* was limited to a single conservation area and single ranch. These results illustrate the widespread presence *Ri. africae* (agent of African tick-bite fever) and the occurrence of *Ri. conorii* (agent of Mediterranean spotted

fever) in Eswatini. This study expands our understanding of the challenges faced by travelers visiting conservation areas and rural communities living close to wildlife and livestock.

0992

SPOTLIGHT REPORT: TICKS AND TICK-BORNE DISEASES OF UGANDA

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The risk posed by tick vectors of tick-borne diseases (TBDs) affecting humans, livestock and wildlife is not well defined in Uganda. Country-wide surveillance data for ticks and tick-borne pathogens is severely lacking. To address this critical knowledge gap, a systematic literature review of peer-reviewed studies of ticks and TBDs in East Africa was performed, augmenting our own surveillance data that is available in VectorMap. Inclusion and exclusion criteria were defined *a priori* and targeted publications reporting tick or tick-borne pathogen distribution data in Uganda, from the year 1901 to 2020. This work is part of a larger effort to examine and compile historical data from several East African countries. Publications were obtained through PubMed, CABI, SCOPUS, and WOS. A total of 1057 publications were screened by title and abstract, of which 33 met our criteria were mined for tick and TBD surveillance data, including but not limited to; tick species, pathogen presence, collection location, collection method and date. Locality descriptions were georeferenced and spatial uncertainty was calculated for each record. These preliminary results identified 33 unique tick species, including several medically important species in Uganda. This data provides a baseline for future studies to further analyze TBD risk in Uganda, and throughout the East African region.

0993

EFFECTIVENESS OF ONE VERSUS TWO DOSE MASS DRUG ADMINISTRATION REGIMENS FOR THE CONTROL OF SCABIES

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Mass drug administration (MDA) is an effective control strategy for scabies in populations where there is a high prevalence. Two-dose ivermectin based MDA has been shown to reduce population prevalence of scabies by around 90%. Current two-dose regimens present barriers to widespread implementation including challenges of cost, logistics in isolate communities, and integration with control of other neglected tropical disease. The Regimens of Ivermectin for Scabies Elimination (RISE) study investigated whether one-dose MDA is as effective as two-dose MDA to control scabies in a high prevalence setting. The study was based in 20 villages in Western Province in Solomon Islands. All residents of the village on the day of the study were eligible to participate. Villages were randomised in a 1:1 ratio to receive either one or two doses of ivermectin based MDA. Topical permethrin was offered to participants who met exclusion criteria for ivermectin. Scabies and impetigo prevalence in each village was assessed at baseline and 21 months after the intervention. We enrolled 5239 participants at baseline and found an overall population prevalence of scabies of 15.0% (95% CI 11.8-19.1) and impetigo prevalence of 5.6% (95% CI 4.2-7.3). Results for the 21-month timepoint will be available in May 2021. We will compare the relative reduction in scabies and impetigo prevalence in each arm. If there is less than a 5% difference in reduction we will consider one dose non-inferior to two doses. The results of this study will guide policy for the dosing regimens of ivermectin for scabies MDA in high prevalence settings.

0994

A COMPARATIVE STUDY OF TWO SAMPLING METHODS ON PYRETHROID RESISTANT MALARIA PARASITE VECTORS IN WESTERN KENYA.

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While long-lasting insecticide-treated nets (LLINs) are widely accepted for controlling malaria, pyrethroid resistance has become widespread among malaria vector populations in Africa. In western Kenya, *Anopheles gambiae sensu stricto (s.s.)* has developed a point mutation in the voltage-gated sodium channel (L1014S) related to knockdown resistance. Resistant *Anopheles arabiensis* and *Anopheles funestus s.s.* enhance the enzymes that metabolize pyrethroid insecticides. Thus, pyrethrum spray catch method (PSC) may underestimate the number of anopheline mosquitoes present during indoor collections. We investigated whether PSC remains effective for monitoring pyrethroid resistant anopheline populations. Specifically, we compared the numbers of female anophelines collected, the species composition and the proportion of blood-fed mosquitoes between PSC and light trap (LT). Cross-sectional data were collected from 10 traditional houses from each of 15 communities between December 2013 and January 2014 in western Kenya. One hundred fifty households sampled once for LT (Day 1) and once for PSC (Day 2). The number of *An. gambiae s.s.* collected was too few to compare the methods. Negative binomial mixed models showed that compared with LT, PSC collected a fewer number of *An. funestus s.s.* [adjusted Risk Ratio (aRR): 0.24, 95% confidence interval (CI): 0.17, 0.35] and *Anopheles rivulorum* (aRR: 0.18, 95%CI: 0.06, 0.53), but the numbers of *An arabiensis* were not significantly different between the two methods (aRR: 0.94, 95%CI: 0.65, 1.38). Generalized linear mixed models for binomial proportions showed that PSC collected higher proportions of blood-fed *An. arabiensis* (aRR: 53.50, 95%CI: 25.10, 114.07) and *An. funestus s.s.* (aRR: 487.28, 95%CI: 224.40, 1059.07) than LT. All *An. rivulorum* had fed blood in PSC. The results suggest that PSC is still effective for monitoring blood-fed pyrethroid resistant anopheline populations, and LT should be used to monitor the number of *An. funestus sensu lato* in particular. Further studies are needed to compare both methods based on the proportion of resistant individuals in the population.

0995

ESTABLISHING A PRACTICAL INSECTICIDE RESISTANCE MONITORING METHODOLOGY FOR ORALLY INGESTED DINOTEFURAN

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Attractive Targeted Sugar Baits (ATSB®) are being evaluated as part of an Integrated Vector Management (IVM) approach. They are likely to be particularly effective against outdoor biting mosquitoes, as well as mosquitoes that are resistant to pyrethroids. An ATSB based on the neonicotinoid dinotefuran has been developed by Westham Co. and is under evaluation in conjunction with IVCC. As with any insecticide-based intervention it will be important to monitor for the possible emergence of vector resistance. While methods for detecting resistance to neurotoxic insecticides via tarsal contact are recommended by the World Health Organization (WHO), these are not applicable for the also neurotoxic but orally ingested dinotefuran, due to its negative log P which hampers tarsal uptake. Sophisticated oral application assays have been developed, able to determine dose response curves among several *Anopheles* strains, including some highly resistant to pyrethroids, by using uranine markers and in a tightly controlled laboratory setting. However, the applicability of those feeding assays in the field is limited. Instead, the WHO method for topical application (TA) bioassays was adapted; applying dinotefuran to the thorax of adult *Anopheles* mosquitoes with organic carrier bypasses lipid cuticle barriers and is more analogous to ingestion than tarsal exposure. The TA dose response is being compared and correlated with the respective response of the oral toxicity bioassays, across several *Anopheles* strains. Should this correlation be fully established, a TA diagnostic dose (DD) can be determined based on WHO guidelines, to be used as a reliable proxy for monitoring the development of resistance in field populations. This DD will be further validated against field populations in three African countries where pilot trials of the bait stations are underway, and used to routinely monitor for the emergence of resistance after deployment of the bait stations. The suitability of the approach and correlation between oral ingestion and topical application across populations with different responses, needs to be evaluated case by case, for other oral toxins.

0996

POST MARKET SURVEILLANCE OF PYRETHROID-PBO NETS: LEARNINGS FROM PERMANET® 3.0

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With widespread pyrethroid resistance, pyrethroid-PBO nets are increasingly becoming a standard, core vector control intervention. Randomized control trials in Uganda and Tanzania indicated pyrethroid-PBO nets were more effective than pyrethroid-only LLINs in preventing malaria even with high level pyrethroid resistance. Knowledge gaps exist in understanding the long term effectiveness of pyrethroid-PBO nets due to differences in design and concentrations of the pyrethroid and PBO. Vestergaard conducts post market surveillance of PermaNet® 3.0 based on adapted methodology from WHO guidelines for evaluation of LLINs. PermaNet® 3.0 were targeted for collection from households 2 to 3 years post distribution and evaluated for bioefficacy, chemical content, and physical integrity. Samples collected from Nigeria, Equatorial Guinea, Sudan, Ethiopia, and Uganda ranged in period of use from 2.5-3.3 years. All samples passed the WHO optimal effectiveness criteria (≥95% knockdown or ≥80% mortality, pyrethroid susceptible *An. gambiae s.s.* Kisumu strain). Deltamethrin content ranged from 1.8-3.5 g/kg; PBO content ranged from 3.5-8.6 g/kg. Further testing was conducted on samples from Uganda using a characterized pyrethroid resistant lab strain with documented target site and metabolic mechanisms including P450 monooxygenase. A new, unused PermaNet® 3.0 and PermaNet® 2.0 were used as the positive controls. After 3.3 years of use in Uganda, PermaNet® 3.0 killed 4.5 times more resistant mosquitoes than PermaNet® 2.0 but reported reduced mortality in bioassay compared to a new PermaNet® 3.0 indicating loss of efficacy, but still significantly more efficacious than a pyrethroid-only net. Post market surveillance of pyrethroid-PBO nets is essential for long term monitoring and addressing data gaps in understanding the insecticidal and PBO availability throughout the lifetime of a pyrethroid-PBO net. There is need to expand the current WHO guidelines to include testing with well characterized pyrethroid resistant strains, in addition to guidance on the post market surveillance of the new types of nets.

0997

COUNTRYWIDE INSECTICIDE RESISTANCE MONITORING IN NIGER: SUPPORT FOR ITN DISTRIBUTION DECISIONS MAKING

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The PMI VectorLink project conducted insecticide resistance monitoring in seventeen sites selected across Niger by the National Malaria Control Programme (NMCP). Since 2018, vector susceptibility to pyrethroids, organophosphate, carbamate, neonicotinoid, and pyrrole were tested every year. The susceptibility status, resistance intensity and synergist assay against the main malaria vector, *An. gambiae* s.l. was determined for alpha-cypermethrin, deltamethrin, permethrin, pirimiphos-methyl, chlorfenapyr and clothianidin. Two-five-day old adult mosquitoes emerged from collected larvae were tested using WHO susceptibility test kits and CDC bottles assay. Insecticide resistance allele frequencies were further characterized using polymerase chain reaction methods. From 2018 to 2020, resistance to all pyrethroids was observed at all sites. Insecticide susceptibility to all three pyrethroids in all sites was below 60% and in some cases as low as 0%. Pre-exposure to piperonyl butoxide (PBO) substantially increased susceptibility to pyrethroids to varying degrees. Mortality against deltamethrin averagely increased in mortality from 30 to70%, for permethrin, 0 to 60%, and for alpha-cypermethrin, 5 to 70%. Susceptibility to clothianidin 2% and chlorfenapyr 100ug/ bottle was recorded in all sites, except for chlorfenapyr in Tessaoua and Magaria where possible resistance was noted. Vectors were susceptible to pirimiphos-methyl in only five sites (Madarounfa, Magaria, Matamey, Say, and Tillabery), moderately resistant in six sites (Agadez, Balleyara, Boboye, Gaya, Madaoua, and Niamey V), and highly resistant in four sites (Guidimouni, Keita, Tchintabaraden, and Tessaoua). Both *kdr*-w and *Ace*-1 mutations were recorded at frequencies between 0.22 - 0.38 and between 0.05 - 0.09, respectively, across the sites. The data collected in Niger supported the NMCP on making decision to purchase Interceptor G2 mosquito nets in fifteen District and PBO LLINs in eight Districts for the 2022 ITN mass campaign

0998

EVIDENCE SUPPORTING DEPLOYMENT OF NEXT GENERATION INSECTICIDE TREATED NETS IN BURKINA FASO BIOASSAYS WITH EITHER CHLORFENAPYR OR PIPERONYL BUTOXIDE INCREASE MORTALITY OF PYRETHROID-RESISTANT ANOPHELES GAMBIAE S.L.

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Pyrethroid resistance poses a major threat to the efficacy of insecticide treated nets (ITNs) in Burkina Faso and throughout sub-Saharan Africa. There are ITNs available treated with alternative insecticides, including piperonyl butoxide (PBO)-based ITNs and Interceptor® G2 (treated with chlorfenapyr and alpha-cypermethrin). Before deploying alternative ITNs on a large scale it is critical to characterize resistance profiles of primary malaria vector species. Larvae from the predominant vector, *Anopheles gambiae* s.l., were collected in 2019 from 15 sites located throughout Burkina Faso and reared to adults for bioassays to assess insecticide resistance status. WHO tube tests were used to determine the intensity of resistance to pyrethroids commonly used on ITNs at 1x, 5x and 10x times the diagnostic dose and to conduct PBO synergist bioassays with deltamethrin and permethrin. Bottle bioassays were conducted to determine susceptibility to chlorfenapyr. Results showed high intensity resistance to deltamethrin and alpha-cypermethrin at all sites. Resistance intensity to permethrin was either moderate or high in 13 sites. PBO pre-exposure followed by deltamethrin exposure partially restored susceptibility in all but one of the remaining sites (often reaching mortality greater than 80%). PBO pre-exposure followed by permethrin exposure partially restored susceptibility in 12 sites but there was no significant increase in three sites. Susceptibility to chlorfenapyr was confirmed at 14 sites. High pyrethroid resistance intensity in *An. gambiae* s.l. is widespread across Burkina Faso and may be a predictor of reduced pyrethroid-only ITN effectiveness. PBO + deltamethrin ITNs would likely provide greater control than pyrethroid-only nets. However, since susceptibility in bioassays was not fully restored in most sites following pre-exposure to PBO, Interceptor G2 may be a better long-term solution, as susceptibility to chlorfenapyr was observed in nearly all sites. This study provides evidence supporting the introduction of Interceptor G2 nets and PBO nets, which were distributed in Burkina Faso in 2019 as part of a mass campaign.

0999

ACCOUNTING FOR HETEROGENEITY IN WILD ADULT SAMPLES TO MEASURE INSECTICIDE RESISTANCE IN ANOPHELES MALARIA VECTORS

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Systematic, long-term, and spatially representative monitoring of insecticide resistance in *Anopheles* malaria vectors is needed to quantify the impact of insecticide resistance on malaria transmission, and to combat failing interventions when resistance emerges. Resistance assays on wild-caught adult mosquitoes offer an alternative to the current protocols, and can be done cheaply, in a shorter time frame, and in the absence of an insectary. We developed a discrete-time deterministic mosquito lifecycle model to simulate different types of insecticide assays and to evaluate the bias in insecticide resistance bioassays using either adult-captured or larval-captured samples. We incorporated non-lethal effects of insecticide exposure that were demonstrated in laboratory experiments. Using output from this model, we simulated assays from either larval-captured or adult-captured samples. We found that the bias in adult-captured assays depended on the level of insecticide resistance in the population, rather than spatial structure of the population or insecticide coverage. Using the model, we compared the results of these assays to true resistance as measured by the presence of the resistance allele, and constructed a correction model that can be used to reduce bias in adult-sampled assays. In a sample of 100 test mosquitoes, simulated 1000 times, we found that compared to adult-captured assays (MSE = 0.0059), larval-captured assays were a better measure of true resistance (MSE = 0.0018). Using the correction model, we were able to improve the accuracy of the adult-captured assay results (MSE = 0.0038). These results show that adult-captured bioassays—which have logistical advantages over the standard larval-captured assays—can be improved using a simple mathematical approach and used to inform resistance monitoring programs.

1000

COLLECTING TRASH FOR PROFIT TO REDUCE VECTOR BREEDING SITES IN SOUTH COAST KENYA

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Dengue virus (DENV), chikungunya virus (CHIKV), and Zika virus (ZIKV) are three important arboviruses that are spread by the same mosquito vector (*Aedes aegypti)*. Recent studies suggest strong link between *Aedes spp*. mosquitoes breeding and plastics. Some of the plastics utilized by *Aedes aegypti* for breeding include water storage like buckets, jerrycans, basins and drums and discarded plastic containers or plastic waste. Efficient management of garbage especially plastic waste has been associated with reduced transmission and risk of both dengue and chikungunya. However, the focus for majority of studies on solid waste is on describing performance of waste management systems and household waste generation behavior and rarely on ways of improving efficiency of waste reduction, recovery and recycling. This study described the relationships among the different actors in informal recycling in Ukunda and Likoni with an aim of increasing the efficiency of recovering plastic wastes and thus minimizing potential *Aedes Spp*. breeding habitats. We determined that the efficiency of the solid waste management in Ukunda and Likoni is very low. No formal plastic waste recovery and recycling was observed. However, a thriving informal plastic waste recycling was observed with waste pickers, yard shop operators and middlemen as key players. This informal recycling channel is able to remove a substantial amount of plastic waste from the environment. However, their efficiency is curtailed by setbacks such market price fluctuations, low value on plastic wastes, plastic transportation issues and government levies and taxes among others. Approaches to address the identified setbacks were developed through a business incubator and the resulting improvements in plastic waste recycling are discussed.

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INTEGRATED VECTOR MANAGEMENT IN PUERTO RICO AND THE CARIBBEAN IN THE MIDST OF A PANDEMIC

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Aedes aegypti is the leading vector for dengue, Zika, chikungunya, and yellow fever, and caused over 3 million cases of dengue fever in the Americas in 2019 alone, the highest number in history for this region (PAHO, 2020). In Puerto Rico, this mosquito has caused over 70,000 cases

of arboviral diseases over the last decade, resulting in the island becoming the USA jurisdiction with the highest number of arboviral diseases and an important source of travel-related cases in North America. Despite this, vector control strategies are becoming limited given the development of insecticide resistance in mosquitoes and the increased environmental and public concerns about pesticides. Novel techniques like Wolbachia and genetically modified mosquitoes are undergoing significant field trials to demonstrate effectiveness and feasibility but require significant community and stakeholder engagement to be implemented. The COVID-19 pandemic severely impacted current vector management activities throughout Puerto Rico and limited the adoption of integrated vector management territory-wide. Limitations arose from government-issued executive orders and policy decisions, citizen concerns that affected community mobilization efforts, concerns about the interaction between some vector control techniques and COVID-19, diagnosis of dengue vs COVID-19, among other complications. This presentation will examine the specific situations in more detail so as to understand how simultaneous outbreaks of dissimilar diseases can be better managed in the future.

1002

INCENTIVIZING MULTIPLE OBJECTIVES IN ACTIVE SURVEILLANCE FOR URBAN DISEASE VECTORS

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Large-scale vector control campaigns have successfully reduced infectious disease incidence around the world. In addition to preventing new infections, these campaigns produce a wealth of information about the distribution and density of insect vectors, which can be incorporated into risk maps. These maps can effectively communicate risk map data to technicians on the ground, although encouraging them to use the data remains a challenge. We carried out a series of rolling trials in which we evaluated risk map use under different incentive schemes. Participants in the studies were trained field technicians tasked with house-to-house surveillance for insect vectors of Chagas disease in Arequipa, Peru. A novel incentive scheme based on poker best achieved a dual objective: to encourage technicians to preferentially visit higher-risk houses while surveilling evenly across the search zone. The poker incentive structure may be well-suited to improve entomological surveillance activities and other complex multi-objective tasks.

1003

IMPROVED GERMLINE PROMOTERS FOR CAS9 EXPRESSION AND INCREASED DRIVE EFFICIENCY IN THE YELLOW FEVER MOSQUITO AEDES AEGYPTI

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The mosquito *Aedes aegypti* is one of the most important vectors involved in the transmission of several arboviruses such as dengue, yellow fever, chikungunya, and Zika virus. To decrease the incidence of these vector borne diseases, the control of mosquito populations plays an essential role and is often the only way to prevent disease outbreaks. New mosquito control approaches based on CRISPR/Cas9 technology are under development and could be a complementary strategy to improve the mosquito control campaigns that nowadays rely mainly on insecticides (i.e., extensive spraying and insecticide-treated bed nets). The 'Homing Endonuclease' gene drive has garnered attention as a means of introducing and driving a trait in a population and recent studies in different dipterans such as *Drosophila melanogaster*, *Anopheles gambiae*, *Anopheles stephensi* and *Ae. aegypti* have demonstrated the feasibility of this system. However, inheritance rates in *Ae. aegypti* have not reached those described in *Anopheles* species, in which inheritance bias approaches 100%. In this study we utilised six germline-specific regulatory elements to express Cas9 and assessed their ability to bias the inheritance of a sgRNA expressing transgene (*kmo*^{gRNAs}). A total of 14 Cas9-expressing isolines were generated and assessed by crossing them to the *kmo*^{gRNAs} line and the trans-heterozygous F_1 were crossed to a wild type mosquito line (Liverpool strain) to determine the inheritance rate of the *kmo*^{gRNAs}. Our results reveal several isolines which have strong (glm; $p < 0.001$) predictors for increased inheritance of *kmo*^{gRNAs}. One insertion using the *sds3* regulatory region biased inheritance of the *kmo*^{gRNAs} element up to 89.8%. Several isolines using the *shutdown* regulatory region could also bias inheritance up to 90.1%. These data present the greatest inheritance bias reported in a gene drive system in *Ae. aegypti* to date. These results suggest that further improvement can be achieved in the development of a gene drive system in yellow fever mosquitoes.

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ASSOCIATION OF VGSC MUTATIONS V410L, V1016I AND F1534C WITH PYRETHROID RESISTANCE IN AEDES AEGYPTI POPULATIONS FROM CENTRAL AMERICA

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Aedes aegypti is the main vector of dengue Zika, and chikungunya in Latin America. Recent outbreaks of Zika (2016) and dengue (2019) in Central America reflect the need for effective vector control. The main strategy for the control and prevention of these *Aedes*-borne diseases is insecticide-based vector control, with a high reliance on pyrethroid insecticides. However, resistance to pyrethroids has been detected in the region, and there limited knowledge of mechanisms underlying this resistance in Central America. Therefore, we genotyped *Ae. aegypti* populations from several sites in Guatemala, Honduras, Nicaragua, Costa Rica, Panama and Dominican Republic to detect the *vgsc* V410L, V1016I and F1534C mutations and associate their frequencies with phenotypic resistance status. We found co-occurrence of these three mutations in all populations. Frequencies for the V1016I and F1534C mutations ranged from 0.42 to 1.0, and 0.69 to 1.0, respectively. The V410L mutation was present in all sites with frequencies ranging from 0.35 to 0.80, representing a widespread presence and the first report of this mutation in field populations in Central America and the Dominican Republic. We observed an association of the V1016I and V410L mutations with pyrethroid resistance in field populations from Costa Rica, Panama and Honduras. The F1534C mutation was found to be fixed in most of the populations, except in three sites from Honduras, where an association between this mutation and deltamethrin resistance was confirmed. Two distinct haplotypes and evidence of selective sweep were found when analyzing the *kdr* region where the 1016 codon is located. We found that these three VGSC mutations are common and widespread in Central America and Dominican Republic, and can partially explain detected pyrethroid resistance.

1005

INSECTARY INFORMATION SYSTEM: BUILDING-BLOCKS FOR REPRODUCIBLE SCIENCE IN VECTOR BIOLOGY

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Data are an extraordinarily valuable resource and there remain gaps in the development of collection methods and analyses to optimize their use for the benefit of public health. For vector-borne diseases, insectaries could benefit from a custom Laboratory Information System (LIS). Although guidelines have been produced to direct and facilitate rearing mosquitoes in insectaries, less effort has been spent on the information that should be systematically collected to implement a successful, long lasting, sustainable, and productive insect colony. The aim of this study is to describe the implementation of an affordable LIS at an insectary housing a colony of the main vector of malaria in Amazonian Peru, *Nyssorhynchus darlingi,* using open-source software to reduce multiple barriers of costs and logistics. This *Ny. darlingi* colony maintains self-contained mosquito generations and was initially established in 2013 at the Amazonia ICEMR laboratory in Iquitos, Loreto, Peru. Implementation of the LIS began at generation 74 and the colony currently stands at generation 90. The LIS included the revision of guidelines for establishing and maintaining insectaries that breed anopheline mosquitoes, mapped the activities in our insectary, identified data inputs, and designed digital forms. The digital forms captured essential mosquito life cycle information and recorded mosquito development and environmental conditions. Two forms were developed, the first to estimate hatch and survival rate of juvenile stages until pupation by following a sample of eggs per each generation, and the second to collect data about the productivity of the colony. In addition, temperature and humidity were recorded in dataloggers at 30 minutes intervals. All the information collected was processed and displayed in a dashboard to visualize key vector biology metrics through interactive figures and tables. Through continuous monitoring of the insectary, this system provided the ability to plan experiments prospectively, and to foresee and prevent adverse scenarios that could affect the colony health.

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ABUNDANCE OF AEDES AEGYPTI FEMALE MOSQUITOES IN SPACES SURROUNDING RESIDENTIAL AND NON-RESIDENTIAL STRUCTURES IN WESTERN AND COASTAL KENYA

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Aedes aegypti is the main vector transmitting human arboviruses like yellow fever, chikungunya, dengue, and Zika. This vector is known not to fly far away from areas it can find both blood-meals and oviposition sites. This study investigated the abundance of *Aedes aegypti* female mosquitoes in spaces surrounding residential and non-residential structures in Kisumu and Ukunda in western and coastal Kenya. Two Biogents (BG)-traps baited with carbon dioxide (CO_2) were placed outdoors, one at a residential and the other at a non-residential structure, within eight selected zones both in Kisumu and Ukunda. Trapped mosquitoes were collected daily at about mid-day for four consecutive days. A total of 517 female mosquitoes were collected: 193 (37.3%) in Kisumu and 324 (62.7%) in Ukunda. Mean density of female *Aedes aegypti* mosquitoes varied in spaces surrounding different structures: yard shops (4.0); garages (1.8); gardens (1.6); open spaces (1.4); banana plantations (1.2); residential houses (0.9); unfinished/uninhabited houses (0.9) and churches (0.3). These abundancies were significantly lower in unfinished/uninhabited houses (P=0.023 OR 0.210), residential houses (P=0.016 OR 0.224), churches (P≤0.001 OR 0.079) and banana plantations (P=0.035 OR 0.292) compared to yard shops. Yard shops are places where trash (glass, metal, plastic) is sorted and informally recycled and often remains uncovered for many months; therefore, providing ample habitat for container breeders like *Aedes aegypti*. In conclusion, the risks of being bitten by female *Aedes aegypti* mosquitoes is higher in areas surrounding yard shops than in the other residential and non- residential structures. Yard shops may be an important place to target vector control in our urban sites in Kenya.

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BEHAVIORAL RESPONSES OF PYRETHROID-RESISTANT ANOPHELES GAMBIAE MOSQUITOES TO INSECTICIDE-TREATED BED NET

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Long-lasting insecticidal nets are an effective tool in reducing malaria transmission. However, with increasing insecticide resistance little is known about how physiologically resistant malaria vectors behave around a human-occupied bed net, despite their importance in malaria transmission. This study assessed the host-seeking behavior of the major malaria vector *Anopheles gambiae s.s,* when an intact human-occupied treated bed net is in place, with respect to their insecticide resistance status under semi-field conditions. Pyrethroid resistant and susceptible females of *Anopheles gambiae s.s* were color-marked with fluorescent powder and released inside a semi-field environment housing a hut which was occupied by a human host. Inside the hut, the occupant slept under an insecticide-treated bed net trap or untreated bed net trap. The window exit trap was installed to catch mosquitoes exiting the hut. A prokopack aspirator was used to collect indoor and outdoor resting mosquitoes in the morning. Clay pots were placed outside the hut to collect mosquitoes resting outdoors. The proportion of resistant females caught in the treated bed net trap was higher compared to the susceptible females (OR=1.445; P<0.00019). Resistant mosquitoes were less likely to exit the house when a treated bed net was present compared to the susceptible mosquitoes. The susceptible females were 2.3 times more likely to stay outdoors away from the treated bed net (OR=2.25; P<0.0001). The resistant mosquitoes showed significantly reduced avoidance behavior compared to the susceptible mosquitoes that were observed to exit the house and remained outdoors when a treated bed net was used. However, further investigations of the behavior of resistant mosquitoes under natural conditions should be undertaken to confirm these observations and improve the current intervention which are threatened by insecticide resistance and altered vector behavior.

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IMPROVING URBAN ENTOMOLOGICAL MONITORING IN MALABO, BIOKO ISLAND

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Malaria transmission on Bioko Island, where historical entomological inoculation rates were record breaking, has been successfully curbed over the past 17 years due to intense vector control. In recent years, as local transmission declined, malaria importation linked to frequent travel between the island and mainland Equatorial Guinea has become more evident and has been pointed as a major challenge for malaria control and elimination. In particular, data suggest that much of the malaria prevalence observed in the capital, Malabo, could be explained by importation. However, it remains unclear how much local transmission actually occurs in the city. Historical entomological sentinel sites continually monitor vector populations, but these sites are located mostly in rural and in some peri-urban areas and this has limited the availability of entomological data in urban Malabo. Here, we expand entomological monitoring in Malabo identifying suitable urban sites where to establish light trap collections (LTC). It is known that malaria vectors are not urban-friendly, hence these sites had to be selected carefully in order to maximize the probability of collecting anopheline mosquitoes. We used a comprehensive spatial decision support system to define clusters and identify the most suitable houses at the center of these clusters. Clusters corresponded mostly to 1x1 km areas and their yolks were defined as houses located within the 200x200 m center, though this configuration varied according to housing distribution. Houses with open eaves and no air conditioning were prioritized for LTC as these characteristics were assumed to increase the chances of mosquitoes entering a house. Outdoor traps were also placed in some of these houses and in specific locations within their neighborhoods. Mosquitoes will be collected once a month in eight houses from April to October. In the same areas, anopheline breeding sites will be identified monthly to complement LTC. The augmented urban entomological data will be used to inform the assessment of local transmission in Malabo and to measure the impact of indoor residual spraying on urban mosquito populations.

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SOME LIKE IT HOT: HOW URBAN MICROCLIMATE ACROSS A TROPICAL CITY IMPACTS AEDES MOSQUITOES LIFE HISTORY

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Urbanization is accelerating and there is considerable concern about its impact on vector-borne diseases. Changes in microclimate associated with urban heat islands may affect the biology of vector mosquitoes. To investigate such impacts, we first quantified microclimate in sites spanning three levels of urbanization, categorized using the normalized different built-up index (NDBI), in Manaus, Brazil, a tropical city that experiences high levels of arbovirus transmission mediated by *Aedes aegypti* and *Ae. albopictus*. High NDBI sites had significantly lower mean, maximum, and daytime-mean humidity than medium or low NDBI sites (DF=2, P<0.02 for all comparisons), and high NDBI sites were 1.2°C hotter than medium or low sites, though this difference was not significant. *Ae. aegypti* were significantly more abundant at high NDBI sites than other sites, whereas *Ae. albopictus* did not show a significant difference in distribution. We experimentally tested the impact of high NDBI microclimate on *Ae. aegypti* life-history by simulating the temperature and humidity of high NDBI or a control (forest-like conditions) in a cycling incubator. We also varied food availability to assess the interaction between the effects of microclimate and nutrition. *Ae. aegypti* larvae reared with *ad libitum* food pupated faster than the food-restricted larvae, showing an independent effect of food availability (P=0.015, DF=1, F=7.35). When food was restricted, high NDBI microclimate decreased the time to pupation relative to control NDBI (P=0.028, DF=6.11, t=-2.36). Time from pupae to adult eclosion, which was only measured in *Ae. aegypti* with food restriction, was faster in larvae reared at high NDBI conditions (P=0.046, DF=5.17, t=2.064). Adult female mosquitoes reared at high NDBI were smaller (P=0.007, DF=1, F=7.72), and adult females reared with ad libitum food were larger (P=0.0001, DF=1, F=17.51). Adult females reared at control settings live significantly longer compared to the high NDBI microclimate (P=0.001, DF=1, X^2=10.28). These results suggest that urbanization and microclimate may lead to greater vectorial capacity in *Ae. aegypti* mosquitoes.

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HUMAN-MOSQUITO CONTACT: A MISSING LINK IN OUR UNDERSTANDING OF MOSQUITO-BORNE DISEASE TRANSMISSION DYNAMICS

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The emergence and intensification of novel and established vector-borne diseases (VBDs) are an increasing threat to global public health. Yet despite the key role that contact between hosts and vectors plays in driving VBD transmission, transmission risk has been primarily studied through the lens of vector density and overlooks host-vector contact dynamics. Thus, we continue to have a limited understanding on how contact dynamics are influenced by various biological and environmental factors that drive mosquito-borne disease transmission, impeding our ability to control them. To fill this gap, we reviewed current knowledge of host-vector contact with an emphasis on mosquito bite rates and risks. We showed that the dynamical contact rate is affected by mosquito and vertebrate factors, as well as other environmental influences, challenging a classic assumption that mosquitoes bite at a fixed rate determined by the duration of their gonotrophic cycle. We also found a lack of standardized description for host-vector contact rates; therefore, we provided a modeling framework based on distinct biological and mathematical definitions of host-mosquito contact rate, blood-feeding rate, and biting rate. We explored alternative ecological assumptions based on the functional response, blood index, forage ratio, and ideal free distribution within a mechanistic host-vector contact model that better reflects our new modeling framework. A renewed focus on contact dynamics between hosts and vectors contributed new insights into the mechanisms behind VBD spread and emergence that are sorely lacking. Our framework identified critical gaps in current knowledge of host-vector contact to guide future research directions that inform VBD prevention and control.

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ECO-BIO-SOCIAL DETERMINANTS OF THE BREEDING AND INSECTICIDE SUSCEPTIBILITY OF AEDES AEGYPTI LARVAE IN ARBOVIRAL FOCI IN ABIDJAN, CÔTE D'IVOIRE

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Our ability to deal with *Aedes* mosquito-borne arboviral outbreaks in Africa, including Côte d'Ivoire, is limited. We explored the ecological, biological and social (eco-bio-social) factors of *Aedes aegypti* breeding, and dengue and yellow fever transmission risk indices in the large city of Abidjan, Côte d'Ivoire. We sampled *Ae. aegypti* immatures (larvae and

pupae) and breeding containers, and household socio-ecological data in six clusters in Abidjan, Côte d'Ivoire from September to October 2020. We calculated *Stegomyia* indices (container index: CI, household index: HI and Breteau index: BI), and pupal counts (pupae/container: PC, pupae/house: PH and pupae/person: PP). Moreover, *Ae. aegypti* larvae were tested against deltamethrin, DDT, bendiocrab, and malathion. We determined insecticide resistance ratio (RR_{50}) and lethal concentration (CL_{50}). The most productive *Ae. aegypti* breeding sites were outdoor water-holding containers; tires (53.7%), discarded cans (25.3%) and uncovered potable water receptacles (17.6%). CI, HI and BI were estimated at 77.2%, 57.1% and 137.2, and PC, PH and PP were of 2.31, 1.45 and 0.93, respectively. *Ae. aegypti* larval infestation and pupal counts were correlated with complex community behaviors related to water and waste management. Breeding sites' positivity and productivity were associated with unmanaged waste, water supply interruptions and long water storage duration. Domestic areas were more favorable for immature production compared with commercial and public spaces, and then schools and religious facilities. *Ae. aegypti* larvae were susceptible to deltamethrin, malathion, bendiocarb, but resistant to DDT. In Abidjan, Côte d'Ivoire, *Ae. aegypti* breeding and larvae indices were correlated with socio-ecological determinants. Moreover, *Ae. aegypti* larvae were susceptible to insecticides. However, the risks of transmission of dengue and yellow fever viruses were above the World Health Organization (WHO)-established epidemic thresholds. Integrated community-based vector control programs coupled with larviciding are recommended to achieve effective arbovirus vector management.

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LONGITUDINAL MONITORING OF MALARIA VECTORS IN GOBU SEYO, ETHIOPIA TO ASSESS TRENDS IN BITING BEHAVIORS OF MALARIA VECTORS FROM 2012-2017

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Entomological surveillance is important to understand bionomics and behavior of malaria vectors. Longitudinal monitoring provides information on seasonality, biting times, and indoor/outdoor biting behaviors. Entomological surveillance was conducted from 2012 and 2017 in Gobu Seyo, located in the Oromia Region of Ethiopia, using human landing collections, pyrethrum spray catches, and CDC light traps for two nights each month during the hours of 6pm-6am. Temperature and humidity were recorded in hourly increments during collections. Analysis of these data addressed longitudinal variations in peak biting times across seasons and between years to inform future timelines for collections and protocol methodologies. Data were analyzed using a negative binomial regression model to assess primary vectors collected, biting times and locations, and seasonality to evaluate inter- and intra-annual variability. Potential covariates were analyzed including: collection location, temperature, and humidity. *Anopheles arabiensis* and *Anopheles pharoensis* were the most collected species both making up 20% of the observations followed by *Anopheles coustani* making up 19% of observations. Other species collected were *An. demeilloni* at 11%, *An. christyi* at 5%, *An. ziemanni* at 7%, and culicine species at 7% of total collections. Initial analysis across years suggested a range of peak biting times across all species occurring between the hours of 8pm-12am, with less biting occurring in the early evening and early hours of the morning. The total number of mosquitoes collected per night decreased over time from 17 in 2012 to 3 in 2017 with large variances. Large variation was likely due to seasonality of the mosquitoes, temperature, and humidity during collections. Smaller variation was noted in species, seasonality, and biting time across years. Total numbers of mosquitoes collected decreased over the years, possibly related to malaria vector control. These data suggest using trends from longitudinal data sets as a method to assist in decision making about the frequency of entomological collections.

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AN EVALUATION OF LONGITUDINAL ANOPHELES STEPHENSI EGG VIABILITY AND RESISTANCE TO DESICCATION

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Anopheles stephensi, an urban vector of malaria endemic to Asia, was detected in Djibouti in 2012 and has since established as an invasive species in Ethiopia, Sudan, and Somalia. This expansion of *An. stephensi* presents a serious threat to malaria control and elimination efforts in Africa. To control the species and halt further expansion, it is critical to understand the biological characteristics and determinants that may have facilitated invasion. In Ethiopia, *An. stephensi* larvae colonize artificial containers, many of which are shared with *Aedes aegypti*. The success of *Ae. aegypti* as an invasive vector is often attributed to its use of artificial containers and the ability of *Ae. aegypti* eggs to remain viable in the absence of water for months. While *An. stephensi* is found in artificial containers, it is unclear whether the eggs can remain viable in the absence of water for extended periods. In 1927, Chalam found *An. stephensi* to remain viable in soil for up to 12 days, but this work has not been revisited since. Here, we evaluate whether *An. stephensi* eggs can resist desiccation and remain viable under a range of temperature, humidity, and temporal thresholds. Batches of ~200-400 eggs from two laboratory strains of *An. stephensi* (STE2 and SDA500) were placed in five environmental conditions mimicking a range of temperature (15°-35° C) and relative humidity (15-75+%) conditions in the Horn of Africa. Egg batches were hatched at 0, 7, 14, and 21 days and larval survival was measured. Under most conditions, no viable larvae emerged after 7 days in the absence of water. However, for both strains in multiple experimental replicates viable larvae were produced after 2 weeks in the absence of water in only one condition (15°C and >75% RH). This suggests that viable eggs may be transported in the absence of water through trade or commerce routes. Persistence of *An. stephensi* eggs in the absence of water should be considered as programs engage in surveillance and control of *An. stephensi* in Africa.

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THE ROLE OF NUTRITION IN RESISTANCE AND TOLERANCE TO BACTERIAL INFECTION IN AEDES AEGYPTI MOSQUITOES

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Mosquito immunity may be separated into two categories. 1) resistance: the ability to directly limit pathogen levels, and 2) tolerance: the ability to limit the deleterious effects of infection. Immune tolerance is a critical but relatively understudied defense strategy utilized by disease vector mosquitoes during infection. Further, factors that may influence tradeoffs in resistance and tolerance are not well understood. Here, we investigated the potential effect of nutrition on resistance and tolerance to a pathogenic strain of *E. coli* in *Aedes aegypti* mosquitoes. We exposed adult female *Aedes aegypti* mosquitoes to four different nutritional regimens that varied in 1) blood meal (presence or absence), and 2) sucrose (1% or 10%), in order to determine how a mosquito's adult diet may influence investment in resistance and/or tolerance. Using this study design, we can also determine whether blood and/or sugar feeding act independently or interact to influence tolerance and resistance. On days 1, 3, and 5 post-infection, we assessed population survival and accompanying average bacterial load by homogenizing and culturing mosquitoes then counting bacterial colony forming units. We built a generalized linear model using blood, sucrose, bacterial load, and time to predict probability of survival. Significant main effects are indicative of individual factors affecting resistance, while any significant interaction between a factor and bacterial load indicates that the factor

affects tolerance. We have collected data from multiple replicates and are actively performing analysis. Our preliminary data analysis reveals that sucrose concentration significantly impacts resistance. Specifically, we have discovered that exposure to 1% sucrose better equips a mosquito to resist *E. coli* infection. Further analysis to determine whether diet impacts tolerance is ongoing. Final results will reveal a previously unknown relationship (or equally important lack thereof) between a mosquito's diet and the balance between resistance and tolerance.

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EPIDEMIOLOGY OF VECTOR-BORNE AND NEGLECTED TROPICAL DISEASES ALONG THE TEXAS-MEXICO BORDER

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Neglected tropical diseases (NTDs) are a group of parasitic and vector-borne infections that disproportionally affect the world's most impoverished populations. Although NTDs and vector-borne diseases have been previously identified in Texas, their prevalence and distribution are not well characterized. Autochthonous cases of Zika, dengue, and parasitic infections have been reported but are rare and often found in the southernmost part of the state near the US-Mexico border. Understanding the burden of vector-borne and parasitic diseases in Texas is critical for prevention, treatment, and control efforts. Our study aimed to determine the epidemiology of vector-borne and NTDs in a cohort of 610 Mexican American adults living in Starr County, Texas along the US-Mexico border. Starr County is the most impoverished county in the state with 32.5% living below the poverty line, putting them at increased risk for NTDs. Each member of the cohort was enrolled and followed for one year between 2018 to 2020, with serial serum samples collected to screen for chikungunya, dengue, West Nile, Zika, and Chagas disease. Stool samples were screened for *Ascariasis lumbricoides, Necator americanus, Trichuris trichiura, Toxocara canis and cati, Strongyloides sterocoralis, Taenia solium, Cryptosporidum sp., Entamoeba histolytica, Blastocystis spp.,* and *Giardia lambia.* We tested serum collected at baseline, 3 months, 6 months, and 12 months for IgG reactivity to vector-pathogens. Helminth and protozoan pathogen testing was conducted using DNA extracted from baseline stool samples using multi-parallel qPCR. We found a seroprevalence of 1.0% for Chagas disease, 0.6% for chikungunya, 15.1% for dengue, 12.8% for West Nile, and 0.9% for Zika. We also identified *Blastocystis spp*. in stool samples. This is the first large-scale cohort study of vector-borne and neglected tropical diseases in this high-risk region. Our findings demonstrate these pathogens are endemic in Texas and could help inform public health response in the region.

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GUT MICROBIOTA IN AEDES TRISERIATUS CONTRIBUTES TO DETOXIFICATION OF TANNIC ACID

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Larval *Aedes triseriatus* mosquitoes live in the water-filled containers where they utilize plant detritus as carbon sources. During the microbial decomposition of leaf litter in container aquatic habitats, the chemical alterations and microbial processing of leaf litter releases compounds such as tannins into the water column, which affects the mosquito output and mosquito vectorial capacity. We tested the hypotheses that tannic acid alters bacterial abundance and community structure in mosquito larval habitats and the specific mosquito-associated gut microbiota contribute to the detoxification of tannic acid. Addition of tannic acid at 0.35mg/ml in the microcosms caused up to 50% larval mortality compared to the negative control without tannic acid. Supplement with kanamycin and tannic acid in the microcosms led to 75% larval mortality compared to the negative control, indicating that microbiota in the rearing system contribute to tannic acid detoxification. The bacteria in water column, on the leaf surfaces, in the larval and adult *A. triseriatus* midguts reared with or without tannic acid addition were studied by sequencing the V4 region of 16S rRNA genes. *Proteobacteria* and *Bacteroidetes* dominated the community [>86.8% of operational taxonomic units (OTUs)]; most taxa were assigned to *Pseudomonas*, unclassified *Enterobacteriaceae*, *Serratia* and *Flavobacterium*. Bacterial communities were significantly different in water column, on the leaf surfaces and in the larval midgut between control and tannic acid addition, indicating that tannic acid altered the microbial structures in mosquito rearing system. However, bacterial communities were similar in adult mosquito midgut fed with sucrose, showing that the microbiome reconstructed in the adult stage. The proportion of *Pseudomonas* unclassified *Enterobacteriaceae* and *Serratia* was significantly higher in water column, on the leaf surfaces and in larval mosquitoes in the tannic acid supplemented microcosms than that in the control without tannic acid addition, indicating that they possibly contributed to the detoxification of the tannic acid.

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MATHEMATICAL MODEL OF THE TRANSMISSION FOR VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) or kala-azar is a protozoan disease caused by parasites of genus Leishmania that affects millions of people worldwide, with a fatality rate comparable to that of malaria. In 2015, the World Health Organization (WHO) classified VL as a neglected tropical disease (NTD) due to relatively minimal granted attention from the public, resulting in high mortality rates (more than 20,000 in 2015) and endemic spreading in poverty-stricken regions around the globe. VL is a vector-borne disease transmitted to humans through a bite of sandflies that have previously taken a blood meal from an infected reservoir host or an infected human. Importantly, VL is mainly caused by two kinds of Leishmania parasites, Leishmania donovani, and Leishmania infantum. After or during the treatment of VL, a varying proportion of cases develop a cutaneous form of leishmaniasis, known as post-kala-azar dermal leishmaniasis (PKDL). In this study, we present a predictive model to forecast the spread of VL in a human population, accounting for the effect of different interventions, the animal reservoir of the parasite, and the vector's population dynamics. The human population is subdivided into susceptible, early infectious, asymptomatic, infected-treated, infected untreated, temporarily immune, early infectious stage of post-kala-azar dermal leishmaniasis (PKDL), treated PKDL, untreated PKDL, and fully recovered individuals. The reservoir animal population is subdivided into susceptible, exposed (infected but not yet infectious), asymptomatic, and infected. The vector population is subdivided into susceptible, incubating, and infectious vectors. The model is exemplified using parameters from the literature based on the underlying biological features of the disease. We analytically calculate the effective reproduction number to investigate the parameter relevant to the disease transmission and control. In the simulation, we consider different scenarios of different interventions to examine which scenario eradicates the disease.

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BRUGIA MICROFILARIAE ENHANCE DISSEMINATION OF ZIKA VIRUS INTO THE HEMOCOEL OF AEDES AEGYPTI MOSQUITOES

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Concurrent ingestion of microfilariae (MF) and arboviruses by mosquitoes can enhance the transmission of virus compared to when virus is ingested alone. After being ingested, MF penetrate the mosquito midgut and

introduce virus into the hemocoel, creating disseminated viral infections much sooner than normal. This is termed MF enhancement of arboviral transmission. We investigated *Brugia* MF enhancement of Zika viral dissemination within *Aedes aegypti* mosquitoes. Blood containing virus (9.5 x 10^4 PFU/ml) plus *B. pahangi* MF was membrane-fed to a batch of *A. aegypti*. Blood containing only virus (9.2 x 10^4 PFU/ml) was fed to another batch of *A. aegypti* mosquitoes. Mosquitoes in the virus + MF group ingested an average of 103 MF. After four days, the viral infection rate was significantly lower in mosquitoes fed virus + MF (38%, 25/65) versus mosquitoes fed virus-only (80%, 64/80). However, viral dissemination within the 25 infected mosquitoes fed virus + MF (28%, 7/25) exceeded that in the 64 infected mosquitoes fed virus-only (3%, 2/64). Earlier studies showed MF enhancement is facilitated by viral adherence to MF, and that MF transport virus across the mosquito midgut. To examine MF affinity to Zika virus, 5 ml of virus (5.7 x 10^5 PFU/ml) was split into two aliquots. Approximately 40,000 MF was added to one and an equivalent volume of diluent added to the other. After 1 h incubation, samples underwent 9 successive cycles of centrifugation and washing, diluting virus beyond the theoretical limit of detection. Residues were assayed for virus by plaque assay and mosquito injection. Plaque assay of the virus + MF residue detected virus at 10^7 dilution, whereas plaque assays of the virus-only residue detected virus at 10^6 dilution. Moreover, 4 of 29 mosquitoes (14%) injected with the virus + MF residue became infected with Zika virus, whereas none of 32 injected mosquitoes from the virus-only group became infected. This suggests that Zika virus adheres to *B. pahangi* MF and that during midgut penetration, *B. pahangi* MF carries Zika virus into the mosquito hemocoel. These studies provide another example of MF enhancement of arboviral transmission.

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ANALYSIS OF DENV2 3'UTR RNA STRUCTURES REVEALS SPECIES-SPECIFIC ROLES IN FLAVIVIRAL BIOLOGY

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The structured 3' UTR of flaviviral RNAs, which accumulates as the subgenomic flaviviral RNA (sfRNA), is involved in multiple virus-host interactions during infection. In the current study, we focused on the dumbbell (DB) RNA structures present in the 3'UTR/sfRNA. Previous work using the DENV2 3' UTR on a reporter mRNA demonstrated that the DB structures are the necessary and sufficient elements for RNA stability and translation. In the current study, we created DB mutants in the DENV2 infectious clone and successfully recovered infectious viruses. DB mutant viruses are attenuated in a cell-type specific manner, and some viruses are incapable of replication in mammalian cells, demonstrating a critical role in trans-species infection and replication. Interestingly, further passage of a DB-deleted DENV2 led to the appearance of a virus population with dramatically increased growth efficiency. Sequencing identified silent mutations in the coding region of the viral genome suggesting a role for RNA structures and possible long-range RNA interactions between the 3' UTR and coding regions. Because the 3' UTR multi-functional, we performed multiple experiments to tease apart the role of DB mutations in viral replication, genome stability, and viral translation and how these mutations differentially regulate growth in insect and mammalian cells. Overall, our study highlights the importance of the DB RNA structures in the viral genomic RNA independent of the sfRNA.

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DENGUE KNOWLEDGE, ATTITUDES, AND PRACTICES: BASELINE DATA FROM THE COESA STUDY

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The cluster randomized controlled trial *COESA* - Sustainable, healthy cities was designed to examine the impact of a community mobilization intervention compared to typical dengue control practices on the risk of dengue virus infection in young children. The study includes knowledge, attitude, and practice (KAP) surveys, which are commonly used in vector-borne disease research to determine acceptability of vector control interventions and identify potential barriers to care seeking. Despite the frequent use of KAP surveys in dengue studies, methods of analyzing this data are highly variable and often result in the loss of data complexity. Alternative analytic methods such as principal component analysis (PCA) and factor analysis can be used to retain the maximum variability in KAP data while reducing the data for use in further analyses. Baseline COESA survey responses were collected from 389 participating households in Fortaleza, Brazil in 2019-2020; survey topics included demographic characteristics and KAP related to dengue and dengue control. Descriptive statistics were calculated from the responses and analyzed thematically. PCA was used with factor analysis to validate the KAP portion of the questionnaire, and to create a reduced KAP index. Participants' survey responses reflected a broad understanding of dengue transmission, fever as a key symptom of dengue, and vector control practices. Participants were generally aware of the severity of dengue, but ambivalent about the capacity of the community to control dengue on their own. KAP was well represented in an index generated through PCA and factor analysis: 3 factors extracted from the KAP data explained 53.8% of the variance. Further regression-based analyses will include seroprevalence data from children in participating households. Despite limited sample size, the analysis allowed us to explore methodologies and provide feedback to the primary study investigators for guiding future research on KAP, and can help inform future planning, implementation, and evaluation of vector control interventions.

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GENETICALLY ENGINEERED DENV PRODUCES ANTIGENICALLY DISTINCT MATURE PARTICLES

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Maturation of Dengue viruses (DENV) alters the structure, immunity and infectivity of the virion and highly mature particles represent the dominant form *in vivo*. The production of highly mature virions principally relies on the structure and function of the viral premature protein (prM) and its cleavage by the host protease furin. We developed a reliable clonal cell line which produces single-round mature DENVs without the need for DENV reverse genetics. More importantly, using protein engineering coupled with natural and directed evolution of the prM cleavage site, we engineered genetically stable mature DENVs without comprising viral yield and independent of cell, host, or passage. Using these complementary strategies to regulate maturation, we demonstrate that the resulting mature DENVs are antigenically distinct from their isogenic immature forms. Given the clinical importance of mature DENVs in immunity, our strategy provides a reliable strategy for the production of stable, high-titer mature candidate DENV live virus vaccines, genetically stabilized viruses for DENV maturation and immunity studies, and models for maturation-regulated experimental evolution in mammalian and invertebrate cells. Our data from directed-evolution across host species reveals distinct

maturation-dependent selective pressures between mammalian and insect cells, which sheds light on the divergent evolutionary relationship of DENVs between its host and vector.

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TRANSCRIPTOMIC PROFILES OF ACUTE DENGUE SPECIFIC CD8 T CELLS

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Accumulating evidence demonstrates that CD8 T cells contribute to protection from severe DENV disease. However, molecular programs associated with DENV-specific CD8 T cell in the acute phase of disease have not been defined. Here, we studied the transcriptomic profiles of human DENV-specific CD8 T cells isolated after stimulation with DENV epitopes from donors that have been admitted to the hospital with either the mild (dengue fever, DF) or severe form (dengue hemorrhagic fever, DHF) of dengue disease and compared the profiles of DENV-specific CD8 T cells from the same donor in convalescent phase of disease. Analyses of these data suggest that while both antigen stimulation and time from infection are associated with clear transcriptomic changes, disease severity is not. This study provides the first transcriptomic analysis of DENV-specific CD8+T cell responses in acute disease demonstrates that there are no qualitative differences as drivers of disease severity.

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IDENTIFICATION OF DISTINGUISHING CLINICAL FEATURES OF COVID-19, DENGUE, AND INFLUENZA AMONG ADULTS PRESENTING TO EMERGENCY DEPARTMENTS AND URGENT CARE CLINICS — PUERTO RICO, 2012-2021

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Dengue, influenza, and SARS-CoV-2 are pathogens of global concern and can present with similar signs and symptoms. Healthcare providers in low-resource settings often make a presumptive diagnosis in the absence of virologic testing. In Puerto Rico, an enhanced surveillance system operating from three emergency departments in southern Puerto Rico and San Juan and an urgent care clinic in Ponce has collected information on signs and symptoms, clinical laboratory results, and blood and nasopharyngeal specimens for pathogen identification from enrolled participants with fever since January 2012, and fever or symptoms of acute respiratory infection since March 2020. We analyzed data from 13,431 adults with polymerase chain reaction confirmed dengue (n=276), influenza A/B (n=2,064), or SARS-CoV-2 (n=303) enrolled from May 2012-January 2021 and assessed symptoms, laboratory results, and days post illness onset (DPO) until presentation. We adjusted for age, region, and DPO and calculated adjusted odds ratios (aOR) and 95% confidence intervals (CI) using logistic regression for clinical characteristics of participants with COVID-19 compared to dengue or influenza. Median DPO was shortest for influenza (2 days; interquartile range [IQR] 1-3), and dengue (3 days; IQR 2-4), and longest for COVID-19 (4 days; IQR 2-7), p<0.01. Cough (aOR 8.4; 95% CI 5.2-13.5) and shortness of breath (aOR 5.5; 95% CI 2.3-13.2) were associated with a diagnosis of COVID-19 compared to dengue. Facial flushing (aOR 20.6; 95% CI 9.8-43.5) and thrombocytopenia (aOR 24.4; 95% CI 13.3-45.0) were associated with dengue compared to COVID-19. Runny nose was associated with influenza compared to COVID-19 (aOR 8.3; 95% CI 5.8-12.1). Symptoms and laboratory findings at presentation can assist clinicians in assessing the likelihood of dengue or COVID-19. Although few symptoms clearly distinguish influenza from COVID-19, patient presentation >4 days after symptom onset might suggest COVID-19. These findings may assist healthcare providers in making time-sensitive decisions related to triage, isolation, and management while pursuing pathogen-specific testing.

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SEROPREVALENCE OF DENGUE AND ZIKA VIRUSES IN A COMMUNITY-BASED COHORT STUDY IN SOUTHERN PUERTO RICO

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Limited dengue virus (DENV) and Zika virus (ZIKV) seroprevalence estimates are available for Puerto Rico, which are needed to inform the potential use of DENV vaccines. A DENV vaccine has been approved but not yet implemented for children 9-16 years old living in endemic areas of the United States who had a previous DENV infection. The last DENV outbreak in Puerto Rico occurred in 2013, followed by a chikungunya outbreak in 2014 and a ZIKV outbreak in 2016. Communities Organized to the Prevent Arboviruses (COPA) is a cohort study initiated in 2018 in Ponce, Puerto Rico, to assess arboviral disease risk and provide a platform to evaluate interventions. We recruited participants aged 1-50 years from households in 38 study clusters. Participants are interviewed and provide a blood specimen annually. Specimens from the first year of COPA were tested for the 4 DENV serotypes and ZIKV using a plaque reduction neutralization assay (PRNT). Neutralizing antibody titers (PRNT50) positive at serum dilutions greater than 1:10 against DENV 1-4 or ZIKV were considered indicative of a previous infection. We used preliminary data to assess the seroprevalence of DENV and ZIKV by age. During 2018-2019, 4,090 participants were enrolled and serology results are available for 552 participants aged 1-16 years. Overall, 49% (n=271) were seropositive for DENV; seroprevalence was 21% (29/138) among children 1-8 years old and 58% (242/414) among children 9-16 years old. Overall, 37% (n=203) had neutralizing antibodies against more than one DENV serotype. A total of 36% were seropositive for ZIKV, including 20% among children 0-8 years old and 41% among children 9-16 years old. Less than half (46%) were negative for both flaviviruses. In this sample of children 1-16 years old in Puerto Rico, about half had evidence of a previous DENV infection and more than one-third with previous ZIKV infection, with increasing seroprevalence by age. These preliminary DENV seroprevalence data from southern Puerto Rico suggest moderate transmission intensity as defined by WHO.

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DENGUE VIRUS INFECTION LOWERED THE PERCENTAGE OF ATYPICAL MEMORY B CELLS IN KENYAN CHILDREN

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Expansion of the peripheral blood atypical memory B cell (aMBC) population has been reported in residents of areas with high endemic malaria transmission and has been associated with chronic malaria exposure. Much remains unknown regarding the prevalence and clinical

impact of this phenomenon, particularly in children. From April to May 2018, we surveyed the composition of peripheral B cells in a cross-section of children residing in Kwale County, Kenya, a coastal area with high endemic transmission of *P. falciparum* malaria. We recruited 33 subjects (median age 5.4 years, IQR 4.3-9.4) who had enrolled in a previous study during a febrile illness. Malaria testing by light microscopic exam of peripheral blood smears, and dengue virus RNA detection by RT-PCR, were performed at the time of the febrile illness. 6 subjects (18%) had dengue virus detected in blood, 13 subjects (39%) had malaria, and 3 of these subjects were infected with both malaria and dengue virus. Blood samples for the present study (U24 AI118648) were collected at a median of 18 weeks (IQR 14.1-50.3) later, and phenotypically characterized using mass cytometry. Overall, $CD27^-CD21^-$ aMBCs comprised 39.5 ±12% (mean ±SD) of peripheral $CD19^+$ B cells, while $CD27^+CD21^+$ classical MBCs made up 5.9 ±2.9%. $CD27^+CD21^-$ activated MBCs made up 6.3 ±3.3%, and $CD27^-CD21^+$ naïve B cells made up 48.3 ±12%. Correlation matrix did not reveal significant associations ($p<0.05$) between the magnitude of the aMBC fraction and age, sex, length of the interval between febrile illness and blood collection for phenotyping, or malaria parasitemia at the time of the previous febrile illness. Curiously, dengue viremia, but not coinfection with malaria and dengue, correlated with lower percentage of aMBCs (Pearson's coefficient -0.43, $p=0.013$). A linear regression model predicted that dengue virus viremia lowered the aMBC population by 13.1% ($p=0.014$). The unanticipated association between dengue virus infection and lower aMBC frequency highlights the need to consider the potential effects of multiple exposures to endemic or epidemic infections when investigating human immunity.

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SAMPLE-SPARING MULTIPLEX SEROLOGIC ASSAY TO DETERMINE FLAVIVIRUS INFECTION HISTORY

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Accurate serodiagnosis of past flavivirus infection is exceedingly difficult due to significant cross-reactivity in the antibody response across flaviviruses, especially in urban settings supporting the cocirculations of multiple flaviviruses. Here, we used a structure-based approach and identified flavivirus-specific epitopes on the envelope protein domain 3 (EDIII) to develop a microsphere bead-based multiplex assay for simultaneously measuring antigen-specific antibodies to multiple flaviviruses. We expressed and purified EDIII antigens from Zika virus, the four serotypes of the dengue viruses, and yellow fever virus in mammalian cells. The recombinant antigens were produced as fusion proteins with self-labeling protein (SLP) to attach a single biotin molecule at a distant site from EDIII. Using this strategy, we were able to control the orientation of EDIII antigens binding to the surface of avidin-coated microspheres. Using this approach, we were able to detect flavivirus-specific antibody concentrations as low as 100 pg/mL in our multiplex assay. When the multiplex assay was used to test a panel of convalescent sera from individuals with neutralizing antibody to just one flavivirus (primary immunity to DENV1-4, ZIKV or YFV), the binding signals were strongly correlated with neutralizing antibody profiles. These data suggest that our antigen panel and assay format is able to accurately establish primary infection history with late convalescent human samples with less than 1/50th of a microliter of sample. Studies are currently in progress to define the performance of the assay for defining exposure to two or more different flaviviruses or flavivirus vaccines.

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EVOLUTION AND GENOMIC EPIDEMIOLOGY OF DENGUE VIRUSIN RURAL AND URBAN COMMUNITIES IN KENYA

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Dengue is a mosquito-transmitted viral infection that poses a significant public health risk, particularly in developing countries. The causative agent, dengue virus (DENV), is an arbovirus whose geographic range covers 128 countries across tropical and subtropical regions of the world, making it the most common human mosquito-transmitted viral disease. Despite the increasing burden of dengue in Kenya, the influences of the virus' evolution, and their interplay with population and global-level factors have not been examined. The objective of this study is to assess the genetic structure of circulating dengue virus and evaluate the extent of gene flow across Kenya as well as migration from outside Kenya. Blood samples from a cohort of acutely ill patients recruited from 4 clinical sites in western and coastal Kenya between 2014 to 2018 were collected and preserved in *Trizol* reagent. Viral RNA was isolated and high sensitivity reverse transcriptase PCR was conducted to test for the presence of dengue virus. Genomic material obtained from DENV positive samples was enriched using a multiplex, tiled-amplicon enrichment protocol with custom primers and complete viral genomes for phylogenetic analysis were obtained via next generation sequencing. 7,563 total patients were recruited into the acutely ill cohort between 2014 and 2018. Of the 2,547 blood samples available for analysis in the study biobank to date, 256 samples were tested for viral presence, and 32 had dengue virus Ct values suitable for sequencing. Preliminary viral sequencing results suggest the circulation of Genotype-II lineage of DENV-2. The evolutionary characteristics of infectious agents like dengue virus drive disease dynamics, and modern advances in genomics and computation allow researchers to model these evolutionary and disease dynamics. Inferences from these models can inform disease preparedness measures in advance of outbreaks, as well as intervention measures during outbreaks, making them invaluable to policy makers.

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EVALUATING VIRAL GENOMICS AND DISEASE SEVERITY USING NANOPORE SEQUENCING: A PROOF-OF-CONCEPT ANALYSIS

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Dengue virus genetic variability provided the heterogeneity of circulating strains in the population, leading to the circulation of different genotypes and an increase in the number of existing strains that may have differences in the potential to cause epidemics. The high genetic variability and the evolution of the disease to lethal forms represent a severe threat to the population, and accurate identification of the genetic variability of the virus is essential to understand the processes of dispersion and virulence, as well as the identification of a variant gene responsible for an epidemic. In 2019, the Brazilian Southeast region reported 65.7% of all dengue cases identified in Brazil, and São José do Rio Preto, located in the northeast of São Paulo state, confirmed 32,727 cases, among 400,856 in the state. Here, we characterize, using a Nanopore portable genomic approach, the genetic diversity of DENV-2 detected in 22 serum samples obtained from patients with infection classified as dengue without warning signs and dengue with warning signs attended at Hospital

de Base of São José do Rio Preto in 2019, during a dengue outbreak. Demographic, epidemiological, and clinical data (symptoms and radiologic and laboratory observations) were also analyzed. The phylogenetic reconstruction and the clinical data showed that in São José do Rio Preto there are the circulation of two strains belonging to the American/Asian genotype of DENV-2: BR3 and BR4 (BR4A and BR4B), these results indicate that we cannot differentiate dengue samples without warning signs from dengue samples with warming signs, since the analyzed samples were grouped with other samples from different DENV-2 lineages. In addition, through this work, it can be proved that the sequencing platform Nanopore MinIon can be used as a methodology for monitoring viral strains during an epidemic.

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MODELLING THE HETEROGENEITY IN DENGUE TRANSMISSION INTENSITY IN SRI LANKA USING AGE-SPECIFIC CASE SURVEILLANCE DATA

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The first dengue cases in Sri Lanka were reported in Colombo, the capital of Sri Lanka in 1962. Since then, it has expanded to, and become established in, other districts following human movement patterns. Colombo has also had the highest incidence of dengue compared to other districts to date. We used dengue case data from the national surveillance system to assess the spatio-temporal heterogeneity of dengue transmission within Sri Lanka by estimating transmission intensity from age-specific incidence profiles in Colombo and other districts where dengue has appeared later; namely Batticaloa district in the east and Kurunegala district in the centre. A catalytic model was fitted to single year age-stratified case incidence data in these three districts for the years 2010-2015, and then seperately for 2000-2016 for Colombo. We estimated the force of infection (λ), the basic reproduction number R0, and probabilities of case detection for primary, secondary, and post-secondary infections and compared age specific reporting for above and below 15 years. Many studies have estimated λ using seroprevalence data, but such data is scarce in Sri Lanka to obtain district level estimates and estimate the spatial heterogeneity across the country. This method , using incidence data, will be expanded to obtain estimates for other districts using aggregate data. Understanding the spatial heterogeneity in dengue transmission intensity can help inform which populations and areas to target in a dengue vaccination strategy.

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COMPREHENSIVE MUTAGENESIS OF DENGUE VIRUS ENVELOPE PROTEINS TO MAP ANTIBODY EPITOPES AND IDENTIFY RESIDES ESSENTIAL FOR FUNCTION

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To characterize the immune response to dengue virus (DENV) infection, we have epitope mapped over 200 anti-DENV monoclonal antibodies (MAbs), using high-throughput, rapid screens of MAb binding to DENV prM/E comprehensive mutation libraries for all four DENV serotypes, 3,380 mutations in total. Each library of individual mutant expression plasmids was transfected into human cells to achieve native protein expression and folding, and immunoreactivity of MAbs to each individual prM/E variant was quantified by high-throughput flow cytometry. The epitopes obtained were both conformational (including quaternary) and non-conformational, were spread across prM and E domains I-III, and many have been correlated with their abilities to protect against DENV infection. A number of anti-DENV MAbs cross-reacted with ZIKV prM/E, predominantly within the fusion loop but also within Domain II of the E protein, identifying critical immunogenic residues shared by DENV and ZIKV. We have also produced DENV virions from all four DENV mutation libraries using a previously developed DENV reporter virus particle (RVP) system, allowing us to screen each individual DENV Env variant protein for DENV particle budding and infectivity. For DENV3, we identified residues whose mutation eliminated virus infectivity but did not impact E protein expression, antigenicity, virion assembly, or particle budding. We identified variants that showed increased DENV virion budding, up to 5-fold above wild-type, indicating the ability to engineer highly expressed, non-infectious DENV variants for use in vaccine design. From these experiments, we have identified neutralizing epitopes in DENV prM/E and specific sites that are critical for DENV infectivity, providing new targets and opportunities for vaccine development. To identify uncharacterized DENV cellular receptors we assayed wild-type DENV RVP infectivity in non-permissive cells expressing our membrane proteome array (MPA) of 6,000 unique human membrane proteins. This has identified candidate membrane proteins that enable DENV infectivity.

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DIFFERENTIALLY EXPRESSED PROTEOMICS PROFILING IN DENGUE REVEALS MULTIPLE UPREGULATION OF INFLAMMATORY PATHWAYS

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Severe dengue is characterized by detrimental inflammation with cytokine storm which jeopardizes endothelial lining integrity leading to multiple complications. The pathogenesis of dengue-associated inflammation and plasma leakage remains incompletely understood. Therefore, a deep understanding of inflammatory proteins regulation during dengue virus infection (DENV) is an important step towards a proper development of diagnostic, and therapeutic measures. We analyzed acute dengue samples (N=43), paired convalescent samples (N=35) and adult healthy volunteers (N=10) using a 386 targeted extensive inflammatory panel from Olink Proteomics AB to determine the differentially expressed proteins (DEPs) and the inflammatory pathways. Our results demonstrated that dengue patients exhibited 242 DEPs consisted of 203 upregulated and 39 downregulated proteins from paired acute vs convalescent samples (N=35). We further validated our finding by comparing acute dengue patients (N=43) versus adult healthy controls (N=10) and demonstrated 219 DEPs consisted of 204 upregulated and 15 downregulated proteins. Next, to further understand the biological relevance of these inflammatory proteins, we stratified the functions of these DEPs using STRING database and observed 7 major important pathways including interferon-gamma (IFNG) pathway, endothelial related markers, dendritic cell and macrophage activation, natural killer cell activation, B-cell activation, chemotaxis pathway and tumor-necrosis factor (TNF) pathway. Several differentially expressed proteins that were unreported previously such as TRIM21, ESM1, GPB2, LAMP3, SIGLEC1, MZB1, CRIM1, CDON and TNFSF11 suggesting the activation of both innate and adaptive antiviral responses. Taken together, our findings provided both validation and novel findings in term of inflammatory host-response against DENV infection.

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LONGITUDINAL ASSESSMENT OF DENGUE 1-SPECIFIC HUMORAL IMMUNE MEMORY IN HUMAN COHORTS FOLLOWING DENGUE-1 INFECTION IN BOTH ENDEMIC AND NON-ENDEMIC TRANSMISSION SETTINGS

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Dengue virus (DENV) is one of the most important vector-borne viral pathogen effecting humans worldwide. Following DENV infection, naïve host B-cells expand, produce, and secrete DENV-specific antibodies (Abs) that recognize viral antigens, specifically epitopes present on the surface of virions and secreted non-structural protein 1 (NS1). After viral clearance, some of these B-cells become antibody secreting long-lived plasma cells (LLPC), while others become memory B cells (MBCs) that remain in circulation, poised to expand on repeat infection. This MBC "founder" population is expected to play a critical role in broader DENV immunity that is established following second DENV infection. Here we quantify DENV-specific MBCs in humans following DENV-1 infection in a longitudinal manner. Using peripheral blood mononuclear cells (PBMCs) from DENV-1 immune donors from endemic (n=15) and non-endemic (n=24) cohorts with times post infection ranging from <1-43 years, we use two complimentary approaches to quantify the MBC population. PBMCs were stimulated *in vitro* to become antibody-secreting cells and the resulting antibodies assessed for DENV-specificity by ELISA. Additionally, a flow cytometry approach was utilized to quantify human MBCs (CD3-CD14-CD19+CD27+IgD-) that directly bind fluorescently labeled DENV.These DENV-specific MBCs can be single cell sorted, screened for functional properties of interest and cloned for monoclonal antibody (mAb) production or sequenced with full isotype resolution using the 10X genomics platform. Using these approaches, we identified DENV-whole virus and NS1-specific MBCs that remain in circulation decades after infection with varying frequencies in subjects from both endemic and non-endemic transmission settings.These experiments lay the foundation to characterize the DENV-specific MBC repertoire overtime and functionally assess the Abs they are programmed to secrete. The results of this project will provide insight into the MBC founder population following DENV infection in boosting and non-boosting environments.

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DEVELOPMENT OF THE INBIOS NORO DETECT™ A LATERAL FLOW IMMUNOASSAY (LFI) FOR MULTIPLEX DETECTION OF NOROVIRUS GENOGROUPS I, II AND IV

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Noroviruses (NoV) are the leading cause of acute viral gastroenteritis. In the US, an annual incidence of 19-21 million infections is reported with 2.3 million seeking medical care. NoV caused 58% of food-borne illnesses in the US at a cost of $2 billion annually. Children under 5 and the elderly are at high risk for severe symptoms and death. NoV spreads easily in contact clusters and about 2500 outbreaks are reported annually. Prevention and early detection are the only measures to control outbreaks. Though there are ten genogroups of NoV, outbreaks are attributed mostly to GI/ GII and GIV, rarely. The ideal detection tool will be useful in near patient settings and sensitive for early detection of suspected genogroups. The gold standard for the detection of NoV is RT-PCR of fecal samples. Though sensitive and specific, RT-PCR is not applicable in near patient settings or during outbreaks when rapid diagnosis is necessary. The only FDA cleared device is the RIDASCREEN®Norovirus EIA, which detects NoV antigens but it is not intended for diagnostic use. To fill the gap in NoV diagnostics, we developed the InBios Noro *Detect*™, a LFI cassette for rapid, sensitive, and specific detection of GI, GII and GIV NoVs from fecal swabs suitable in near patient settings. The LFI incorporates pan- NoV reactive antibodies raised using virus like particles (VLPs). In preliminary studies, the LFI could detect 5 GI, 8 GII and a GIV NoVLPs including known and suspected causes of outbreaks, at the level of ≤ 10 ng when spiked in fecal samples. The LFI is amenable for use with direct application of fecal swabs without the need for additional sample processing. This will allow safe handling of infected samples and improve utility for rapid detection. Direct application resulted in a LOD of 6-12 ng/mL on average for GI and GII VLPs compared to 10-15 ng/mL for pre- solubilized sample. Notably, for GII.4 which is associated with most outbreaks, direct sampling yielded a LOD of 3 ng/mL, by far the most sensitive detection of this important NoV. With planned development and FDA clearance, the InBios Noro *Detect*™ will be the first test to offer rapid, near patient diagnosis of NoV.

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EVIDENCE OF RIFT VALLEY FEVER VIRUS CIRCULATION IN CATTLE PARKS IN SLAUGHTERED OF MAN DISTRICT (CÔTE D'IVOIRE)

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Rift Valley Fever Virus is a viral zoonosis caused by an arbovirus transmitted by Culicidae. This arbovirus can seriously affect different species of domestic ruminants, causing severe outbreaks, economic losses and humans' mortality. This study was designed to investigate RVFV infection in cattle and mosquito in the slaughterhouse of Man city, western Côte d'Ivoire. Blood samples were collected from two cattle parks during dry seasons. Mosquito were collected during rainy and dry seasons. Mosquitos were trapped using six light traps, installed in general at 6:00 pm and visited the following morning at 6:00 am during four consecutive days. Molecular diagnosis of RVFV was carried out by real-time RT-PCR with specific primers; Forward: 3'-TGAAAATTCCTGAGACACATGG-5', Reverse: 3'-ACTTCCTTGCATCATCTGATG-5' and Probe: FAM -CAATGTAAGGGGCCTGTGTGGACTTGTG-TAMRA for L segment. Blood samples were analysed for RVF IgM and IgG detection using Enzyme-Linked immunosorbemt assay. Forty blood samples from animals and 634 adults mosquitoes belonging to five species: *Anopheles gambiae* (1.7%), Culex cinereus (6.6%), Culex quinquefasciatus (91%) Culex nebulosis (0.5%) and Mansonia sp. (0.2%) were collected. No IgG and IgM antibodies against RVFV were found in any of 40 sera. More than 70% of Culex quinquefasciatus regardless of site and season was observed. RVFV was successfully detected in 50% of Culex cinereus' pools and 6% of Culex quinquefasciatus' pools. In conclusion, this study diagnosed for the first time RVFV from mosquito in western country. It was found in Culex (Culex quinquefasciatus and Culex cinereus) which represents the most abundant genera in study site.

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ASSESSING THE EFFECTS OF MEASLES INFECTION ON CHILDHOOD INFECTIOUS DISEASE MORTALITY IN BRAZIL AND THE U.S.

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Despite an effective vaccine, measles infections remain a public health concern globally, causing hundreds of thousands of deaths each year, predominately in children. A danger of measles infection stems from its facilitation of other infections. By damaging preexisting immunological memory through the infection of immune memory B, T, and plasma

cells, the measles virus causes "immune amnesia" that increases one's susceptibility to other infections, potentially for years. This effect can lead to a positive association between measles and other infectious diseases in epidemiological records. Examining this hypothesis in distinct settings could help evaluate measles' contribution to childhood infectious disease burdens. Here, we analyzed the annual mortality of children age 1-9 in Brazil from 1980 to 1995 and children age 1-4 in the U.S. from 1921 to 1937. In Brazil, linear regressions between annual non-measles infectious disease mortality (e.g., respiratory and diarrheal diseases) and measles mortality showed a high correlation (R^2=0.944). This correlation could be confounded by a general decrease of all-cause mortality across years that reflects overall improvements in healthcare. To control that, we 1) modeled the time trends using change-point analyses and extracted residuals of mortality (i.e., "detrending"), and 2) calculated mortality differences between every two consecutive years (i.e., "difference"). With both methods, in Brazil, measles mortality remained significantly positively correlated with non-measles mortality (detrending R^2=0.523, difference R^2=0.700). Similar results were also observed separately in São Paulo and Rio de Janeiro. In the U.S., although original mortality data showed a low correlation (R^2=0.088), removing time trends revealed significant correlations between measles and non-measles mortality (detrending R^2=0.304, difference R^2=0.459). These results support that measles dynamics partially drove the variations of other infections, and emphasize the disproportionate benefits of measles vaccination in reducing childhood infectious disease mortality.

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EMERGING ROUTE OF SARS-COV-2 IN BANGLADESH: AN IN-SILICO APPROACH

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Severe acute respiratory syndrome coronavirus-2, the etiological factor behind coronavirus disease 2019 (CoVID-19), has afflicted most countries and territories of the globe - infecting millions followed by the extirpation of hundreds of thousands. Commensurate with what is perceived as the race of sequencing and annotating the coronavirus genome, a number of research institutions hailing from Bangladesh have delved deep into this matter - having sequenced a good few isolate of the virus collected from Bangladesh. Here, we performed comparative analysis of publicly available 1254 'Spike Protein' sequences derived from submitted Bangladeshi sequences. Phylogenetic analysis presumably revealed that the virus gained entrance to Bangladesh from multiple countries. The viruses isolated from Chattogram were closely related to strains from Saudi Arabia whereas those in Sylhet were similar to those from United Kingdom and South Africa. Missense mutations mostly had weak effects on the pathogenesis. Molecular docking analysis to evaluate the effect of the mutations on the interaction between the viral spike proteins and the human ACE2 receptor, though no significant difference was observed. This study provides some preliminary insights into the origin of Bangladeshi SARS-CoV-2 isolates, mutation spectrum and a possible emergence of Bangladeshi strain.

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DEVELOPMENT AND APPLICATION OF IMMUNO-FOCUS-BASED MICRO ASSAYS TO MEASURE INFECTIOUS BETACORONAVIRUS AND INFLUENZA A VIRUS UNDER BIOSAFETY LEVEL-2 CONDITIONS

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Infections with enveloped, RNA respiratory viruses, such as the current pandemic Betacoronavirus, severe acute respiratory syndrome coronavirus-2 (SARS-CoV2), and seasonal Influenza A viruses are significant global health threats. In the wake of this pandemic, numerous exposure-prevention and therapeutic technologies have been developed, such as metal ion-embedded surface materials, disinfectants, germicidal Ultraviolet light-emitting devices, antiviral drugs, and vaccines. However, the field is hampered by the lack of rapid, and reliable *in vitro* bioassays that can measure infectious virus particles in a high or semi-high throughput manner within safe biosafety level (BSL)-2 facilities. Such assays are essential for evaluating the efficacy of antiviral and infection prevention technologies. *In vitro* assays with SARS-CoV2 require high-containment BSL-3 facilities, are costly, unsafe, and limited to developed countries. To this end, we adapted simple, efficient, and robust cell line-based assay platforms, based on previously published assays for dengue viruses (Rodrigo, W. W. S. I. et al., Am. J. Trop. Med. Hyg. 2009 Jan; 80(1): 61-5. PMID: 19141841), employing a Betacoronavirus (HCoV-OC43) and Influenza A virus [A/PR/8/34 (H1N1)] that may be performed within BSL-2 facilities. These immuno-focus/spot-based quantitative assays were compared to classical plaque assay methodologies. We used human RD cells for HCoV-OC43 and MDCK cells for the Influenza A virus. We further optimized various cell- and virus-based assay parameters including the cell integrity, focus staining, various testing methodologies, signal-to-noise ratio, linearity, detection limits and robustness. Our new methodology is rapid (detection within 16-24 hours), may be miniaturized from 24-wells to 96-wells, and is easily adapted to various test articles, methodologies, cell lines and virus strains. These assays may be adopted for serum neutralization end-point determinations during preclinical or clinical stage vaccine evaluations, antiviral screening, diagnostics, and plasmid-based virus reverse genetics.

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HEPATITIS B VIRUS INFECTION IN NIGERIA: A SYSTEMATIC REVIEW AND META-ANALYSIS OF DATA PUBLISHED BETWEEN 2010 AND 2019

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HepatitisB virus infection results in substantial human morbidity and mortality worldwide. In Nigeria, the burden of this infection is significant, due to increase in complications of cirrhosis and liver cancer attributable to HBV.However, estimates of HBV prevalence vary widely in literature, and the use ofroutine pathology tests to enhance early detection in vulnerable populations remain largely unknown. We conducted a systematic review and meta-analysis.PubMed, Advanced Google Scholar and African Index Medicus were searched between January 2010 and December 2019. A random-effect model based on proportions wasused to estimate the population-based prevalence of HBV and identify pathologyassays and/or bio-markers that are used to support diagnosis and clinical management in the Nigerian population. Heterogeneity was analysed and sub-group analyses were performed. The final analyses included 47 studies with 21702 participants. We obtained a pooled prevalence of 9.5% in the Nigerian population. Aprevalence estimate above 7% in a population is classified as high. Sub-group analyses revealed the highest HBV prevalence in rural setting (10.7%). The North West region had the highest prevalence (12.1%) among Nigeria's six geopolitical zones/regions. Among routine pathology assays, Full Blood Counts(FBC) accounted for the highest prevalence estimate of 16.5%. The estimate of total variation between studies indicated substantial heterogeneity. These variations could be explained by setting, geographical region and pathology assay. We provide an up-to-date prevalence of HBV in Nigeria. Understanding true disease prevalence will provide critical data to optimise and assess theimpact of current prevention and control strategies in Nigeria, including disease surveillance and diagnoses, vaccination policies and management for those infected.

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HOST GENETIC FACTORS AND ROTAVIRUS INFECTIONS IN A VACCINATED BIRTH COHORT OF NICARAGUA

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Nicaragua introduced the rotavirus Rotateq vaccine into the national immunization program in 2006 and Rotarix in 2015. The vaccine coverage has been approximately 90%. Despite that, a proportion of vaccinated children remain susceptible to rotavirus acute gastroenteritis (AGE). Studies have shown that histo blood group antigens (HBGAs) influence Rotarix vaccine take, with non-secretor and/or Lewis "a" children having less seronconversion and less vaccine strain shedding after vaccination. However, whether this has an impact on vaccine effectiveness is unclear as these children are also less susceptible to wild type rotavirus of same genotype. To assess rotavirus AGE and the association with HBGAs in a vaccinated cohort, a total of 444 Nicaraguan children were weekly followed weekly from birth until 3 years to investigate the incidence of rotavirus AGE by RT-qPCR. Of 1,491 episodes of AGE reported from June 2017 until February 2021, 123 were rotavirus-positive (8%). The overall incidence of rotavirus AGE was 11.7/100 child-years, and this was higher in secretor compared to non-secretor children (13.1 vs. 3.3/100 child-years, *p=0.002*). After stratification by Lewis phenotype, the incidences of rotavirus-AGE was 12.9, 11.7 and 3.3/100 child-years for Lewis b, Lewis negative and Lewis a, respectively. No strong association between ABO blood group and rotavirus incidence was observed. Forty-six samples were successfully genotyped. Of these, 16 (35%) were highly homologous with the monovalent rotavirus vaccine strain G1P[8], followed by G8P[8] (11, 24%) and Equine-like G3P[8] (11, 24%). Interestingly, most genotyped wild-type rotavirus occurred after 6 months of age (21/27) as compared to prior to vaccination (4/27). This study suggests that secretor vaccinated children have higher risk of rotavirus P8, and warrants further studies on susceptibility to genotypes variants.

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ANTIBODY RESPONSE TO SARS-COV-2 INFECTION OVER SIX MONTHS AMONG NICARAGUAN OUTPATIENTS

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New information is emerging about SARS-CoV-2 epidemiology and immunity, but little of this 4 information comes from low- and middle-income countries or from patients receiving care in the 5 outpatient setting. The current study investigated the SARS-CoV-2 infection status and antibody 6 responses in 157 patients seeking care for a respiratory disease suggestive of COVID-19 in 7 private healthcare clinics during the first wave (June–October 2020) of infections in Nicaragua. 8 We examined nasal swabs for the presence of viral RNA via RT-PCR and longitudinally 9 collected sera for the changes in SARS-CoV-2 Spike antibody levels over six months. Among 10 patients with confirmed SARS-CoV-2 infections, we evaluated if clinical symptoms were 11 associated with age, hematological parameters and co-morbidities. The combination of PCR and 12 paired serology identified 60 (38%) of the 157 outpatients as acute COVID-19. While both PCR 13 and serology identified the majority (n = 38, 64%) of the acute infections, a notable number of 14 outpatients were identified by RT-qPCR (n = 13, 22%) or by serology (n = 9, 14%) only. During 15 the longitudinal study, we identified 6 new infections by serology among the 97 non-COVID-19 16 subjects. In conclusion, this study report that more than one third of the outpatients seeking care 17 for acute respiratory disease during the first epidemic wave of SARS-CoV-2 in Nicaragua had an 18 acute mild COVID-19 infection that correlate with prolonged humoral response. This immune 19 response to the RBD antigen, more likely IgG dependent, significantly increased between the 20 acute to convalescent and decay in the late convalescent but still remained seropositive.

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EVALUATION OF SARS-COV-2 CIRCULATION AND IMMUNOLOGICAL CHARACTERIZATION IN A HOSPITAL COHORT IN SAO JOSE DO RIO PRETO, SAO PAULO, BRAZIL

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In a global context COVID-19 is the biggest health threat in the present days. In just one year, SARS-CoV-2 has infected more than 126 million people around the world, reaching more than 2.7 million deaths. In Brazil, COVID-19 was responsible, until the beginning of April 2021, for almost 12.5 million cases and 300,000 deaths. Since epidemiological studies are used to reveal the extent of viral spread and to direct control measures to contain the disease, this project consists in the study of a hospital cohort in Sao Jose do Rio Preto, located in the northwest region of the state of Sao Paulo, Brazil, to evaluate the circulation of SARS-CoV-2 variants and the impacts in a clinical level during the pandemic period. The monitoring of patients at the *Hospital de Base* of the Faculty of Medicine of Sao Jose do Rio Preto (FAMERP) has been carried out. Nasopharyngeal and blood samples collected from these patients were used in the screening of SARS-CoV-2 variants and evaluation of immunological aspects, respectively. Until now, 183 nasopharyngeal samples, from November 2020 and March 2021, were screened by a Sanger sequencing approach that can detect mutations in the RBD region of SARS-CoV-2 spike protein. This method can effectively track most of the variants of concern (VOC) described until now, like B.1.1.7, B.1.351, P.2 and P.1. Our data shows that, in November of 2020, the distribution was composed by 42% of P.2 variant (derived from B.1.1.28) and 58% of other variants (that cannot be discriminated by this approach), while in December the scenario was similar (35% P.2 and 65% others). However, in January of 2021, P.1 variant was detected (5%) and P.2 was, at that moment, the most frequent in the population (68%). After this point, a clade replacement between P.1 and P.2 was observed, where in March 2021, P.1 was detected in more than 90% of the analyzed samples, while P.2 was present in less than 5%. To confirm these data, a subset of samples were also submitted to complete genome sequencing by NGS methods, and to observe the impact of these variants in a clinical level, we will analyze clinical data, pro-inflammatory cytokines and PBMCs will be analyzed from the subjects.

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HYPOGLYCEMIA IN EBOLAVIRUS DISEASE

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Ebolavirus disease (EVD) is associated with multisystem organ failure and high mortality. Severe hypoglycemia is common, life-threatening, and correctable in critically ill patients, but glucose monitoring may be limited in Ebola Treatment Units (ETUs) because of infectious risk and/or resource limitations. We conducted a retrospective review of consecutive patients admitted to two ETUs in Butembo and Katwa, Eastern DRC. Glucose measurements were done at the bedside using a handheld glucometer, or using the Piccolo xpress Chemistry Analyzer (Abaxis, Union City, CA). Between 30 March and 1 Oct 2019, 418 patients were admitted, of whom 376 (92%) had at least one glucose measurement, and were included in the analysis. The median age of patients was 30 years (IQR 20 to 45) and 56% were female. 6,360 glucose measurements (median of 11 per patient (IQR 4 to 22) over the hospital admission) were recorded. Severe (≤2.2mmol/L) and moderate (2.3 to 3.9mmol/L) hypoglycemia were recorded at least once during the ETU admission in 94 (25%) and 127 (34%) patients, respectively. 1,973 infusions of glucose-containing intravenous solutions were administered to 301 patients (78%), including 18 patients (4.7%) who received D5W, 122 (32%) D10W, 59 (15%) D50W boluses, and 254 (66%) Ringer's lactate with 5% dextrose. The total corrective dose of intravenous glucose administered over the admission was median (IQR) 100g (3.6-200), 150g (50-430), and 190g (51-430) in patients with no, moderate, and severe hypoglycemia, respectively (p<0.0001). Patients with hypoglycemia had higher peak viral load (p=0.0079), creatinine (p=0.00051), AST (p<0.0001), and ALT (p<0.0001). The case fatality rate was 48%. Compared to patients without hypoglycemia, severe hypoglycemia was associated with an increased hazard of death (HR 1.5 (95%CI 1.02-2.2), p=0.039) whereas moderate hyperglycemia was not (HR 1.1 (95%CI 0.73-1.6), p=0.71). In summary, hypoglycemia is common in patients with EVD, requires repeated correction with intravenous dextrose solutions, and is associated with organ injury and mortality.

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SARS-COV-2 VIRAL LOAD PEAKS PRIOR TO SYMPTOM ONSET: A SYSTEMATIC REVIEW AND INDIVIDUAL POOLED ANALYSIS OF CORONAVIRUS VIRAL LOAD FROM 66 STUDIES

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The kinetics of SARS-CoV-2 viral loads within individuals is important in understanding its transmission dynamics in populations. Viral load likely contributes to the transmission potential of the virus, but current findings around the temporal viral load dynamics and shedding, particularly the peak of transmission potential, remain inconsistent across studies. To characterize the temporal viral load dynamics and the duration of viral shedding of SARS-CoV-2 and other human coronaviruses, we conducted a systematic review and meta-analysis in which we collected and analyzed individual-patient data. We searched PubMed through June 8th, 2020 and collated unique individual-patient data from papers that reported novel data concerning quantitative viral load or the duration of viral shedding of coronaviruses. We analyzed viral load trajectories using a series of generalized additive models that treated paper ID as a random effect, and we analyzed the duration of viral shedding by fitting log-normal survival analytic models which accounted for interval censoring. We obtained individual-patient data from 66 papers, representing 1198 patients across 14 countries. We found that SARS-CoV-2 viral load peaks prior to symptom onset and remains elevated for up to three weeks, while MERS-CoV and SARS-CoV peak after onset. SARS-CoV-2, MERS-CoV, and SARS-CoV had median viral shedding durations of 4.8, 4.2, and 1.2 days after symptom onset, respectively. Disease severity, age, and specimen type all had an effect on viral load, but sex did not. Using a pooled analysis of the largest collection of individual-patient data on coronavirus viral load to date, we are the first to report that SARS-CoV-2 viral load peaks prior to - not at - symptom onset. Peak viral loads occurring before the onset of symptoms make syndromic surveillance and symptom-based isolation policies challenging. Detailed estimation of the trajectories of viral load and virus shedding can inform modeling of transmission dynamics and clinical interventions for coronavirus infections.

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OROPOUCHE VIRUS IN VITRO REPLICATION IS INHIBITED BY ACRIDONE

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Over the decades, arboviruses have been responsible for important public health issues, Oropouche orthobunyavirus (OROV) is an emerging arbovirus associated with a fever illness called Oropouche fever in the Amazon region of South and Central America, is neurotropic in animal models and can cause encephalitis and meningitis in humans. However, the pathogenic determinants associated with neurological involvement is not fully understood and there is still no antiviral therapy specified for the treatment of these infections. In this study, we report the inhibition of virus replication in Vero E6 cells by plaque reduction assay and immunofluorescence. Cells were infected with 25 PFU of OROV for 1h at 37°C, following overlay with MEM+1% carboxymethylcellulose (CMC) with or without four different acridones (FAC16, FAC20, FAC21, FAC22). Treated cells showed efficient inhibition of the viral replication at concentrations that presented minimal toxicity to the cells. The assays showed that the acridone FAC20 and FAC22 completely inhibited viral replication with no plaque formation, whereas FAC21 showed a 90% reduction, while FAC16 reduced approximately 75% plaque formation with no effect on cell viability. The virucidal activity was performed with the compounds to identify a possible mechanism of action. In this assay, FAC20 completely inhibited plaque formation, whereas FAC16 and FAC21 inhibited approximately 85% plaque formation and FAC22 showed a 90% reduction in plaque formation. Our results suggest that these are promising molecules to be studied with potential antiviral activity against the Oropouche virus.

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RAPID SPREAD OF SARS-COV-2 INFECTION IN THE COMMUNITY OF SOTUBA, A PERI-URBAN AREA OF BAMAKO, MALI, FROM JULY TO JANUARY 2021

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The Mali Ministry of Health, the Malaria Research and Training Center and the Laboratory of Malaria Immunology and Vaccinology conducted a longitudinal seroprevalence study in the peri-urban community of Sotuba, Bamako to understand local COVID-19 epidemiology. A total of 587 individuals ≥6 months of age provided blood samples tested for antibodies against SARS-CoV-2 spike and receptor binding domain proteins using an assay qualified for use in Mali. Samples were collected between July and

October 2020 and December 2020 and January 2021. No participant or a household member reported a previous COVID-19 diagnosis at enrollment. 89.9% (528/597) of participants completed study visits. The seropositivity rate was 13.1% (95% CI: 10.4-15.9) at Survey 1 and increased markedly over the study period to 44.9% (95% CI: 40.7-49.1) at Survey 2. After adjusting for community age-distribution and assay performance, the seroprevalence was 19.0% (95% CI: 14.2-23.8) at Survey 1 and 70.4% (95% CI: 56.8-84.1) at Survey 2. This represents a daily infection rate of almost 1% of the population every two days over the study period. The seropositivity rate increased with age group at both visits. At Survey 2 the adjusted seropositivity rate was 38.8% (95% CI: 28.9-48.7) in participants aged <10 years, 63.7% (95% CI: 49.5-77.9) aged 10-17 years, and 77.2% (95% CI: 61.5-92.9) in adults aged ≥18 years. Seropositivity rates did not differ by sex.These results suggest rapid community spread of SARS-CoV-2 in Bamako Keywords: SARS-CoV-2, COVID-19, Seroprevalence, Peri-urban, Mali, West Africa.

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AGGRESSIVE COMMUNITY AND WORKPLACE SPREAD OF SARS-COV-2 B.1.1.7 AND SEVERE ILLNESS ON A LARGE UNIVERSITY CAMPUS

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Active and passive surveillance among employees and students at one of the largest universities in the United States was stood up in August 2020 to closely monitor and mitigate SARS-CoV-2 infections on campus. In February 2021, the COVID-19 Surveillance team was alerted to the first local case of the B.1.1.7 SARS-CoV-2 variant infection, which was in a campus member. An outbreak investigation using existing health surveys and prospective interviews was immediately launched. The investigation revealed a cluster bridging community and campus transmission; 16/34 (45%) students and campus employees (median age 29 yrs (range 23-40); 44% male) tested positive for SARS-CoV-2 over a short, 9-day period. Campus cases initially emerged from community spread, where 6/18 (33%) party attendees (5 of whom were campus members) tested positive, some with unknown test results. Only 1 case underwent viral typing, done at a private laboratory through standard statewide recommended protocols, and 2 domestic animals linked to the cluster were the first to be reported with confirmed B.1.1.7 illness. Viral transmission was estimated to occur up to 18 hours before symptoms manifested in the source cases, with incubation period 3 (2-5) days. All cases exhibited symptoms, mainly (82%) mild, though at least 1 experienced long-term respiratory dysfunction and depleted oxygen saturation levels. Community infections crossed quickly into the campus environment, owed to pre-symptomatic spread, and burned rapidly through a workplace environment on campus during close contact in low airflow environments while single-masked and other rigorous public health measures in place. Since this investigation, a higher rate of B.1.1.7 infections has been observed in the campus setting compared to the community setting. Local health departments and academic institutions should partner for multifaceted surveillance and mitigation. Genomic surveillance, particularly tracking emergence and spread of SARS-CoV-2 mutants of potential concern should inform recommendations and guidelines and can be tailored to phenotypes, such as high transmissibility, of local viral strains.

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CHARACTERIZATION OF THE COVID-19 DISEASE IN CARTAGENA DE INDIAS: AN EFFICIENT PREVENTION AND CONTAINMENT STRATEGY

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The Severe acute respiratory syndrome type 2 (SARS-CoV-2) is the causal agent of the coronavirus disease 2019 (COVID-19) which was first described in Wuhan, Hubei province (China). SARS-CoV-2 causes a respiratory disease and is mainly transmitted from person to person through aerosols generated by coughing, breathing and talking. Because short-distance transmission in closed and non-ventilated spaces is the route that describes the majority of currently confirmed cases, therefore it is necessary and priority the characterization the disease epidemiology and identify areas with a high rate of cases. A data-driven response to COVID-19 allow to reduce the transmission through effective public health approaches that mitigate the impact on the population. The retrospective study of the epidemic allows the characterization of the dynamics and groups that were greatly affected by COVID-19. Hence, in the context of COVID-19 surveillance this lack of information translates into a lack of evidence that potentially could be used to improve public health intervention and policies especially in low-income countries with reduced availability of vaccines. UNIMOL was the first laboratory that worked in COVID-19 diagnosis in Cartagena, Colombia. Therefore, we have processed 107,127 samples to date, which corresponds to approximately 90% of the data reported for Cartagena-Colombia. We reconstructed the epidemic curve from April 2020 when the first COVID-19-case was reported to April 2021 using historical laboratory results. Furthermore, epidemic curves were constructed according sex, age groups, occupation and local residence to explore differences across the time. We tracked cases and deaths across the time using heat maps considering correction by population sizes. Finally, we evaluated the correlation between the daily positivity percentage and the epidemic curve in order to validate its use as a metric to measure COVID-19 dynamic. Our study illustrated the occurrence of COVID-19 cases considering demographic characteristics in Cartagena that was one of the Colombian cities with the lowest occurrence of cases and deaths related COVID-19.

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CD47 REGULATES PARASITE BURDEN AND PROMOTES PATHOGENESIS IN MURINE MALARIA MODELS

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CD47 is an anti-phagocytic (don't eat me) signal that inhibits programmed cell removal of self and loss of this molecule by aging erythrocytes is associated with increased susceptibility to clearance by macrophages. We have investigated the role of CD47 in malaria immunity and pathogenesis in murine malaria models. Previously, we demonstrated that absence of CD47 confers resistance to infection with *Plasmodium yoelii* 17XNL, a murine malaria that exhibits an aged-based preference for young erythrocytes. Next, we established that CD47 blockade with an anti-CD47 monoclonal antibody promotes survival and reduces the pathologic features of cerebral malaria during *Plasmodium berghei* ANKA (*Pb–A*) infection in C57BL/6 mice, a murine model of experimental cerebral malaria (ECM). To delineate the immunological mechanism of CD47

regulation of ECM pathogenesis, we present studies comparing *Pb–A* infection in wildtype (WT) versus CD47 KO C57BL/6 mice. In CD47 KO mice, absence of CD47 resulted in partial but highly significant ($p<0.001$, log-rank) resistance to ECM; following infection with *Pb–A* parasites, 22/23 (95.6%) WT mice developed ECM by day 10 post-infection. In contrast, only 13/23 (56.5%) of CD47 KO mice succumbed to malaria during the cerebral phase of infection. Through flow cytometric analysis of brain sequestered and splenic immune cell subsets and cytokine profiling of serum, we show that absence of CD47 during *Pb–A* malaria is associated with a significant reduction in brain sequestered $CD8^+$ T cells which are pathogenic during ECM and alteration of a small subset of cytokines. In addition, comparative analysis of WT versus CD47 KO brain tissue by immunohistology demarcates clear differences in pathologic features such as hypertrophied endothelial cells, presence of parasite hemozoin, macrophage infiltration, vascular lesions, and ring hemorrhages. A further understanding of the mechanism of anti-CD47 antibody-mediated protection from ECM may open avenues for novel immunologic-based treatment options against cerebral malaria in African children.

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QUANTIFICATION OF THE SPOROZOITE INOCULUM FROM MOSQUITOES WITH HIGH AND LOW SALIVARY GLAND LOADS

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Plasmodium parasites cycle between their mosquito and mammalian hosts, and both transmission stages are associated with severe bottlenecks in parasite numbers. Thus, the transmission stages have been recognized as an excellent target for interventions that could reduce the likelihood of successfully infecting a new host. We recently found that most infected mosquito bites did not result in a blood stage infection in the rodent malaria model, *Plasmodium yoelii* in Swiss Webster mice. Importantly, the likelihood of success was strongly correlated with the salivary gland sporozoite load of the infecting mosquito, with a steep increase in infection likelihood when mosquitoes had salivary gland loads greater than 10,000 sporozoites. In this study, we set out to determine how sporozoite inoculum size relates to salivary gland sporozoite load. Previous studies on this topic are conflicting, with the majority finding no association between gland load and inoculum size. We hypothesized that the high biological variability inherent in these data, combined with the use of mosquitoes whose sporozoite loads were biased towards either the low or the high end, could account for the lack of consensus on this topic. To test this, we manipulated culture conditions to generate mosquitoes with a range of salivary gland sporozoite loads that include the 4 to 5 log span observed in the field. We then allowed single *P. yoelii*-infected mosquitoes to probe on individual mouse ears and collected that ear tissue for quantification of injected sporozoites using a novel and highly sensitive qPCR assay that amplifies genomic DNA from a region of the mitochondrial genome. This work supports our previous finding that parasite loads in the mosquito impact the likelihood of a blood-stage infection. Further, we provide new information about the quantity of sporozoites transmitted during probing, allowing us to make connections between the salivary gland sporozoite load, the number of sporozoites deposited in the skin, and the potential for a subsequent blood stage infection.

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EXPERIMENTAL INFECTION OF PIGTAILED MACAQUES WITH PLASMODIUM KNOWLESI MALARIA PARASITES

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Plasmodium knowlesi (Pk) parasites can infect a range of non-human primate host species with varied disease outcomes. Pigtailed macaques (*Macaca nemestrina*) are one of several non-human primate reservoir hosts for Pk in Southeast Asia. As wild pigtailed macaques do not show signs of Pk blood stage carriage, infections in this host species are believed to be benign. Mild infections have also been reported for other related macaque species originating from areas where Pk is endemic. By contrast, experimental infection of non-natural hosts typically results in more severe disease outcomes. The best studied example is the rhesus macaque (*M. mulatta*), where Pk infection frequently causes severe disease that can be fatal if untreated. Severe disease has also been reported for other host species originating from outside the geographic range of Pk parasites. These observations suggest that pigtailed macaques have evolved traits that limit Pk disease severity, and because of this, may offer promise as a new potential model for chronic malaria. However, no previous studies have characterized Pk infection and disease in pigtailed macaques in a well-controlled experimental setting. Here we report results from a pilot Pk infection study in captive pigtailed macaques. Adult male macaques were infected intravenously with 2500 purified, cryopreserved Pk sporozoites (PkSPZ) and blood stage parasitemia was assessed daily beginning 6 days post-infection by Giemsa-stained thin smears and 18S rRNA RT-PCR. Infections were found to be highly reliable, with all animals becoming RT-PCR positive on days 6-7 and patent by Giemsa smear by day 11. Remarkably, blood stage parasite burdens plateaued at day 12-13 and remained just above the limit of detection by Giemsa smear until chloroquine treatment was initiated 2-3 weeks later. Despite this ongoing blood stage infection, animals did not display signs of disease, anemia or any other hematological abnormalities. These findings confirm that pigtailed macaques can be reliably infected with PkSPZ in an experimental setting and sustain low-level blood stage parasite burden for multiple weeks without apparent symptoms.

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DYNAMICS OF PLASMODIUM FALCIPARUM IN THE NON-HUMAN PRIMATE AOTUS NANCYMAAE DURING PREGNANCY

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Plasmodium falciparum sequesters in the placental intervillous space, causing placental malaria (PM) and leading to poor prognoses for both mother and child. VAR2CSA, the PfEMP1 variant surface protein, facilitates placental sequestration by binding to chondroitin sulfate A (CSA) expressed on syncytiotrophoblasts, causing severe pathologies. Women acquire antibodies against VAR2CSA over subsequent pregnancies, which is correlated with improved outcomes. We have established a novel model of PM in the non-human primate *Aotus nancymaae*, which is characterized by sequestration of late-stage *P. falciparum* CS2 parasites in the placenta that express VAR2CSA and bind to CSA. Further, these *Aotus* develop antibodies against VAR2CSA over subsequent pregnancies that inhibit the binding of maternal field isolates to CSA. We have previously reported the use of this model for VAR2CSA-based vaccine studies. Here we provide

extensive data on *P. falciparum* infections in the *Aotus* model from nearly one hundred infections during pregnancy. We have tested five different strains in the model, with varying outcomes. The more virulent strains quickly develop high parasitemias, increasing the risk of miscarriage, while others, including CS2, do not. We present data on the course of infection in these strains, as well as VAR2CSA-specific immune responses. We also assess the effect of gravidity and the length of infection on parasitemia. Finally, we analyze pregnancy outcomes and the birth weight of the neonate in the context of PM. Our advances with the *Aotus nancymaae* PM model demonstrate its value in understanding PM infection and immunity. They also provide a foundation for future vaccine studies, including heterologous challenge in pregnancy malaria.

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IN VITRO GROWTH PHENOTYPES OF SINGLE PARASITE LINEAGES CLONED FROM MULTICLONAL MALARIA ISOLATES

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Measurement of malaria parasite proliferation in cultured erythrocytes is critical for elucidating key determinants of phenotypes, including drug susceptibility, virulence, and fitness. Multiple parasite lineages with different proliferation rates or fitness may coexist within a clinical isolate, resulting in complex growth interactions and variations in phenotype. We measured proliferation rates of three *Plasmodium falciparum* isolates, including Cambodia IPC_3445 (MRA-1236), IPC_5202 (MRA-1240), IPC_6403 (MRA-1285), and parasite lineages previously cloned from each of these isolates by limiting dilution. Following synchronization, *in vitro* cultures were maintained over four consecutive asexual parasite life cycles, with parasite sampling at the end of every cycle to estimate parasitemia and growth rate. Cell cycle duration was measured by monitoring the development of 0 to 3 hr post-invasion rings from 36 to 52 hrs. In parallel with isolates and component parasite lineages, growth rates and cell cycle times were measured for parasite lines 3D7 (MRA-102) and DD2 (MRA-150) as controls. We observed significant differences in parasite growth rate (GR), fold-change in parasitemia (FC), and cell cycle duration between parasite isolates and clonal lineages that make up each isolate. For example, while parental isolate IPC_5202 exhibits similar a proliferation rate to one of its constituent lineages, MRA1240-hap1 (GR: 1.02±0.04 vs. 0.97±0.03; FC: 65±14 vs. 61±2), two other constituent lineages differ markedly, MRA1240-hap2 (GR: 0.95±0.02; FC: 51±3) and MRA-1240-hap3 (0.93±0.03; FC: 46±2). We observed that the most abundant parasite haplotype often dominates the growth phenotype, masking the effect of minority haplotypes akin to recent observations from drug susceptibility testing. Our results also show diminished proliferation of isolate MRA-1236 (GR: 0.90±0.05; FC: 38±7) relative to the component lineages MRA1236-hap1 (GR: 1.14±0.02; FC: 90±20) and MRA1236-hap2 (GR: 1.02±0.01; FC: 82±5) suggestive of competitive suppression. All parasite lines will be available through BEI Resources.

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PRO AND ANTI-INFLAMMATORY CYTOKINE RESPONSES DURING PREGNANCY ASSOCIATED MALARIA IN MANGALURU, INDIA

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Malarial infections during pregnancy results in placental parasite sequestration leading to maternal anemia, low birth weight, intrauterine growth retardation and infant mortality, especially during *Plasmodium falciparum* infections. However, the complications of *P. vivax* have not been well characterized. The Severe Malarial Anemia (SMA) not only results due to dyserythropoiesis, destruction of infected and non-infected red blood cells, but also due to imbalanced inflammatory cytokines response. In this study, we analyzed the burden of malarial anemia, inflammatory cytokine profiles and the associated complications during uncomplicated and severe malaria in the southwestern coastal city of Mangaluru, India. Among 105 pregnant individuals in the malarial infected group, 39% were non-anemic, 29.5% had mild anemia, whereas 23.8% and 7.6% had moderate and severe anemia, respectively. Among SMA patients, mixed infections were the highest (23.1%), followed by *P. falciparum* (10%) and *P.vivax* (8.3%). The hemoglobin levels decreased significantly in mixed (8.1 g/dL), *P. falciparum* (8.6 g/dL) and *P.vivax* (9.3 g/dL) infections. A highly significant increased pro-inflammatory response and decreased anti-inflammatory cytokine responses were observed with increase in anemic intensity, especially during *P.falciparum* infections ($P<0.002$). Further, positive correlation was observed between TNF-α and IL-6 levels in patients with *P.vivax* infections during moderate anemia ($r=0.594$, $p=0.025$), severe anemia ($r=0.005$, $p=0.0012$) and during *P. falciparum* with severe anemia ($r=0.005$, $p=0.0015$). The IL-6 and IL-10 levels showed a significant inverse relationship in patients with severe anemia during *P. vivax* ($r=-0.167$, $p=0.0014$) and *P. falciparum* ($r=-0.245$, $p=0.0012$) infections. Pregnant women with SMA suffered from complications such as jaundice (12.5%), hematuria (12.5%), acute renal failure (25%), hepatomegaly (25%) and metabolic acidosis (25%). In conclusion, although *P.vivax* is considered to be benign, our data indicates its role in SMA, cytokine imbalance and its associated complications as observed in *P. falciparum* infections.

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VAR GENES TRANSCRIPTION PROFILING OF PLASMODIUM FALCIPARUM FROM AN INDUCED-MALARIA EPISODE IN AOTUS MONKEYS

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Cytoadherence of *Plasmodium falciparum*-infected erythrocytes (iE) to host cells is a major pathogenic factor mediated by the highly polymorphic *P. falciparum* erythrocyte membrane protein-1 (PfEMP1). The *var* genes-encoded PfEMP1s bind diverse receptors, including CD36, intercellular adhesion molecule 1 (ICAM-1), and endothelial protein C receptor (EPCR), as well as mediate rosette formation with uninfected red blood cells. Whereas CD36 binding has been linked to mild malaria, parasite adhesion to ICAM-1, EPCR, and rosetting have been associated with severe malaria. *Aotus* monkeys represents an important *in vivo* model system in the study of *Plasmodium* biology. *Aotus* has been used to study *P. falciparum* sequestration and may provide information useful in understanding the parasite ligands involved in the bone marrow niche or anemia. However, little is known about the parasite-host interactions in the *Aotus* monkey model. In this study, we analyzed the transcriptional pattern of PfEMP1/*var* genes of FVO-strain *P. falciparum* isolated from an induced-malaria episode in *Aotus* monkeys. The animals were assessed daily for 27 days for parasitemia. Parasitized erythrocytes were cryo-conserved and RNA preparations were obtained at days 0, 5, 13, 20 and 22. We observed a reduction in Hb levels by day 22 when parasite density had reached ~6% (Hb: day 0, 20.8g/dL; day 22, 15.1 g/dL) at which point monkeys were treated with 20 mg/kg mefloquine. Optical microscope examinations of blood samples revealed a large proportion of *P. falciparum*-infected erythrocytes bound to uninfected erythrocytes, a cytoadhesive phenotype known as rosseting. Transcriptional profiling of *var* genes identified higher transcript levels of specific *var* genes associated to rosseting, and others unknown. Our study indicates that *Aotus* monkey model can be used to investigate the rosetting virulence trait and has the potential to study other PfEMP1 subsets for their interaction with specific vascular locations.

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FUNCTIONAL CHARACTERIZATION OF A PUTATIVE PHOSPHATIDYLETHANOLAMINE BINDING PROTEIN OF MALARIA PARASITE

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Raf Kinase Inhibitor Protein (RKIP), a member of the phosphatidylethanolamine binding protein (PEBP) family, is a classical regulator of MAPK signaling pathway in mammalian cells. Malaria parasite, *Plasmodium falciparum* RKIP (PfRKIP) is a putative PEBP that shares limited similarity in protein sequence with HsRKIP, the *Homo sapiens* homolog of PfRKIP. Interestingly, the classical components of MAPK pathway are not expressed in malaria parasite. The physiological function of RKIP in *P. falciparum* lifecycle remains unknown. Here we show that PfRKIP is a *bona fide* PEBP. We have generated a transgenic parasite line wherein the endogenous PfRKIP is tagged with a V5 epitope tag. We show that RKIP is expressed in the asexual stages of *P. falciparum* by probing the PfRKIP-V5 parasite lysate with anti-V5 antibodies in Western blot. The V5 tagged protein in PfRKIP-V5 parasite lysate was further verified by specific antibodies generated against recombinant PfRKIP protein. Mutant PfRKIP recombinant proteins differing from WT protein in amino acid residues in the PEBP binding domain have been generated to elucidate key molecular determinants that facilitate PfRKIP-lipid interaction. Importantly, lipid interaction studies with recombinant PfRKIP and HsRKIP show qualitative differences in lipid interaction profiles. Ongoing studies on complete and conditional knock-out of endogenous *pfrkip* will help in functional characterization of the gene in parasite biology. Moreover, qualitative differences in the lipid interaction profile of PfRKIP and HsRKIP suggests that specific inhibitors of PfRKIP may be designed that may be used as anti-malarial compounds for malaria control.

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HOST INFLAMMATION AND PARASITE VAR EXPRESSION ASSOCIATED WITH CEREBRAL MALARIA IN KENYAN CHILDREN

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Cerebral malaria primarily afflicts children and is the leading cause of malaria deaths. However, the pathogenesis of cerebral malaria is not fully understood. A unique feature of *Plasmodium falciparum* (Pf) is the ability to sequester in vascular tissues through binding of Pf Erythrocyte Membrane Protein 1 (*PfEMP1*) to specific endothelial receptors (e.g. ICAM-1, EPCR). *PfEMP1* is encoded by approximately 60 *var* genes with the extracellular head region of *PfEMP1* composed of cysteine-rich interdomain regions (CIDRs) and Duffy-binding-like (DBL) domains. Endothelial activation and possible damage by cytoadherent Pf contributes to immune mediated pathogenesis in addition to systemic inflammation characteristic of malaria. A better understanding of the molecular pathways involved in cerebral malaria may inform new adjunct therapies to reduce malaria mortality and morbidity. We aim to identify biomarkers of systemic inflammation and *PfEMP1* variants associated with cerebral malaria in Kenyan children. We enrolled 34 children presenting with cerebral malaria and 17 children presenting with uncomplicated malaria. We measured pre-treatment plasma levels of cytokines indicative of systemic inflammation (IFN-γ, TNFα, IL-6, IL-10, IL-21, IL-8, and IL-27) and biomarkers of endothelial cell activation (Eotaxin, PAI-1, MPO, neutrophil elastase, VEGF, and angiopoietin-2). Total Pf biomass was estimated by Pf Histidine Rich Protein 2 (HRP2), and *var* expression of specific head variants was assessed by qPCR. Preliminary analysis based on feature selection by random forest cross-validation indicates that 7 (of 45 tested) feature variables distinguish cerebral malaria from uncomplicated malaria with a 9.8% out-of-bag error rate. These 7 features in order of decreasing variable importance are plasminogen activator inhibitor 1, neutrophil elastase, IL-21, DBLα1.7 of DC13 (EPCR binding), vascular endothelial growth factor, DBLβ (ICAM-1 binding), and HRP2. These results suggest that both inflammation and sequestration lead to cerebral malaria.

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ASEXUAL PARASITE AND GAMETOCYTE DYNAMICS FOLLOWING 3-DAY OR 5-DAY ARTEMETHER-LUMEFANTRINE TREATMENT FOR PLASMODIUM FALCIPARUM MALARIA IN UGANDAN CHILDREN

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Parasite clearance after antimalarial treatment is often used to assess therapeutic response, drug efficacy, and in the context of artemisinin-based combination therapy, artemisinin resistance. Microscopy has been the gold standard for monitoring parasite clearance and recurrent infections. However, the development of ultrasensitive RNA and DNA-based detection can be used to study parasite dynamics at densities below the limit of detection of microscopy or traditional PCR. Recent studies with 14-day follow-up using newer diagnostic approaches have detected persistent submicroscopic parasitemia up to 14 days after artemether-lumefantrine (AL) treatment, though the significance of persistent detection remains unclear. We conducted a randomized PK/PD study of standard 3-day (6-dose) versus 5-day (10-dose) AL for the treatment of uncomplicated malaria in HIV-infected and HIV-uninfected children in Uganda. Ultrasensitive RT-PCR and parasite genotyping using amplicon deep sequencing is underway to determine the dynamics of clonal parasites in 72 HIV-infected and 234 HIV-uninfected children over 42-days of follow-up. We are further assessing gametocyte prevalence and clearance following treatment. Preliminary RT-PCR showed high correlation with microscopically determined parasite densities on day 0 in HIV-uninfected children (n = 202, R = 0.71, p < 2.2e-16), and was able to achieve a limit of detection of 5-50 parasites/mL. While 42% of children had microscopically detectable parasites on day 42, nearly 70% of children had 18S-RNA detectable at every time point throughout 42 days of follow-up. Median parasite density at day 0 was 3,224 parasites/μL, which decreased to a median of 0.1 parasites/μL by day 14 before increasing to 38 parasites/μL by day 42. Our final analysis, which will be presented, will determine whether improved AL exposure more effectively clears parasites after treatment or prevents earlier recurrence. Furthermore, we will use extended follow-up and sequencing data to further explore if persistent parasitemia extending out to day 14 and beyond potentially represents viable asexual parasites.

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CHRONIC AOTUS NANCYMAAE MODEL OF PLACENTAL MALARIA

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Placental malaria (PM) infection is characterized by sequestration of *Plasmodium falciparum* parasitized erythrocytes (PE) that bind to chondroitin sulfate A (CSA) in the intervillous spaces of the placenta. This interaction is mediated by VAR2CSA, a large variant antigen expressed on the PE surface. We previously reported a model for acute human PM using *P. falciparum* infection of pregnant *Aotus nancymaae* for up to a week, and observed sequestration of CSA-binding parasites within the intervillous spaces of the *Aotus* placenta and the acquisition of broadly neutralizing antibodies following multiple pregnancy malaria episodes, as seen in malaria-exposed pregnant women. However, this model does not replicate the intervillositis (IVS) seen in chronic human PM. Here, we

have optimized this model to allow infections of greater duration and collected placentas to examine parasite sequestration and IVS. Pregnancy was detected and dated by ultrasound and *Aotus* were inoculated with *P. falciparum* strain CS2 blood-stage parasites at ~11 (mid) or ~17 (late) weeks gestation, followed by Cesarean section 7, 12, 13, 14 or 17 days post-inoculation. To assess the impact of chronic infection on the protective antibody (Ab) response, antibodies against VAR2CSA were measured by ELISA and against PE surface antigen by flow cytometry. Functional Abs with the ability to block PE binding to CSA were measured by a binding inhibition assay. We found that animals with infections for 13 or more days developed IVS, while animals with 7 or 12-day infections did not. ELISA titers were also higher after longer infections versus the shorter 7-day infections. These results suggest that the chronic model with a 13+ day infection duration has a pathology similar to that of chronic human PM and may provide a valuable tool for testing future vaccines.

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AN ECOLOGICAL ANALYSIS EXPLORING THE IMPACT OF SEASONAL MALARIA CHEMOPREVENTION IN BURKINA FASO AND NIGERIA USING NATIONAL HOUSEHOLD SURVEYS (2010-2018)

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In 2012, the World Health Organization (WHO) issued a policy recommendation for the use of Seasonal malaria chemoprevention (SMC) in the Sahel and sub-Sahel regions of Africa as an effective malaria control strategy for children ages 3-59 months. Malaria Consortium has supported the SMC programme in Burkina Faso and Nigeria since 2014. Clinical trials have shown the intervention to prevent around 75% of clinical malaria episodes. Results of previous analyses to assess impact of the intervention under routing programmatic conditions have varied markedly between countries. This can be due to methods of measurement and/or quality of data. A previous analysis using population based household surveys (Demographic Health Surveys from 2010; Malaria Indicator Surveys from 2014 and 2017) in Burkina Faso showed strong evidence of a decrease in odds of malaria and anemia in districts with SMC. The analysis overlaid the household data with SMC programme data, geographic data from the Burkina Faso Mapping Institute, and rainfall data from the Burkina Faso Meteorological Institute. Adjustments were made to this model to investigate the decaying effect of impact of SMC as well as any effect of treatment seeking behaviour. The analysis was replicated using household survey data from Nigeria in 2010, 2015, and 2018. A mixed effects logistic regression with random intercepts for district was conducted to estimate the level of impact of SMC during the implementation period and up to 5 months after. A cubic spline was fitted to adjust for seasonal factors other than rainfall. Preliminary results indicate there is strong evidence that after controlling for a variety of factors that there is a decrease in odds of malaria confirmed by rapid diagnostic test in districts with SMC in Burkina Faso (OR 0.26, 95% CI: 0.20-0.33, P<0.001) and Nigeria (OR 0.39, 95% CI: 0.29-0.51, p<0.001). Further adjustments are being made to the model and results will be presented. The results from this analysis will be compared to data from routine health information systems in both countries.

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PROFILING TRENDS IN SUSCEPTIBILITY TO FRONTLINE ANTIMALARIALS IN KENYA BETWEEN 2008-2021 THROUGH SUSTAINED REGIONAL SURVEILLANCE

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Antimalarial drug resistance has been a hindrance to achieving a malaria free world since the first case of chloroquine resistance was recorded in South Eastern Asia (SEA) in 1950s. Consequently, evaluating the association of the scarce frontline antimalarials sensitivity with molecular markers is a top priority for efficient monitoring of drug failure and guiding treatment policy in the country. A total of 255 clinical *P. falciparum* isolates collected from subjects presenting with uncomplicated malaria at six hospital sites in Kenya between 2008 and 2021 were tested for immediate *ex-vivo* and *in-vitro* susceptibility to selected antimalarials using SYBR Green I fluorescence-based assay. Drugs tested include; piperaquine (PPQ), dihydroartemisinin (DHA), lumefantrine (LM), artemether (ART) and chloroquine (CQ). W2 and D6 reference clones were tested in parallel as the assay and test controls. Additionally, each isolate was assessed for drug resistance markers in *Pfcrt, Pfmdr1, Pfdhps, Pfdhfr, Pfpm2/3, Pfexo* and *Pfk13* genes using qPCR and mass ARRAY platform. Lumefantrine median IC_{50}s inclined significantly between 2008; 11.0 nM, n=54 (IQR 2.7 nM to 26.9 nM) and 2021; 30.55 nM, n=51 (IQR 3.2 nM to 47.7 nM) (p<0.05). A steady statistically significant decline in median IC_{50} for CQ was observed between 2008; 7.9 nM, n=36 (IQR 3.9 nM to 15.8 nM) and 2021; 4.6 nM, n=62 (IQR 3.1 nM to 8.2 nM) (p<0.05). However, the PPQ susceptibility during the study timeline was stable, median IC50 of 16.8 nM, n=34 (IQR 10.8 nM to 23.1 nM) in 2008 and 14.1 nM, n=42 (IQR 6.4 nM to 21.3 nM) in 2021. There were varying trends in response of naturally acquired infection clinical isolates between 2008 and 2021 as depicted by increasing sensitivity to CQ and impaired response to LM. These findings underscore the paucity of the drug resistance profile trends and warrant continued surveillance in order to support effective treatment.

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MFR4 MEDIATES DRUG RESISTANCE TO THE ANTIMALARIAL HALOFUGINONE VIA THE ADAPTIVE PROLINE RESPONSE IN PLASMODIUM FALCIPARUM

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Halofuginone (HFG) is a potent antimalarial drug that targets the *Plasmodium falciparum* cytoplasmicprolyl tRNA synthetase (*Pfc*PRS). Previously, we had shown that parasites selected for an extended timewith increasing concentrations of HFG develop high-level resistance conferred by mutations in the *pfcprs*target (mutant EC50 = 180nM, Dd2 wildtype (WT) EC50 = 0.7nM). In contrast, short-term exposure to HFGyielded parasites with moderate resistance yet no mutation in the *pfcprs* locus (EC50 = 18-24nM). Previousstudies showed that exposure to HFG triggers

a novel mechanism of drug tolerance, termed the AdaptiveProline Response (APR), wherein intracellular proline levels increase twenty-fold. Whole genomesequencing of these parasites identified mutations in the major facilitator superfamily related protein 4 gene(*pfmfr4*, PF3D7_0914700) resulting in predicted frameshift and nonsense mutations. This led to thehypothesis that APR mediated HFG-resistance was due to a loss of *Pf*MFR4 function. To test thishypothesis, we used CRISPR/Cas9 to disrupt the locus and generate MFR4 knockout parasites (Dd2-ΔMFR4). Interestingly, Dd2-ΔMFR4 parasites showed a moderate HFG-resistance phenotype similar tothat observed in APR parasites (Dd2 WT EC50 = 0.7nM, Dd2-ΔMFR4 EC50 = 14nM). However, theseparasites were not previously exposed to HFG. To further understand the role of MFR4 in modulatingintracellular proline levels, we performed metabolomic studies in the Dd2-ΔMFR4 parasites. Metaboliteprofiling of Dd2-ΔMFR4 parasites showed an over 20-fold increase in intracellular proline levels relativeto Dd2 WT parasites. These findings reveal a new drug resistance mechanism in *P. falciparum* thatmodulates metabolic homeostasis, which has implications for both parasite biology and antimalarial drugdevelopment.

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UPDATE ON MULTIDRUG RESISTANT PLASMODIUM FALCIPARUM MALARIA: TREATMENT OPTIONS IN THE CONTEXT OF CHANGING DRUG SUSCEPTIBILITY PROFILE IN CAMBODIA

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Multi-drug resistant (MDR) strains of *Plasmodium falciparum* (*P.f*) continue to pose a significant threat to malaria elimination efforts in the Greater Mekong Subregion (GMS). The introduction of new drug combinations targeting multi-drug resistant strains of *P.f* was proposed for areas where conventional artemisinin-based combination treatments (ACTs) are losing their effectiveness. To guide optimal treatment options, we investigated anti-malarial drug susceptibilities of 638 *P.f.* samples collected from 2015 to 2020 including both military and civilian populations. In Northern Cambodia, we observed a rise in IC90 values from 12.3 nM to 64 nM (p = <0.001) for mefloquine (a component of first line ACT treatment). During the same period, there was corresponding decrease in piperaquine IC90s, from 987 nM to 32.8 nM in Northern Cambodia. A similar decrease in mefloquine drug susceptibility was observed in Eastern Cambodia but without significant changes in PIP susceptibility (IC90s <100 nM). There were no 'triple mutant' isolates with K13 mutations, amplified *pf*mdr1 and increased PM2 copy number despite concerns about the emergence of these strains. Samples from Northern Cambodia demonstrated a pattern of drug susceptibility similar to isolates from the neighboring Thai province. Utilizing whole genome sequencing, the relatedness of these parasites is being explored. The samples were also tested against 13 additional antimalarials and found to be highly susceptible to tafenoquine (IC50 138 nM) and pyronaridine (IC50 6.4), the 2 latest antimalarials approved for treatment. Corresponding treatment outcome data are available for 92 isolates collected as part of multi-center clinical trial (APACT study) in which volunteers received treatment with single-dose primaquine and one of the following: artesunate-pyronaridine, artesunate-pyronaridine plus atovaquone-proguanil, or artesunate-mefloquine plus atovaquone-proguanil. The treatment outcomes for these combinations and the effect of the partner drugs on the metabolism of primaquine will also be presented to help guide optimal drug combinations in the GMS.

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HOST INFLAMMATORY, MATRIX METALOPROTEINASE AND CELL DEATH RESPONSES TO ACUTE MALARIA TREATED WITH MEFLOQUINE AND THE ROLE OF CURCUMIN SUPPLEMENTATION

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Pro-inflammatory signaling cascade, cell death and activation of matrix metalloproteinases are sepsis events in *Plasmodium* infection. It is however, not known if treatment with mefloquine (MF), an antimalarial drug with curcumin (CM) supplementation will modulate this effect. Malaria was induced using chloroquine sensitive (NK 65, study 1), and chloroquine resistant (ANKA, study 2) strains of *Plasmodium berghei* in thirty male Swiss mice (n=5). Following confirmation of parasitemia, animals received 10mL/kg distilled water (infected control), MF (10mg/kg), MF and CM (25mg/kg), MF and CM (50mg/kg), CM (25mg/kg) and CM (50mg/kg). Five mice (not infected) were used as control. After treatment, the animals were terminated via cervical dislocation. Liver mitochondria were isolated via differential centrifugation. Serum Interleukins 1 beta and six (IL-1β and IL-6), Tumour Necrosis Factor alpha (TNFα), C-reactive protein (CRP), Immunoglobulins G and M (IgG and IgM), caspases 3 and 9 (C3 and C9) were assayed using ELISA techniques, mitochondrial membrane permeability transition (mPT), F_0F_1 ATPase and lipid peroxidation (mLPO) were determined spectrophotometrically. Matrix metalloproteinases 2 (MMP2) and 9 (MMP9) expressions were determined eletrophoretically. CM supplementation (25mg/kg) significantly decreased serum IL-6, TNFα and CRP compared with MF only. In the resistant model, CM prevented mPT pore opening, significantly decreased F_0F_1 ATPase activity and mLPO. MF activated caspase 3 while supplementation with CM significantly decreased this effect. CM supplementation increased immunoglobulin levels. MMP2 and MMP9 were selectively expressed in the susceptible groups only. Malarial treatment elicits different cell death response mitigated by curcumin supplementation.

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MOLECULAR MARKERS OF ANTIMALARIAL DRUG RESISTANCE IN UGANDAN CHILDREN WITH SEVERE MALARIA AND RISK OF READMISSION OR REPEAT ILLNESS

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We investigated the presence of anti-malarial drug resistance markers in *P. falciparum* samples from a cohort of Ugandan children with severe malaria (cerebral malaria or severe malarial anemia) and asymptomatic community children (CC) enrolled from 2008-2013. 159 children with severe malaria (SM) and 49 CC with asymptomatic parasitemia were tested and followed up for 2 years. Children with SM were treated with parenteral quinine or artemether before 2011, and after 2011 with either of these drugs or artesunate, each followed by oral quinine or artemether-lumefantrine. From enrollment filter paper blood spots, we evaluated polymorphisms in genetic markers of resistance associated with response to chloroquine *(pfcrt*); chloroquine, quinine, and lumefantrine (*pfmdr1)*; or artemisinin

(*pfkelch13*). In children with SM, prevalence of the mutant *pfcrt* K76T allele was 96%, indicating widespread chloroquine resistance. For *pfmdr1,* prevalence of mutant or mixed alleles for N86Y, Y184F, and D1246Y, were 71%, 50%, and 75%, respectively. Parasite genotypes were similar in asymptomatic CC. After hospital discharge, children with SM infected with the mutant *pfmdr1* 1246Y allele had an increased risk of readmission (age adjusted hazard ratio 2.44 [95% CI, 1.10, 5.41], p=0.03) and a higher incidence rate ratio (IRR) of outpatient visits for illness (age adjusted IRR 1.85 [95% CI, 1.00, 3.44], p=0.05) than children infected with only the WT D1246 allele. For *pfkelch13*, prevalence of mutations upstream of the propeller domain in children with SM and asymptomatic CC was 56% and 51% respectively. We identified two infections with A578S and one with S522C mutation in the propeller domain of *Pfkelch13*. The study data support the need for continued surveillance of markers associated with resistance to antimalarial drugs, and highlight the possibility of drug resistance leading to an increased risk of repeat infections or readmission for SM.

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EMERGENCE AND EXPANSION OF PLASMODIUM FALCIPARUM WITH PFK13 POLYMORPHISMS POTENTIALLY ASSOCIATED WITH ARTEMISININ RESISTANCE IN UGANDA

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For over a decade, artemisinin-based combination therapies (ACTs) have been the mainstay of treatment of uncomplicated malaria in Africa. Resistance to artemisinins, mediated by several polymorphisms in the propeller domain of the *P. falciparum* Kelch protein (PfK13), is widespread in southeast Asia. Recent reports suggest that previously rare PfK13 polymorphisms associated with artemisinin resistance are now emerging in parts of Africa. We are continuing surveillance for key resistance associated polymorphisms at 16 health facilities across Uganda, including dideoxy and molecular inversion probe deep sequencing of *pfk13* and analysis of 8 microsatellites flanking the *pfk13* gene. Preliminary results for samples collected in 2020 are reported here. Out of 952 samples with available data from dideoxy sequencing, we observed 22 PfK13 polymorphisms, 4 with prevalences above 5% at individual sites. Two polymorphisms, 469Y and 675V, which are candidate markers for artemisinin resistance based on results in southeast Asia, had prevalences of 4.2-30.5% and 1.2-12.5%, respectively, with results similar to those seen in samples from 2018-19, but with spread to additional sites. Two other polymorphisms, 469F and 442L, were seen at sites in south-western Uganda at prevalences up to 29.7% and 7.1%, respectively. Considering older samples, analysis of flanking microsatellites in samples from 2018-19 suggested local emergence and clonal expansion of mutant isolates. Next generation deep sequencing of 2020 isolates, a more sensitive assay for minority variants, and assessment of prevalences of other drug resistance-associated polymorphisms, is underway. Our results provide evidence of emergence of *P. falciparum* with genetic polymorphisms that may diminish the efficacy of ACTs in Africa.

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CARDIAC SAFETY MONITORING OF ARTEMISININ-BASED COMBINATION THERAPY IN HIV-INFECTED AND HIV-UNINFECTED UGANDAN CHILDREN

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Artemisinin-based combination therapy is recommended as first-line treatment for uncomplicated malaria in sub-Saharan Africa where there is high HIV and malaria coinfection. The impact of antiretroviral (ART) drug interactions on antimalarial exposure may lead to cardiotoxicity via corrected QT (QTc) prolongation, raising the risk of fatal arrhythmias. In the context of two clinical trials, we assessed the cardiac safety of artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP) in children with and without HIV. The first trial, EXALT (Extended Duration Artemether-Lumefantrine Treatment for Malaria in Children), is a randomized, open-label, prospective pharmacokinetic (PK) and pharmacodynamic (PD) study of extended duration AL conducted in Busia, Uganda. EXALT enrolled HIV-infected children on an efavirenz-based ART and HIV-uninfected children, both with uncomplicated malaria. Participants were randomized to 3-day or 5-day treatment groups. The second trial, DPART (DP in the context of antiretroviral therapy), is an open label, prospective PK study in Kampala and Busia. The trial is enrolling HIV-infected children on lopinavir/ritonavir-, efavirenz-, or dolutegravir- based ART regimens, compared with HIV-uninfected children, all receiving a single or 3-day course of DP. ECG monitoring for QTc prolongation is ongoing at baseline, pre/post-last dose, 24-hours post last dose, and at the end of follow-up. To date, we have completed enrollment and follow-up with ECG analysis in EXALT on 42 HIV-infected children, and 107 HIV-uninfected children. For DPART, we have enrolled 30 children on efavirenz-based ART, 20 on lopinavir-ritonavir, and 30 on dolutegravir-based ART, with other groups being enrolled currently. Preliminary data suggests no significant increase in QTcF between the 5-day and 3-day AL treatment groups, no significant prolongation of QTcF in HIV-infected children receiving DP in the context of efavirenz-based ART, or single-dose lopinavir-ritonavir. Results for all groups will be presented, providing a comprehensive dataset on the use of DP with several HIV regimens, and extended duration AL.

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A SECONDARY LOCUS ON CHROMOSOME 6 INTERACTS WITH PFCRT TO CONFER HIGH LEVEL CHLOROQUINE RESISTANCE

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Is chloroquine (CQ) resistance simple or complex? Mutations in *pfcrt* have a large effect on CQ resistance. Additional transporters, such as *pfmdr*, have been shown to influence levels of CQ resistance in some genetic backgrounds. Utilizing human liver-chimeric mice infused with human red blood cells (the FRG huHep/huRBC mouse), we can efficiently generate crosses using recent patient-derived parasite isolates to explore how loci interact and contribute to drug resistance phenotypes. We utilize a cross between lab-adapted NF54 (CQ-susceptible) and NHP4026, a recent CQ-resistant Southeast Asian isolate to search for additional loci. Initially, we used a bulk segregant approach, pressuring uncloned progeny populations with CQ followed by bulk sequencing. This reveals a strong QTL on Chr 7 (coinciding with pfcrt) as expected, but we also see a QTL on Chr 6. The Chr 7 NHP4026 *pfcrt* allele is in strong LD with the Chr 6 QTL allele from NHP4026. To uncover the specific genetic architecture of these QTL, we chose 16 cloned progeny bearing distinct combinations of parental alleles at the Chr 6 and 7 QTL and measured CQ IC_{50}. These QTL interact epistatically to control variation in CQ IC_{50} of individual progeny: the *pfcrt* locus controls 60% of the variation in CQ IC_{50} and the Chr 6 QTL modulates resistance controlling 16% of the variation primarily in resistant progeny. The Chr 6 QTL contains an outstanding candidate locus - the amino acid transporter *pfaat1* - which shows strong signals of selection and strong interchromosomal LD with *pfcrt* in natural parasite populations. This locus was identified in genome wide association analyses examining CQ-resistance, and underlies CQ-resistance in the *P. chabaudi*. CRISPR/Cas9 gene editing studies are in progress to examine the functional role of two SNPs that differentiate NF54 and NHP4026. These results (i) demonstrate the utility of identifying QTL using rapid bulk segregant analyses and determining how these QTL interact to determine phenotypes using a subset of individual progeny and (ii) reveal that CQ-resistance is oligogenic involving at least three loci - *pfcrt*, *pfmdr1* and the chr 6 locus identified here.

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EFFECTIVENESS OF RECTAL ARTESUNATE IMPLEMENTED AS PRE-REFERRAL TREATMENT FOR SEVERE MALARIA IN CHILDREN IN THREE AFRICAN COUNTRIES

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Parenteral artesunate followed by an oral artemisinin-based combination therapy (ACT) is the recommended treatment for severe malaria. In remote settings, where most malaria deaths occur, parenteral treatment is rarely available. In a clinical trial, pre-referral treatment with rectal artesunate (RAS) reduced case fatality in children with signs of severe malaria who delayed reaching a referral facility. Regardless of potential, the real-world impact of RAS is expected to be lower due to health system constraints. In a multi-country observational study, we assessed the health status and treatment seeking of children below 5 years with suspected severe malaria 28 days after they presented to a community health worker or primary health centre ('community enrolments'), or to a referral facility. Enrolment started 10 months before and continued 15 months after the roll-out of RAS in the Democratic Republic of the Congo (DRC), Nigeria and Uganda. We included 11,952 children in the analysis (6,216 community enrolments). The case fatality in the pooled sample was 3.9% (95% CI 3.5, 4.4) in community enrolments and 2.6% (95% CI 2.3, 3.1) in referral facility enrolments. An additional 13.3% and 12.3%, respectively, were alive but sick after 28 days. RAS use reduced the risk of dying in Uganda (adjusted risk ratio [aRR] = 0.27, 95% CI 0.09, 0.77) but only the risk of illness in DR Congo and Uganda. Completing referral did not consistently reduce the risk of dying, but administration of an ACT in Uganda (aRR = 0.23; 95% CI 0.09, 0.58) or injectable artesunate plus ACT in DRC (aRR = 0.40; 95% CI 0.23, 0.68) were protective. Oral ACT was not consistently administered following RAS and injectable artesunate treatment. This is the largest community-based study of severe febrile illnesses in highly malaria-endemic areas. A functioning continuum of care is essential for RAS to have a noticeable impact. With fluctuations in RAS use, health systems constraints and secular trends, the roll-out of RAS did not reduce case fatality in children with suspected severe malaria in the project countries, despite some evidence of a positive health effect of RAS at an individual level.

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DISSECTING THE RELATIVE CONTRIBUTIONS OF PFCRT AND PLASMEPSIN II/III COPY NUMBER ON PIPERAQUINE SENSITIVITY USING A GENETIC CROSS

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Piperaquine (PPQ) is widely used in combination with dihydroartemisinin (ACT) as a first-line treatment of malaria infection. Resistance to this ACT has emerged in Cambodia and is threatening to spread to other malaria endemic regions. Multiple drivers of PPQ resistance have been proposed, including mutations in the *Plasmodium falciparum* chloroquine resistance transporter (PfCRT) and increased copies of plasmepsin II/III (PM). Genetic crosses offer a unique opportunity to quantify the relative contributions of different mutations to phenotypes and have the distinct advantage of assaying the effects of naturally evolved genetic variation in genome-wide context. We crossed KH004-2-019, an isolate from a rapidly spreading multi-drug resistant Southeast Asian lineage to a generally drug susceptible Malawian isolate, Mal31. Mal31 harbors a wild-type PfCRT and one copy of PM, while KH004 has a chloroquine-resistant PfCRT and a mean of 4 copies of PM. Neither parent carried known PPQ resistance SNPs in PfCRT. We isolated 103 unique progeny from this cross and focused on a targeted set of progeny representing all possible combinations of PfCRT and PM alleles for their contribution to the PPQ Survival Assay (PSA) phenotype, standard dose-concentration effect curves, and head-to-head fitness competitions. Importantly, progeny inheriting the KH004 PM inherited a range of copies (1-5), giving us a unique opportunity to directly assess the contribution of increasing copies on the phenotypes. We find that progeny inheriting both PfCRT and PM alleles from KH004 are PPQ resistant for both drug response phenotypes, and the inheritance of KH004 PfCRT is required for resistance. Isogenic progeny that differed only in PM copy number showed that increased copies generate higher resistance, a trend not observed in progeny inheriting Mal31 PfCRT. Ongoing studies will determine whether PfCRT can, by itself, generate PPQ-R in this cross as well as the effect of copy number on competitive outcomes among these progeny clones. These data indicate that the relationship between PPQ-resistance and PM copy number is not simple and may be conditional on PfCRT genotype.

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MARKED DECREASE IN ANTIBIOTIC USAGE FOLLOWING INTENSIVE MALARIA CONTROL IN A COHORT OF UGANDAN CHILDREN

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Malaria control interventions reduces all-cause child mortality and hospitalizations. Acute bacterial infections are common in malaria endemic settings, and the widespread use (and overuse) of antibiotics to treat these infections, particularly in outpatient settings, drives the antimicrobial resistance. This observational study of two sequential cohorts of children aged 6 months through 10 years in Tororo District, Uganda assesses the association between malaria control and outpatient prescriptions of antibiotics from 2011 to 2019. Indoor residual spraying (IRS) beginning in December 2014 was associated with a dramatic decline in the incidence of malaria treatments, from 2.68 treatments per person-year (ppy) before IRS to an average of 0.05 ppy in years 4-5 after IRS was instituted. Antibiotic prescriptions also dropped from 4.13 ppy prior to IRS, to 1.26 ppy in years 4-5. This corresponds to a 70% decrease in the IRR (95% CI: 66% to 73%; p<0.001), and 2.88 fewer antibiotic treatments per person-year (95% CI: 2.54-3.21; p<0.001). To explore potential mechanisms for this finding, data were stratified by fever and parasitemia status at the time antibiotics were prescribed. Compared to pre-IRS, there was a small but significant increase in antibiotic treatments among children who were afebrile without parasitemia. All other categories experienced significant reductions, with the most marked declines in children who were febrile with either sub-microscopic parasitemia (IRD=1.15, 95% CI: 1.02-1.27, p<0.001) or clinical malaria (IRD=1.12, 95% CI: 0.99-1.25; p<0.001). Upper respiratory infections (URI) were the most common diagnostic category, but both annual incidence and proportion of antibiotics declined over time. Amoxicillin was the most common antibiotic prescribed throughout the study and it also declined in both incidence and proportion over time. These results demonstrate that successful efforts to control malaria in highly endemic settings may have additional benefits for child health beyond malaria with additional implications for health systems and the spread of antimicrobial resistance.

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MAPPING PARTNER DRUG RESISTANCE TO GUIDE ANTIMALARIAL COMBINATION THERAPY POLICIES IN SUB-SAHARAN AFRICA

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Resistance to artemisinin-based combination therapies (ACTs) threatens the global control of *Plasmodium falciparum*. Although ACTs remain widely effective in sub-Saharan Africa, long-standing circulation of parasite alleles associated with reduced partner drug susceptibility may contribute to the development of clinical resistance. Molecular markers provide rapid information on landscapes of resistance to ACTs and can protect the efficacy of artemisinin by informing regional selections of longer-acting partner drugs. We fit a hierarchical Bayesian spatial model to data from over 500 molecular surveys to predict the prevalence and frequency of four key markers in transporter genes (*pfcrt* 76T, *pfmdr1* 86Y, 184F, and 1246Y) in first-level administrative divisions in sub-Saharan Africa, from the uptake of ACTs (2004-2009) to their widespread usage (2010-2018). Our models estimated that the *pfcrt* 76T mutation decreased in prevalence in 90% of regions, the *pfmdr1* N86 and D1246 wild-type genotypes increased in prevalence in 96% and 82% of regions, respectively, and there was no significant directional selection at the *pfmdr1* Y184F loci. Rainfall seasonality was the strongest predictor of the prevalence of wild-type genotypes, with other covariates including first-line drug policy and transmission intensity more weakly associated. We lastly identified regions of high priority for additional surveillance that could signify decreased susceptibility to the local first-line ACT. These results can be used to infer the degree of molecular resistance and magnitude of wild-type reversion in regions without survey data to inform therapeutic policy decisions. Working with global partnerships and local National Malaria Control Programs, our work can assist in efforts to sustain the use of ACTs as first-line therapies and improve disease surveillance strategies more broadly.

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ANALYSIS OF THE DISCREPANCY BETWEEN MICROSCOPY AND RAPID DIAGNOSTIC TESTS (RDTS) FOR THE DETECTION OF PLASMODIUM FALCIPARUM IN TWO DIFFERENT ENDEMIC SETTINGS OF DANGASSA AND KOILA, MALI

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In malaria-endemic areas, early and accurate diagnosis of malaria is essential for both effective disease control and surveillance as misdiagnosis can be associated with significant morbidity and mortality. The present study aimed to analyze the discordance between the microscopy and the rapid diagnostic test (RDT) results, in two ecological settings with different transmission patterns (Dangassa and Koila in Mali). This study includes a cohort of 1400 persons followed in each of the two villages. Microscopy and RDT (Malaria HRP2/pLDH Pf) were performed on all patients received at the community health center with suspected malaria symptoms during the study period (2013-2016). Frequencies with 95% confidence intervals (CI) with the Wilson-Brown method were used for categorical variables. Discrepancies between the two methods were analyzed and estimated by odds ratio (OR) with 95%CI regarding age, parasite load, season, and geographical settings. A total of 4,666 samples tested by microscopy and RDT, were analyzed, with respectively 3,616 and 1,050 samples for Dangassa and Koila from 2013 to 2016. The average cumulative incidence per 1000 persons*year was 34 cases ± 8.7 in Dangassa and 9 cases ± 8.06 in Koila. Compared to Dangassa, RDTs to Koila had higher sensitivity (90.17; 95% CI 87.66-92.21 vs 83.07; 95% CI 81.50-84.53, respectively). The same trend was observed with the specificity (83.8; 95% CI 79.82-92.46 vs. 63.16; 95% CI 60.47-65.78, respectively). The Likelihood Ratio (LR) showed a moderate effect in Koila (LR= 5.56) versus a weak effect in Dangassa (LR= 2.25). During the rainy seasons, 95% susceptibility was observed in Koila vs. 86% in Dangassa, with twice the disease incidence in Dangassa. Regression analysis showed an increase in discrepancy with increasing age, rainy season, and parasite load. This study showed the presence of diagnostic discrepancies between RDT and microscopy. These discrepancies can probably be influenced by factors such as age, seasonality, or malaria endemicity. However, further studies are needed to better estimate the discrepancies between all malaria diagnostic methods.

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LABORATORY DIAGNOSTIC CAPACITY AND PROFICIENCY IN MALARIA MICROSCOPY IN KENYA: IMPLICATIONS FOR ROLLOUT OF QUALITY ASSURANCE PROGRAM

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Quality malaria case management (CM) requires accurate diagnosis, which depends on well-trained microscopists, functional equipment, supplies, quality assurance, and consistent supervision. To assess malaria diagnostic capabilities in Kenya, Impact Malaria (IM) supported the Division of National Malaria Program (DNMP) to conduct a national cross-sectional assessment of 169 public health facilities (17 county hospitals, 47 sub-county hospitals, 100 health centres) in 43/47 counties in September 2020. A standard checklist was used to identify capacity gaps, and proficiency testing was conducted to assess performance on malaria parasite detection, species identification, and parasite quantification with a validated slide set (3 negative, 3 positive with pre-quantified parasitemia ranges, 4 species identification). Less than 50% of health centres and sub-county hospitals participated in quality assurance programs compared to 100% for county referral hospitals. Standard operating procedures were available in a third of health centres, about half of sub-county hospitals, and in all county referral hospitals. WHO-guided proficiency testing of 112 microscopists with 568 positive and 460 negative slides identified 17% false negatives and 7% false positives. Compared with the WHO-informed 80% national standard, microscopists achieved 76% agreement (A) with a Kappa (K) value of 0.52 on parasite detection (0.44 in health centres; 0.70 county referral hospitals) and A: 48%; K: 0.29 on species identification (0.25 in health centres; 0.36 county referral hospitals). Parasite quantification results were available for 125/1,028 (12%), due to lack of timers (56%) for staining and tally counters (43%) for quantification. Multi-level diagnostic inadequacies, mostly in lower level facilities where most malaria cases present, reduce malaria CM quality and can affect quality of diagnosis results. To address the gaps, IM will support the DNMP, Department of Public Health Laboratory, and Counties to improve diagnostic quality through supply chain improvements, staff training, supportive supervision, and quality assurance programs.

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LEVERAGING GEOSPATIAL MAPPING TO OPTIMIZE THE CONTINUITY OF MALARIA CARE DURING COVID-19 PANDEMIC BY COMMUNITY-BASED HEALTH WORKERS IN MASHONALAND EAST, ZIMBABWE

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In Zimbabwe, existing gaps in access to health services have been worsened by the COVID-19 outbreak. COVID-19 related travel restrictions and inadequate personal protective equipment (PPE) threatened normal health service delivery. Community Health Workers (CHWs) substantially contribute to primary health services delivery, including providing 30% of national malaria tests. The peak malaria transmission season for 2020 coincided with the emergence of COVID-19, creating a challenge for CHWs managing malaria due to the similarity in symptoms of malaria and COVID-19 and limited PPE availability. As a result, the National Malaria Control Program (NMCP), working with partners, mobilized resources to train CHWs on the safe delivery of services during the COVID-19 outbreak and provide the required PPE for use by the CHWs. In January 2021, supportive supervision visits were conducted in four districts in Mashonaland East province to map 1,258 CHWs and understand gaps in CHW needs and accessibility at a time of increased demand for services. A data collection tool was developed to measure access to CHW services by the communities at high risk of malaria, provision of COVID-19 support by CHWs, and availability of malaria case management commodities for CHWs testing and treating for malaria. Data were collected using Survey CTO and analyzed in SPSS and QGIS. Using drive-time to the nearest health facility as a measure of access to healthcare service for the population in the CHW catchment area, the analysis showed that 37% of CHWs were operating within 15 minutes of the nearest health facility, 31% within 30 minutes to a health facility and 32% in areas over 30 minutes away from the nearest health facility. The proportion of malaria cases tested in the community in the four districts increased steadily after the COVID-19 related lockdown, resulting in CHWs contributing 45% of 232,420 malaria rapid diagnostic tests and treating 50% of the 99,469 positive malaria cases recorded in 2020. The data also reflected gaps in the availability of PPE and commodity stock out on RDTs and ACTS, primarily driven by the unexpected surge in patient attendance at the CHW level.

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ADHERENCE TO CASE MANAGEMENT GUIDELINES BASED ON RESULTS OF MALARIA RAPID DIAGNOSTIC TESTS IN SUB-SAHARAN AFRICA FROM 2010-2020: A SYSTEMATIC REVIEW

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In 2010, the WHO updated case management guidelines for uncomplicated malaria, recommending parasite-based diagnosis in endemic areas. As a result, the annual number of suspected malaria cases that received a diagnostic test across sub-Saharan Africa (SSA) from 2010 to 2019 increased from 51 million to 235 million, respectively. This was driven by scale-up of malaria rapid diagnostic test (RDT) use, which increased in SSA from 21 million instances in 2010 to 195 million in 2019. This scale-up was vital towards improving the quality of malaria case management, yet provider adherence to guidelines based on test results ("adherence") has not been systematically assessed across SSA. To evaluate provider adherence, determinants of provider adherence, and patient-client factors, we followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines to conduct a systematic search using Embase and Scopus, and five search engines to identify articles published from January 2010 to August 2020. Search terms included "malaria," "rapid diagnostic test," and "adherence," combined with "Africa". Bibliographies of relevant studies were checked for other publications. Of 1,463 articles identified, 324 were reviewed and results from 76 were analyzed. This review synthesizes trends of RDT adherence across SSA over the past decade and highlights key factors driving adherence issues. Adherence when RDTs were positive was universally high; adherence when RDTs were negative varied. Improved adherence for negative RDT results was associated with training and supervision, trust in RDT quality, accepted practice norms, manageable patient caseloads, and adequate RDT stock. Persistent poor adherence when RDTs are negative contributes to overuse of antimalarials, distorts malaria surveillance data, may deprive patients of treatments for non-malaria fevers, and may obscure detection of other febrile illness with pandemic potential. Information about adherence to RDTs and drivers of adherence can inform program interventions and improved case management practices and guide future studies on identified gaps.

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A TABLET-BASED HEALTH NETWORK QUALITY IMPROVEMENT SYSTEM (HNQIS) FOR DATA-DRIVEN DECISION MAKING AND TARGETED OUTREACH TRAINING SUPPORTIVE SUPERVISION (OTSS+) AT LOW PERFORMING SITES IN THE DEMOCRATIC REPUBLIC OF CONGO (DRC)

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Outreach Training Supportive Supervision Plus (OTSS+) is a quality improvement approach aimed at enhancing the quality of malaria clinical and diagnostic service delivery. During OTSS+ visits to health facilities (HF), a checklist and on-site training are used to ensure procedures follow national guidelines. Diagnostic service delivery components are assessed, such as malaria microscopy (MM) skills, stock management, human resources, and quality assurance processes. PMI Impact Malaria supports the National Malaria Control Program to implement OTSS+ in nine provinces in DRC. The paper checklist used for field data collection was inefficient and did not allow for rapid corrective actions identified during field visits. The digital checklist, HNQIS, is an Android-based application which works on a DHIS2 platform. It enables clinical and laboratory (lab) data to be collected and generates competency scores in real-time, allowing supervisors to provide immediate feedback and take corrective actions. HNQIS was first used in DRC during a lab OTSS+ round in March 2020, during which 109 HF were visited. Action plans based on competency scores were developed during each visit and follow-up responsibilities were assigned. Based on the March 2020 results, 24 of the 109 (22%) HF were identified as low performing. Identified gaps included low MM skills and a lack of internal quality control processes. These HFs were targeted for additional visits in August 2020. Of these 24 HF, 16 HF (67%) showed improvement in parasite detection (PD) which ranged from 33% to 100%. The average PD scores of these 24 HFs was 62% during the initial visit and 75% after being re-visited. The 8 HF that did not show improvement had recently graduated lab technicians who have not participated in OTSS+. It is expected that new technicians will increase their MM skills with OTSS+ and Malaria Diagnostic Refresher Trainings. The feedback loop of HNQIS enabled quick turnaround of results, which were useful in making data-driven decisions to revisit low-performing facilities. HNQIS helps supervisors prioritize low-performing HFs to visit more regularly.

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IMPROVING MALARIA CASE MANAGEMENT PRACTICES AND REPORTING IN THE PRIVATE SECTOR IN UGANDA

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Coverage of fever case management interventions remains low across sub-Saharan Africa, including Uganda. While many caregivers seek treatment for symptoms of fever in the private sector, private sector outlets may not have adequate fever diagnostics, training, waste management, and first line quality assured treatments to ensure appropriate case management. The National Malaria Control Division together with partners have prioritized private sector engagement by ensuring that quality of services offered by private providers is improved and they report into DHIS II. An Assessment of malaria case management in licensed/accredited private outlet types including drug shops, pharmacies, clinics and hospitals was conducted, and participating members received training on integrated case management for febrile illnesses, supportive supervision, access to quality assured malaria rapid diagnostic test kits, subsided ACTs and self-regulation coordination mechanisms. Baseline and follow-up assessment through integrated support supervision were conducted in 2020 and follow on in 2021 to assess improvement in malaria case management in 20 districts in Uganda. Key results include overall change in reporting comprising number, completeness, timeliness and accurateness of data by private providers; Effect on the availability of quality guidelines and protocols in private outlets to provider behavior; coordination mechanisms of private sector providers at all levels and changes in case management of those who reportedly received treatment in the private sector. Implications for scaling up case management coverage through strengthening services provided by private sector providers focusing on the different outlet type will be discussed.

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DETECTING AND CLASSIFYING CELLS WITH A MULTI-CLASS MASK R-CNN IN THIN SMEAR MICROSCOPY FOR MALARIA DIAGNOSIS

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Malaria is a leading cause of death worldwide. The parasitic infectious disease can be transmitted easily to humans through mosquito bites that result in over 200 million infections and 400 thousand deaths every year, with children under five accounting for the majority of all malaria deaths. Due to the high infection rate, pathologists need to read millions of blood smears for malaria diagnosis. This manual process is time-consuming, costly, and subject to human error. Reporting accurate reading is essential for treatment, assessing effective drug doses, and determining disease severity. Computer-assisted algorithms have become a mainstay of biomedical applications to automate, simulate and standardize medical interpretations and quantification. In this study, we design a single multi-class classifier for detecting and classifying cells in thin blood smear images. We adapt a state-of-the-art instance segmentation network (Mask R-CNN) to delineate cells accurately and identify infected and uninfected red blood cells, and white blood cells. It segments hundreds of cells, in a matter of seconds and with a low memory footprint. Specifically, this approach is scalable to large blood smear images (5312×2988 pixels) featuring high cell densities, where cells are relatively small objects compared to the overall image size. We tested the method on an archived collection of human malaria smears with nearly 200,000 labeled cells across 965 images from 193 patients, acquired in Bangladesh. The cell detection accuracy is very promising and outperforms state-of-the-art methods in the literature. Our software implementation is a crucial step towards automated malaria diagnosis. It can assist microscopists in the field and in resource-poor primary care settings as a fast and affordable method for blood smear interpretation. Furthermore, it can assist researchers in major hospitals and institutes allowing a fast analysis and accurate quantification of their research experiments. Several organizations and institutes are currently helping to field-test the software developed in this study.

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MALARIA DIAGNOSIS QUALITY CONTROL AT HEALTH FACILITIES IN RWANDA

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Although significant progress has been made, malaria remains one of the most common diseases in Rwanda, with more than 2 million cases and more than a hundred deaths per year. To achieve Rwanda Malaria Control Program goals as a way to contribute to the socio-economic development through elimination of malaria burden, malaria diagnosis external quality assessment, quality control (EQA/QC) is conducted every quarter to evaluate and strengthen quality of diagnosis and treatment.The EQA/QC was conducted in 42 hospitals where 15 Slides were randomly collected at each hospital and retested at Rwanda Biomedical Center/ National Reference Laboratory Division by microscopists accredited by WHO (ECAMM) in Malaria microscopy. After retesting, a prompt feedback is provided so that actions can be taken to correct errors.For 1917 slides collected in 42 district hospitals in which EQA/QC of blood smears was

conducted, 2019-2020 EQA/QC was noted compared to the previous fiscal year with an improvement in overall results from 4.12% to 1.74% which remain above the cut off 5% acceptable range with 4/42 hospitals (Kabutare (8,89%), Masaka (6,67%), Muhororo (6,67%), Mibilizi (6,67%)) exceed the acceptable range. Findings from the EQA/QC has revealed that laboratory technicians at hospitals in Rwanda have high (>98%) proficiency testing -a continuous capacity building is needed to ensure and sustain accurate diagnosis for appropriate case management. Thus, Mal & OPDD will continue to work closely with NRL to correct reported discrepancies in district hospitals through formative and refresher training during the next fiscal years.

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MAGNETO-OPTICAL RESPONSE TO MAGNETIC FIELD ACROSS ALL HUMAN MALARIAS SPECIES USING GAZELLE PLATFORM

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All malaria parasites create the by-product Hemozoin as a result of Red Blood Cell digestion. However, because of the different pathways and speed of creating the Hemozoin, the resulting iron containing crystals have slightly different sizes and shapes which theoretically translates to a different response to presence and absence of a magnetic field. Here we detect the orientation and disorganization of the Hemozoin crystals from all the human malarias; *P. falciparum, P. vivax, P. malariae, P. ovale,* and *P. knowlesi* from short term cultures. Frozen samples of each of the species from BEI (Pf/Pv/Pk) or patient samples (Pm/Po) were thawed and placed in cultures of RPMI 1640 CMCM (Pf) or RPMI 1640/Waymouths +10 % Human AB (Pk, Pf, Po, Pm) and grown in 5% C02 for 24hr to allow for the creation of hemozoin. Samples were then tested on the Hemex Health Gazelle platform. The platform continuously measures the transmitted light passing through blood samples as the magnetic field is repeatedly applied and removed. As the magnet is applied the hemozoin aligns blocking an amount of light equivalent to the parasitemia. As the magnetic field is removed the hemozoin becomes disorganized via Brownian motion. Analysis of the output data reveals that the Hemozoin from Pv and Po showed a much faster disorganization upon removal of the magnetic field than the other species. Other work on adjoining posters has demonstrated that this difference reliably allows for the delineation of pure Pv from Pf infections from one another in field samples. This work could allow for the diagnosis of all forms of malaria as well as treatment with Primaquine to eliminate the hypnozoites in the liver created by the Po and Pv parasites to prevent relapse infections.

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HEALTHCARE PROVIDERS CHALLENGE TO CORRECTLY INTERPRET MALARIA RDT RESULTS IN NIGERIA

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Malaria rapid diagnostic tests (mRDTs) has become the widely used tool for the parasitological confirmation of suspected malaria cases in Nigeria. The potential of mRDTs in expanding access to malaria testing remain incontestable as it accounted for about 85% of malaria tests performed in public health facilities in Nigeria in 2019. Challenges with mRDTs have been underscored but the contribution of weak/faint lines in mRDT interpretation is unknown. Our facility, a WHO designated mRDT lot verification Centre in Lagos, Nigeria, observed during mRDT lot assessments high frequency of "faint, very faint and very very faint lines" [weak (<1+)/very weak (<1++), extremly weak(<1+++)] in some mRDTs lots that were difficult to interpret. We hypothesized that if these test lines posed a challenge in interpretation at the level of our operations, correct interpretation by health care providers (HCPs) in downstream health facilities may be a serious challenge in effective malaria case management. Instruction for use (IFUs) for mRDTs merely require weak test lines should be read as positive without categorization. A total of 677 HCPs in three states of Nigeria namely, Katsina, Gombe, and Osun states, were assessed for correct interpretation using 21 standardized picture images of mRDTs (mRDT EQA card). Overall, 102 (15.1%) and 109 (16.1%) of the 677 HCPs correctly interpreted 15 and 16 RDT results respectively. Remarkably, 16.1% of the 677 HCPs only correctly interpreted less than 10 RDT images. HCPs that correctly interpreted above 17 of 21 RDTs in the states were: Gombe, 37(20.3%), Katsina, 36 (18.3%) and Osun, 31(10.4%). Analyses of capacity to correctly interpret mRDT test lines showed that HCPs had little challenge with clear (bold) positive tests (90.3%), but had challenge with very weak positive tests (<1++)(34.7%), All positives (27%); very faint positives [extremely weak tests(<1+++)(3.5%), and negative tests (54.1%). The consequence was that HCPs were likely to interpret most "weak" positive tests as negative. This user challenge in RDT test interpretation with false negative and false positive tests are to be identified and addressed urgently.

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DEVELOPMENT OF A RAPID DIAGNOSIS TEST FOR PLASMODIUM VIVAX

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The issue with current Rapid Diagnosis Test for *Plasmodium vivax* (RDTs) is that they are often not sensitive enough to detect low parasitemia or mixed infection which requires additional medication to fully eradicate liver-stage parasites. In the Bosch Lab, we have selected five *P. vivax* proteins that were expressed, refolded, and purified in the lab using self-assembled protein nanoparticles (SAPNs) to be used as antigens for the generation of antibodies in rabbits for the development of a sensitive, *P. vivax*-specific RDT. These protein antigens were chosen due to their high affinity and selectivity for *P.vivax* as opposed to other Malaria species. The majority of our work thus far has consisted of the evaluation of the polyclonal antibodies using multiple lab techniques to determine that they recognize *P.vivax*-infected cells and have limited cross-reactivity with infected cultures of other Malaria species. Initial findings using Imagestream analysis indicate the antibodies recognize and bind to infected-*P.vivax* cells at various stages of the parasite life cycle while showing low binding affinity for uninfected cells. Additionally, paper-based and ELISA assays with mixed *P.vivax* and *P.falciparum* cultures suggest all five antibodies have a higher affinity for *P. vivax* antigens as opposed to *P .falciparum*. With these initial assays, we have determined a proposed limit of detection for *P.vivax* significantly lower than that of current RDTs used in the field today. We plan on continuing these evaluation assays using flow cytometry to examine double positive red blood cells (Hoechst and antibody stain) between *P. vivax*, *P. falciparum*, and *P. knowlesi* Malaria species. Results from these experiments will be analyzed to determine which of our polyclonal antibodies have the highest specificity to then generate monoclonal antibodies to be used in a new RDT that will be designed using laminar flow or an electrophoresis based device. By developing a more sensitive and specific RDT for *P. vivax*, we in the Bosch Lab hope to bridge a gap in current endemic Malaria control efforts by designing a means by which to identify and treat an often overlooked yet devastating form of Malaria disease.

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THE STANDARD G6PD TEST (SD BIOSENSOR) SHOWS GOOD REPEATABILITY AND REPRODUCIBILITY IN A MULTI-LABORATORY COMPARISON

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The introduction of short course, high dose primaquine and single dose tafenoquine for the radical cure of *Plasmodium vivax (P. vivax)* presents a great opportunity for more effective control of *P. vivax* malaria. Roll out of these novel regimens will require a reliable point-of-care diagnostic that can identify individuals with intermediate or severe G6PD deficiency. SD Biosensor (Republic of Korea) has developed a handheld quantitative G6PD screening device (the STANDARD™ G6PD), that demonstrated an accuracy of around 90% in field studies for identifying individuals with intermediate or severe deficiency. However, the device can only be considered for routine care if repeatability and reproducibility of the assay are high. Commercial controls (ACS Analytics, USA) with high, intermediate, and low G6PD activities were tested with ten Biosensor devices 20 times and compared to the reference method spectrophotometry (Pointe Scientific, USA). Each device was then dispatched to one of ten different laboratories with a standard set of controls. Each control was tested 40 times at each laboratory by a single experienced user and compared to spectrophotometry results. When tested at one site, the mean coefficient of variation (CV) was 11%, 17% and 26% for high, intermediate and low controls across all devices respectively; combined G6PD Biosensor readings correlated well with spectrophotometry (r_s= 0.859, p<0.001). When subsequently tested in different laboratories, correlation was lower (r_s=0.604, p<0.001) and the results determined by Biosensor for the low and intermediate controls overlapped. Conversely, Biosensor G6PD readings did not differ significantly between sites (p=0.436), but readings differed markedly by spectrophotometry (p<0.001). The use of lyophilized samples derived from human blood rather than fresh blood for which the device was developed may have affected these findings. Repeatability and inter-laboratory reproducibility of the Biosensor are significantly better than those of spectrophotometry. Clinical studies are required to assess the performance of the test in clinical practice.

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DETECTION OF A SUBSTANTIAL NUMBER OF PFHRP2 GENE DELETIONS AMONG SUBMICROSCOPIC INFECTIONS IN ETHIOPIA

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Apart from parasites resistance to drugs and mosquitoes resistance to chemicals, respectively, nowadays parasite escaping detection has challenged efforts to test and treat malaria cases. HRP2-based RDTs have been challenged by *P.falciparum* deleting the gene that encodes for HRP 2 and 3 proteins leading to HRP2-based RDTs are unable to detect parasite undergoing *pfhrp*2 and 3 gene deletion. A total of 218 quantitative PCR-confirmed *P. falciparum* positive samples were included for analysis of *pfhrp 2 and 3* genes deletion. The prevalence of *pfhrp*2 exon 2 gene deletions was 17.9% (39/218) and that of *pfhrp3 exon 2* was 9.2% (20/218). The deletions extended to the downstream (55.0%) and to the upstream (42.7%) regions in *pfhrp2*. Of eighty-six PfHRP2 RDT negative samples, thirty-six lacked the main HRP2 coding region, *pfhrp2 exon 2*. Five PfHRP2 RDT negative samples had double deletions in *exon 2* of both *pfhrp2* and *pfhrp3* genes. Of these double deletions, only two of the samples were positive by the microscopy. Three samples with intact *pfhrp3 exon2* but with deleted *pfhrp2 exon2* were tested positive by PfHRP2 RDT and microscopy. Of the 39 *P.falciparum* isolates without *hrp* 2 and 3 genes, 36 of them were submicroscopic while three of them are microscopic infections. In conclusion,this study confirms the presence of deletions of *pfhrp 2 and 3* genes including the flanking regions. *Pfhrp 2and 3* gene deletions results in false-negative results undoubtedly affect the current malaria control and elimination effort in the country. Assessment of *pfhrp* 2 and 3 gene deletions should take account of both microscopic-and submicroscopic-infections to avoid underestimation of the true prevalence of gene deletions. *Pfhrp*2 deletions were compensated by the intact *pfhrp*3 regions.

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INTEGRATING G6PD POINT-OF-CARE TESTING INTO MALARIA CASE MANAGEMENT TO SUPPORT RADICAL CURE: AN ASSESSMENT OF HEALTH WORKER SKILLS AND KNOWLEDGE IN LAOS AND VIETNAM

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The lack of tests for glucose-6-phosphate dehydrogenase (G6PD) deficiency, an inherited condition common in malaria-endemic countries, has resulted in both an underuse of radical cure due to safety concerns in some areas and the unsafe use of these drugs with no G6PD screening in others. Recently commercialized diagnostics for G6PD deficiency have the potential to improve health outcomes, align diagnosis and treatment options in remote settings with globally recommended clinical practices, and reduce transmission through the expanded use of radical cure treatments, including Kozenis (tafenoquine). Adoption of these tests has been limited in part due to concerns around product usability in malaria case-management settings. Malaria control programs and research partners in Vietnam, and Lao PDR have undertaken operations research studies to assess the feasibility of integrating G6PD testing into malaria case management. The aim of this study is to assess whether health workers that currently diagnose and treat *P. vivax* can also use G6PD tests, and what training practices enable their effective use. District hospital laboratory and health center staff at 23 facilities in malaria-endemic regions of Laos PDR and Vietnam were trained in the use of a point of

care G6PD biosensor-based diagnostic. A mixed-methods approach to assessing user knowledge and competency was developed, which included a quantitative questionnaire and an observational component, where users completed the diagnostic workflow and interpreted the test result using control reagents. These data were collected immediately after the training and four to six months after training. Results from these assessments indicate that health workers ability to run and interpret a G6PD test varies widely across settings and is mediated by the quality of the training and by the volume of malaria patients that are seen by the health worker over time. These data provide insights into what changes to routine care, training, and supervision will be needed to accommodate G6PD testing as well as recommend specific supplementary training materials to accompany the introduction of the test.

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PAST EFFECTS OF CLIMATE AND INTERVENTIONS ON MALARIA IN UGANDA

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Studies have estimated the impact of malaria control interventions on malaria incidence although few have explored the differential impact due to environmental conditions. We aimed to evaluate the influence of temperature, rainfall, humidity, and vegetation, on the relationship between insecticide-treated bednets (ITNs) and indoor residual spraying (IRS) and malaria. A longitudinal analysis was conducted, based on episodes of laboratory-confirmed malaria from a dynamic cohort of children followed from 2011 to 2017 in Uganda. Children less than 10 years were randomly selected from low, medium, and high transmission sub-counties. Participants were provided ITNs, and four rounds of spraying were conducted in one subcounty. Environmental variables were extracted from remote sensing sources and include enhanced vegetation index (MODIS), rainfall (ARC2), minimum and maximum temperature (ERA5), humidity (ERA5), averaged over different time periods. General linear mixed models were constructed based on a log-binomial distribution, accounting for repeated measures and clustering by household and non-linear associations with malaria incidence were explored. Over six years, 1,090 children were followed with a mean of 35 visits and 5 malaria episodes per individual. The best fit model was with the meteorological measures averaged over 90 days prior to the date of clinic visit. Controlling for the interventions, humidity and precipitation presented non-linear relationships with malaria incidence while increasing minimum and maximum temperatures were associated with a linear reduction of malaria risk. IRS was associated with a significant risk reduction (OR=0.21, 95% CI 0.17-0.26). An increased risk of malaria associated with increased minimal temperatures was only observed for individual who did not sleep under an ITN, demonstrating an interaction effect. Effect of minimal temperatures on malaria risk is modified by ITN. Non-linear relationship and modifying effect of climate by anti-vectorial interventions need to be considered when assessing the epidemiology of malaria.

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LONGITUDINAL GENETIC EPIDEMIOLOGY SURVEILLANCE WITH A SNP-BASED MOLECULAR BARCODE DETECTS WIDESPREAD COTRANSMISSION OF PLASMODIUM FALCIPARUM MALARIA PARASITES IN THIÈS, SENEGAL

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Genomic epidemiology surveillance shows great potential for monitoring transmission dynamics and informing targeted public health interventions to reduce infectious disease burden. In *Plasmodium falciparum*, these surveys consistently identify a large number of polygenomic (multi-strain) infections whose complexity of infection (COI, number of strains per infection) is greater than one. While polygenomic infections in high transmission settings tend to have high COI, it is unclear to what extent COI correlates with transmission intensity. This uncertainty stems from two competing hypotheses regarding either superinfection, which predicts a positive correlation between COI and transmission due to the introduction of unrelated strains from multiple infectious bites, or cotransmission, which predicts a weak correlation due to the introduction of multiple, genetically related strains from a single bite. To address this issue, we examined a set of 2,203 infections (23.6% polygenomic) collected from Thiès, Senegal between 2006 and 2019 and genotyped at 24 single nucleotide polymorphisms (SNPs). To determine whether polygenomic infections were comprised of related strains and the result of cotransmission, we calculated an F-statistic that measures an inbreeding coefficient based on comparing the heterozygosity from single-strain and polygenomic infections. We found that polygenomic infection heterozygosity was depressed by an average factor of 0.38 (0.36, 0.40). Most polygenomic infections showed evidence of inbreeding, suggesting they were the result of at least one cycle of cotransmission. Genomic epidemiology studies should supplement COI with estimates of polygenomic relatedness to account for cotransmission when evaluating parasite transmission intensity. This study also highlights the usefulness of field-deployable SNP assays for conducting simple genomic surveillance data to inform transmission dynamics and assess the impact of interventions on parasite population structure toward malaria burden reduction.

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MALARIA IN THE PERIPARTUM PERIOD AND ASSOCIATED CLINICAL OUTCOMES FOR MOTHER AND BABY IN A HIGHLY ENDEMIC REGION OF THE BRAZILIAN LEGAL AMAZON, ACRE STATE

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Deforestation in Amazonia has recently resulted in a resurgence of malaria in this region of the world, with 75% of all 2018 Latin American malaria cases occurring in Brazil and Venezuela. In Brazil, the disease burden predominantly lies in the Brazilian Legal Amazon region. Pregnant women in this region are particularly susceptible to malaria infection and clinical disease. Malaria infection in the peripartum period can lead to a range of negative maternal-child outcomes including, maternal anaemia, congenital malaria, preterm birth, and stillbirth. This study sought to understand the clinical outcomes of malaria during pregnancy on mothers and neonates in a hospital in Acre state. We enrolled 2,809 women at delivery or within 3 days post-delivery from the Women and Children's Hospital of the Juruá River Valley in Cruzeiro do Sul county of Acre state. Blood samples were taken from mothers to assess malarial status and haemoglobin levels,

questionnaires administered, and birth outcomes assessed via clinical charts. Questionnaires sought to assess maternal demographics and preventive measures including the use of DEET and insecticidal bed-nets. Further, maternal *Plasmodium* species was determined following malaria diagnosis. We found 7.2% of mothers were infected with malaria during pregnancy, and 1% were diagnosed in the perinatal period. We found similar rates of *Plasmodium falciparum* and *P. vivax* in mothers who were infected. Nearly half of mothers had been diagnosed with malaria in a previous pregnancy. Nearly half of enrolled women presented with perinatal anaemia, and those with anaemia were more likely to be infected with *P. falciparum* ($p = 0.004$). Living in a rural area and diagnosis of malaria in a previous pregnancy were significantly associated with malaria diagnosis at the time of the current pregnancy ($p < 0.001$, $p < 0.001$). Perinatal anaemia was significantly associated with lower APGAR scores in neonates ($p = 0.004$). Outcomes of this study will inform preventive efforts and clinical case management for maternal malaria infection in a particularly susceptible population in a highly endemic rural region of the Brazilian Amazon.

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UnMASC: UGANDA MALARIA ACTIONABLE SURVEILLANCE IN THE TIME OF CORONAVIRUS

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As the COVID-19 epidemic progresses in Uganda, its repercussions may affect healthcare-seeking behavior, case management, and control interventions for malaria. Improved malaria surveillance will be essential to fully understanding the effects of COVID-19 on malaria transmission, burden, and care. Our study will conduct cross-sectional surveys in communities surrounding a subset of 64 malaria reference centers (MRCs) that serve as an enhanced malaria surveillance network. Households in the catchment area of 12 MRCs have been enumerated and are currently being randomly surveyed with questionnaires on healthcare seeking behavior and household-level malaria control interventions (such as long-lasting insecticide treated net availability and use from the 2020-2021 national distribution campaign). We plan to enroll 50 households with at least one child 2-10 years of age from each catchment area. We will obtain a thick smear for microscopy and collect dried blood spots from all children in enrolled households, allowing us to calculate parasite prevalence and conduct precision serosurveillance for malaria and COVID-19 using a multiplex Luminex assay. In 2020, we recorded 952,270 outpatient visits from 64 health facilities, with 72.1% of those attending suspected to have malaria. From all 64 MRCs, we will report changes over time in the number of visits and standard malaria indicators from March 2020-October 2021 compared to prior years' data. From the subset of 12 MRCs, we will report 1) changes in healthcare-seeking behavior and household-level malaria control interventions in the context of the COVID-19 pandemic, 2) estimates of parasite prevalence using microscopy and precision serosurveillance, allowing us to contextualize the malaria indicator data collected from MRCs (which may be biased by pandemic-related factors such as health facility attendance) and 3) estimates of COVID-19 prevalence in children. Combining data from both health facilities and cross-sectional community-based surveys will allow for a robust assessment of the effects of the COVID-19 pandemic on routine malaria care and prevention.

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ASSESSING THE UTILITY OF ANTENATAL CARE SURVEILLANCE IN TANZANIA FOR MONITORING COVERAGE OF MALARIA CONTROL INTERVENTIONS

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Estimates of malaria burden and intervention uptake in Africa are primarily based upon nationally representative household (HH) surveys. However, their expense and infrequency limits their utility for timely analysis for operational action by malaria programs. We assessed whether population surveillance via women attending antenatal care (ANC), consisting of collecting data on malaria prevalence using rapid diagnostic tests and on coverage of malaria interventions among women and their households using a short questionnaire during 1st ANC visits (ANC1) could provide relevant data to guide decision-makers. Malaria prevalence from routine ANC-based surveillance in 40 health facilities (HF) in Geita Region were compared with those in children under 5 (U5) from a cross-sectional HH survey conducted in the same HF catchment areas in November-December 2019. Data on coverage of malaria control interventions and care-seeking behavior was collected routinely from women at ANC1 starting March 2020. A second cross sectional HH survey is planned for summer 2021. We fitted a linear relationship between the log odds of both ANC1 prevalence and HH prevalence with a random effect for each health facility catchment. The model showed a statistically significant positive relationship ($p<0.005$), though this relationship was non-linear ($p<<0.0001$): malaria prevalence during ANC1 exceeded that in U5 in areas where U5 prevalence was low (<10%), while prevalence among U5 increasingly exceeded ANC prevalence in higher prevalence areas, where multigravida have acquired substantial immunity. Prevalence in younger women (<20 yr) appeared nearly uniformly high in all HF regardless of U5 prevalence, making them and their newborns a core malaria risk group. Findings on the utility of ANC surveillance for measuring coverage of malaria control interventions and care-seeking behavior will also be presented. Our study suggests that malaria prevalence estimates derived from ANC surveillance correlate well with estimates derived from HH surveys and might be useful in informing prevention efforts when more resource-intensive HH surveys are not feasible.

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THE MALARIA ATTRIBUTABLE FRACTION OF FEVER IN A UGANDAN COHORT STUDY

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Fever is a common reason for patients to seek medical care in sub Saharan Africa. In malaria endemic settings, fever is often caused by infection with *Plasmodium falciparum*. However, quantifying the fraction of fever attributable to malaria has been challenging. We used well characterized cohorts of children and adults followed from 2011 to 2019 in a historically high transmission area of Uganda to estimate the malaria attributable fraction of fever (MAFF). Standard clinic evaluations during clinic visits included documentation of a history of fever in the last 24 hours and measurement of tympanic temperature. Over the 8-year observation period, 2 rounds of universal distribution of long-lasting insecticidal nets (LLIN) and 7 rounds of indoor residual spraying of insecticide (IRS) were deployed, resulting in dramatically reduced malaria vector populations, infection and disease. We estimated and compared MAFF for self-reported vs objective fever and background vs clinical fever in younger children (<5 years old), older children (5-10 years) and in adults (over 18 years old). The study enrolled 814 participants, 32.8% younger children, 38.3% older children and 28.9% adults. Objective fever was lower than self-reported fever. After intensive vector control interventions, the MAFF for self-reported fever during scheduled visits reduced by 94%, 95% and 99% in younger children, older children and adults respectively. During the unscheduled visits, it reduced by 50%, 65% and 80% in younger children, older children and adults. During scheduled visits, objective fever was extremely rare in adults. After intensive vector control interventions, the MAFF for objective fever reduced by 72% in younger children with no significant reduction in older children. Malaria accounted for 88% of objective fever in younger children, 75% in older children and 91% in adults. In this area where IRS was deployed alongside mass LLIN distribution, the MAFF was remarkably high and fever was rare in the absence of malaria. Effective control of malaria could lead to improvement in health beyond traditional measures of symptomatic malaria.

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IS ZOONOTIC TRANSMISSION OF MALARIA A RISK FOR BLOOD DONATION AND ORGAN AND TISSUE TRANSPLANTATION?

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Zoonotic transmission of malaria is described in several regions. Asymptomatic humans infected by *Plasmodium cynomolgi* and *P. knowlesi* were reported in the Asia. Human cases of *P. vivax/P. simium* and *P. malariae/P. brasilianum* were detected in the Brazilian coast, where monkeys of the genus *Alouatta* and *Callithrix* act as reservoirs of *Plasmodium*. Asymptomatic infections pose a challenge for elimination and represent a risk for blood donation and transplantation. Although guidelines recommend screening candidates from endemic areas or those with a history of malaria, the risk of asymptomatic individuals infected in areas of low endemicity with zoonotic transmission requires attention. This study was conducted in two blood centers and in hospitals in the state of Sao Paulo, Brazil. We processed 431 blood samples from candidates with displacement to areas of low endemicity with malaria zoonotic transmission, using thick blood smear (TBS), two genus-specific protocols (in house qPCR and Alethia® Malaria LAMP) and RealStar® Malaria Screen & Type PCR for determination of species. Eleven samples from donors/recipients of organs/tissues and 525 samples from blood donors who traveled to endemic regions were screened for *Plasmodium*, following the Brazilian guidelines. Among the 431 samples, two donors (0.5%) were positive for *Plasmodium* by qPCR, LAMP and TBS, both confirmed as *P. malariae*. One sample showed a single parasite-like structure in the TBS, but it was negative in all molecular tests. No positive samples were detected among the 11 donors or recipients of organs/tissues and the 525 blood donors from endemic areas. Epidemiological screening in blood banks considers displacement to endemic regions to prevent the transmission of malaria. If infected, this population would probably present symptoms, a hypothesis corroborated by our findings, in which no donor was positive. According to our results, the risk was detected in blood donors from areas of low endemicity, where zoonotic transmission of malaria was reported. The detection of *P. malariae* coincides with reports of non-human primates infected with *P. brasilianum* in this region.

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EFFICACY OF ARTEMETHER-LUMEFANTRINE FOR UNSUPERVISED TREATMENT OF UNCOMPLICATED MALARIA IN LOW AND HIGH TRANSMISSION AREAS IN GHANA

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The Artemisinin combination therapy continues to be effective in the supervised treatment of uncomplicated malaria in Ghana. In the hospital setting however, the ACTs are administered to individuals with malaria without strict supervision of drug intake. This may not provide a true reflection of the efficacy of the ACTs under such circumstances. This study determined the response to unsupervised treatment with Artemether lumefantrine (AL). Fifty-nine individuals with uncomplicated malaria were recruited from Lekma Hospital in Accra (low transmission area; N= 23), and King's Medical Centre in Tamale (high transmission area; N= 36). Participants were followed up on day 1, 2, 3, 7, 14, 21 and 28. Presence of parasites on follow up days were determined by microscopy. Sub microscopic infections were determined by nested PCR of the *18s rRNA* gene of *Plasmodium falciparum*. Clearance of malaria parasite clones was assessed by MSP 1 and 2 genotyping. Two out of the 36 (5.56%) participants from the high transmission area showed delayed parasite clearance on day 3, but they did not have any parasite on day 7. In all prevalence of delayed parasite clearance for all study participants was 3.39%. One participant who had parasites on day 7 had no parasites on day 14 by microscopy. There was presence of parasites by microscopy on day 21 in 11.11% of participants from the high transmission area. On day 28, only one of these individuals still had parasites by microscopy. Also, one other participant whose parasites were cleared by day 1 had recurrence on day 28. Prevalence of sub microscopic infections was 16.67% in participants from the low transmission and 21.74% from high transmission areas respectively. Malaria parasite genotyping by MSP 1 and 2 indicated that all parasite clones were cleared equally after drug administration. To this end, Artemether-lumefantrine, taken under unsupervised conditions in Ghana, continues to be efficacious in the treatment of uncomplicated malaria in Ghana. However, sub-microscopic persists after treatment. This could serve as reservoirs of parasites that could be transmissible.

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IMPACT OF SIX-MONTH TRAVEL MORATORIUM ON PLASMODIUM FALCIPARUM PREVALENCE ON BIOKO ISLAND, EQUATORIAL GUINEA: A DIFFERENCE IN DIFFERENCES ANALYSIS

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Malaria control on Bioko Island, Equatorial Guinea, has substantially reduced the prevalence of *Plasmodium falciparum (Pf)* malaria, but it is still ~19%. Residents of Bioko frequently travel to the mainland, where *Pf* prevalence is much higher (>40%). Analyses suggest that imported infections from the mainland contribute to sustained prevalence on

Bioko Island, especially in urban areas where travel is common. Between March and September 2020, travel on major airlines and passenger ferry boats between the island and mainland was suspended in response to the Covid-19 pandemic. This, ostensibly, would have also halted the importation of *Pf* infections, and provides a natural experiment to evaluate the contribution of imported infections to *Pf* prevalence in Bioko. Data from the 2019 and 2020 malaria indicator survey (MIS) was used to evaluate whether the change in *Pf* prevalence before the travel restriction (2019) to after the travel restriction (2020), was greater in areas with a high proportion of reported travelers compared to the change in areas with a low proportion of reported travelers using a difference in differences model. Quartiles of travel prevalence were used to categorize sampling areas, and the areas in the highest and lowest quartiles were compared. The change in *Pf* prevalence over time in each travel group was compared. On average, there were 2900 individuals from 820 households in the low-travel areas per year, and 6800 individuals from 1685 households in high-travel areas. In low travel areas, *Pf* prevalence increased 5% from 2019 to 2020 (8% to 13%). Over the same time, the prevalence in high-travel areas decreased 2% (14% to 12%), for an unadjusted difference in differences of 7% (p<0.005). This suggests that imported infections do contribute to the overall malaria burden in areas where travel is frequent. We plan to use a weighted regression model that accounts for time-varying household and community level covariates with an interaction term for year and travel group, to estimate the reduction in malaria prevalence due to the restriction of travel and importation of malaria infections.

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CAN HOUSING IMPROVEMENTS FOR MALARIA CONTROL ALSO REDUCE ACUTE RESPIRATORY INFECTION AND NON-MALARIA FEVER? A COHORT STUDY IN UGANDA

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Housing improvements may support malaria control and elimination in sub-Saharan Africa by reducing house entry by malaria vectors, but concurrent effects on other causes of child morbidity and mortality are poorly understood. We tested the hypothesis that improved housing is associated with reductions in malaria, acute respiratory infection (ARI), non-malaria fever and diarrheal disease in Nagongera, Uganda. Data were analyzed from a cohort study that followed all residents (n=531) from 80 randomly selected households for 24 months from October 2017 to October 2019. Houses were classified as modern (cement, wood, or metal walls, tiled or metal roof, and closed eaves) or traditional (all other homes). Analyses are ongoing; results will be presented on associations between house type and human biting rate (HBR) , proportion of blood fed *Anopheles* (measured every two weeks using CDC light traps in all rooms where participants slept), and between house type and malaria parasitemia, incidence of ARI, incidence of non-malaria fever and incidence of diarrheal disease (measured every four weeks using active surveillance for all enrolled participants).The findings of this study will provide important insight into the potential health benefits of housing improvements beyond reductions in malaria in rural Sub-Saharan Africa.

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CEREBRAL MALARIA AND SEX-BASED DIFFERENCES IN RISK OF IN-HOSPITAL MORTALITY, POST-DISCHARGE MORTALITY, AND POST-DISCHARGE READMISSION IN YOUNG AFRICAN CHILDREN

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While studies report a male bias in clinical malaria among adults living in endemic regions, often attributed to behavior or sex hormones, the bias in children is less defined. We report findings from three cohort studies of Ugandan children admitted with cerebral malaria in 2003-2006 (cohort 1, n=92), 2008-2015 (cohort 2, n=269), and 2014-2016 (cohort 3, n=69). In each cohort, males were disproportionately affected (58%, 59%, and 56% in cohorts 1, 2, and 3, respectively, while national census data showed proportion of males age 0-14 years to range from 49-51% during these periods). In each cohort, there were no significant sex-based differences in length of symptoms prior to seeking care, primary caregiver, or socio-economic status. To evaluate if sex related to clinical outcomes in children with cerebral malaria, we analyzed in-hospital mortality, post-discharge mortality, and readmission among children with cerebral malaria in each cohort by sex. Using data from all cohorts, overall in-hospital mortality was lower in males than females (12% vs 17%, p=0.10) but these differences were not statistically significant. Across the 3 cohorts, there was also no significant difference by sex in post-discharge mortality (2% males, 3% females, p=0.72). In cohort 3, males had a 3-fold increased risk of malaria readmission compared to females (IRR [95% CI]: 2.9 [1.2, 7.0], p=0.01). Among Ugandan children, males are more often admitted for cerebral malaria than females, and have a greater risk of post-discharge readmission. Future studies should investigate the reasons for these sex-based differences in cerebral malaria morbidity.

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PREVALENCE OF ASYMPTOMATIC MALARIA IN SOUTHERN BENIN

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With the renewed interest in malaria eradication around the world, strategies targeting the human parasite reservoir are needed to continue to reduce transmission and achieve elimination in malaria endemic area. Asymptomatic carriers of *Plasmodium* are important sources of infections for malaria vectors. The objective of this study was to assess the asymptomatic malaria case rates, during dry season, in the general population in southern Benin.From February to March 2020, 1064 inhabitants of the village of ADJRAKO, in the south of Benin, were screened for malaria, by three diagnostic methods: Rapid Diagnostic Test-RDT-, microscopy, Polymerase Chain Reaction-PCR.Demographic data, vital signs, search for antecedent of taking antimalarial were recorded. Symptomatic malaria prevalence across all village in dry season was 6,3% (67/1064)The frequency of asymptomatic carriage of plasmodium was 19,3% (182/944); 18,1% (171/944); and 39,8% (376/944) by RDT, microscopy and PCR respectively.The predominant parasite is plasmodium falciparum with a proportion of 32.4 % (306/944).Stratification by age group (&lt 5 years, 5-15 years, &gt15 years), shows respectively, a frequency of carriage of microscopic malaria: *3,7% (35/944); 10,6% (100/944); 3,8% (36/944)*The percentage related to asymptomatic submicroscopic malaria is respectively: *1,6% (15/944) ; 9,4% (89/944) et 11% (104/944)*.Whatever the diagnostic method used (microscopic, or PCR), the average quantitative values are higher in the age group of children under 5 years old (mean QPCR : 1720) compared to the groups of children from 5 to 15 years (mean QPCR: 710), as well as those over 15 years (mean QPCR: 108), and this significantly (Kruskal Wallis ch2 =

60.212; p &lt 0.001).Our findings show a low prevalence of clinical malaria episodes with a significant proportion of asymptomatic carriersTherefore, the group of children under 5 years of age has a higher average parasitaemia rate than the older age groups despite a lower frequency of asymptomatic malaria

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SPATIAL HETEROGENEITY OF THE SUBMICROSCOPIC MALARIA RESERVOIR IN RURAL BAGAMOYO DISTRICT, TANZANIA

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Submicroscopic malaria reservoirs are composed of asymptomatic parasite carriers who areinvisible to standard malaria diagnostics yet likely contribute to malaria transmission. Theproportion of the asymptomatic reservoir that is submicroscopic varies by malaria prevalence – inareas of lower malaria endemicity, much of the malaria burden is submicroscopic, whereas in hightransmission areas, asymptomatic malaria is more commonly patent (positive by microscopy orRDT). Since malaria transmission is highly heterogeneous even at local scales, we investigatedwhether variable malaria prevalence between villages in eastern coastal Tanzania translates todifferences in the size of the microscopic reservoir. Malaria screening data including from rapiddiagnostic tests (RDT), blood smears, and 18srRNA-based real-time PCR (qPCR) from more than4,300 participants (median age 23.8, range 6 to 100 years) collected from October 2018 to March2021 from 112 villages throughout rural Bagamoyo district, Tanzania, are used in this analysis.Villages include homes primarily constructed of sheet metal roofs (87%) and bamboo with mudhousing material (68%). To identify differences in the submicroscopic reservoir at the local scale,we employed ArcGIS to map the ratio of RDT and blood smear positive cases to qPCR positivecases in 17 of the 112 villages (those containing >50 screened participants). The proportion ofsubmicroscopic infections ranged from 26%-84% and, similar to trends identified on largergeographic scales, was lower in the villages with higher overall malaria prevalence. We willinvestigate individual risk factors associated with submicroscopic parasite carriage (age, gender,malaria prevalence in village) as well as village-level risk factors (altitude, season/rainfall,proximity to water, and distance to nearest health center) associated with the prevalence of malariaand submicroscopic carriage. While our study sample mainly draws from two locales and theirsurrounding villages that are just 30km apart, significant spatial heterogeneity is present in the sizeof the submicroscopic reservoir.

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EVALUATING THE EFFECT OF EXTRACTIVE INDUSTRIES ON MALARIA TRANSMISSION IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Extraction of natural resources through mining and logging activities provides revenue and employment across Sub-Saharan Africa, a region with the highest burden of malaria globally. The extent to which mining and logging influence malaria transmission in Africa remains poorly understood. Using malaria testing results and demographic information from 16,277 adults in the Democratic Republic of the Congo sampled across 489 nationally representative clusters in the 2013 Demographic and Health Surveys, we evaluated the association between exposure to mining and logging concessions (residence ≤15km from concession) and prevalence of malaria infections. We stratified analyses by urban vs. rural and employed hierarchical regression models that a) controlled for intra-cluster correlation alone, and b) additionally incorporated a spatially varying intercept to adjust for residual spatial confounding. We found that, without adjusting for spatial confounding, individuals in rural areas residing in a cluster exposed to mining had a malaria prevalence odds of 1.82 (95% uncertainty interval (UI): 1.35, 2.44) compared with residents of unexposed clusters. Adjusting for space, the effect of living close to a mine moved close to the null with an odds ratio of 0.93 (95% UI 0.69, 1.26). In urban areas, mining was unassociated with malaria prevalence regardless of adjusting for space. Logging was unassociated with malaria in urban and rural areas when controlling for intra-cluster correlation alone and when adjusting for space. For both mining and logging, models incorporating a spatially varying intercept fit the data better than models with a random intercept alone. These findings suggest that while mines are often located in areas of high transmission, mining itself may not drive the high prevalence; when controlling for spatial confounding, mining and logging operations are not associated with malaria prevalence.

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MALARIA IN REFUGEE CHILDREN IN A HOLOENDEMIC REGION OF ZAMBIA: A RETROSPECTIVE COHORT AND CROSS-SECTIONAL STUDY

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Malaria due to *Plasmodium falciparum* is a leading cause of morbidity and mortality in children in highly endemic areas in sub-Saharan Africa. Refugees are at increased risk of various infectious diseases. Knowledge about the malaria risk in refugees compared to the general population is limited. This retrospective observational study was conducted in a high-transmission region of northern Zambia hosting Congolese refugees. They first were situated in a transit center during the acute phase of the refugee crisis, and then transferred to a refugee settlement (post-acute phase). We analyzed hospital records from October 2017 to May 2020 of children below 16 years of age from refugee sites and local villages with severe malaria, and malaria surveillance data of health centers from October 2018 to February 2021 to examine differences in demographic and clinical characteristics and identify risk factors. In-hospital mortality was assessed in multivariable logistic regression models. 2 197 hospitalized children had severe malaria. Of those 3% were refugees from the transit center (n=63) and 5% from the post-acute settlement (n=110). The overall in-hospital mortality was 13%, and was significantly higher in children from the refugee settlement (25%) than in children from the surrounding villages (12%) and from the acute phase transit center (14%). It was independently associated with being refugee (OR 1·7, p value=0·049) after adjustment for age, sex, and road distance from the hospital. Referral rate

from the settlement health center to the hospital was lower in refugee than in local children (0·4 *vs* 1·1%). The findings suggest that refugee children were referred earlier in the acute than in the post-acute phase, when the transit center was situated closer to the hospital. In the post-acute phase, the in-hospital mortality in refugee children with malaria was higher, what may be explained by late access to health care. Interventions tailored to the refugee context encouraging earlier healthcare-seeking and access to health care are required to improve malaria outcomes in this population.

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PREVALENCE OF MULTIPLE PLASMODIUM SPECIES IN UGANDA - TIME TO CONSIDER SUBMICROSCOPIC INFECTIONS AND NON-FALCIPARUM MALARIA IN THE CONTROL AND TREATMENT STRATEGIES?

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The World Health Organization lists Uganda as one of the five countries that collectively suffers>50% of all malaria cases globally. *Plasmodium falciparum* is the predominant malaria species inUganda, but the prevalence of *P. malariae*, *vivax,* and *ovale* infections remain unclear. In thisstudy, we investigated the prevalence of multiple *Plasmodium* species in blood samplescollected for a prospective observational study of neurodevelopmental outcomes in children (6months - 4 years of age) with severe malaria (SM) conducted between 2014-2018 in Uganda. The study included participants with SM (n=600) and asymptomatic community controls (CC,n=120). Presence of malaria at enrollment was confirmed by microscopy for all species or*Pf*HRP2 or pan-lactate dehydrogenase rapid diagnostic test (RDT). We later performed nestedPCR (nPCR) testing on whole blood samples, targeting the 18S small subunit ribosomal RNA ofthe *Plasmodium* genus, followed by species-specific primers for amplification of *P. falciparum*,*malariae*, *ovale*, and *vivax*. In children with SM, prevalence of *P. falciparum* infections was 73%,100% (for Pf or pan-malaria), and 96% by microscopy, RDT or nPCR, respectively. Inasymptomatic CC, prevalence of *P. falciparum* infections was 8%, 24% (for Pf or pan-malaria),and 28% by microscopy, RDT or nPCR, respectively. The prevalence of *P. malariae* infectionsdetected by microscopy in SM group was 0.3% and 0.8% in the asymptomatic CC group. nPCRfound a prevalence of *P. malariae* in 1.2% in SM group and 1.7% in asymptomatic CC group.Testing is ongoing for *P. ovale* and *P. vivax.* Our preliminary findings suggest a need forimproved detection of non-*falciparum* infections in malaria endemic countries as well as a needto understand prevalence of all-species sub-microscopic infections, which will be key in malariatransmission reduction goals.

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ELUCIDATING MALARIA PLASMODIUM SPECIES COINFECTIONS ACROSS ZAMBIA USING MOLECULAR AND GENOMIC APPROACHES

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Malaria continues to be a major public health problem in sub-Saharan Africa, including in Zambia, where the majority of population is at risk. In Zambia, the *Plasmodium falciparum* parasite causes the overwhelming majority of malaria cases, but a study from Eastern and Luapula provinces found that *Plasmodium spp*. coinfections accounted for 10.6% of analyzed samples. As Zambia heavily relies on rapid diagnostic tests (RDTs) for malaria diagnosis which only detect *P. falciparum*, we hypothesize that non-*falciparum* malaria infections are under-diagnosed, and their prevalence underestimated. This study aimed to assess the prevalence and spatial patterns of coinfections with *P.falciparum*, *P.ovale*, *P.malariae* using molecular and genomic data. We analyzed data from the Zambia 2018 Malaria Indicator Survey (MIS) with light microscopy (LM), PET-PCR, and RDT results for dried blood spots. The overall prevalence of *P. falciparum* by PET-PCR was 19.6% (747/3817). Of the PET-PCR *Pf*-positive samples, 3.7% (28/747) were positive for *P.ovale*/*P.malariae* by LM, although this is likely higher due to 13.5% of samples lacking *P. ovale*/*P.malariae* microscopy results. To assess whether we can untangle *Plasmodium spp*. coinfections by mining available WGS *Pf* genomic data, we selected a subset of samples coinfected with *P.ovale*/*P.malariae* and *P.falciparum*, and samples only *Pf*-infected, and mapped reads to 4 reference genomes (*P.falciparum*, *P.malariae*, *P.ovale curtisi*, *P. ovale wallikeri*). Coinfected samples yielded a higher percentage of apicoplast mapped reads ($p<0.05$) for *P.ovale w.* when comparing apicoplast mapped reads for coinfected vs. *Pf*-infected samples across species. Also, *P.ovale w.* and *P.malariae* had a significant difference ($p<0.05$) between percentage of bases with >5X read coverage for coinfected relative to only *Pf*-infected samples, indicating these samples are likely coinfected with *P.malariae*/*P.ovale w.* These results improve our understanding of prevalence and spatial distribution of coinfections across Zambia. Further analysis with more samples is needed and we will assess how coinfections impact anemia and parasitemia levels.

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ENVIRONMENTAL RISK OF MALARIA INFECTION ALONG THE MOZAMBIQUE AND ZIMBABWE BORDER

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Mozambique currently has the 5th highest malaria prevalence globally. Malaria incidence varies within country, with it being lowest in the south and highest in the north. The central area experiences sustained high incidence and prevalence and has heterogeneous environments and ecologies. Sussundenga District in Manica Province directly borders Zimbabwe and has a varying ecology which impacts malaria transmission at the community level. We conducted a community cross-sectional survey from December 2019-February 2020 to determine the malaria prevalence, risk factors, and impacts of severe weather events. *P. falciparum* malaria prevalence in this area was high at 31%. We collected MODIS environmental data from NASA including land surface temperature (LST), evapotranspiration (ET), normalized difference vegetation index (NDVI), land use, and elevation. We joined survey data with time static environmental data (land-use and elevation) by spatial location of the household collected during the survey. We joined time varying environmental data (LST, ET, and NDVI) data to survey data by spatial location with a 2-week lag in time from survey data collection. We used mixed effects logistic regression models to estimate associations between environmental factors while controlling for individual risk factors. We investigated the impacts of malaria control interventions in varying ecologies in the area. Our analysis was done at the community level, with direct implications for identifying transmission hotspots. Along the Mozambique, Zimbabwe border differing malaria control policies are in place with heterogeneous environments, while already experiencing early impacts of climate change. Our findings will show how these control policies and environments impact malaria transmission in this setting.

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SEROLOGICAL MARKERS OF PREVIOUS EXPOSURE TO PLASMODIUM FALCIPARUM AND P. VIVAX IN WESTERN ETHIOPIA

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Sub-Saharan Africa sustains a disproportionate share of the global malaria burden. Malaria cases and deaths in Africa have historically been attributed almost exclusively to *Plasmodium falciparum* infections, as *Plasmodium vivax* was previously thought to be absent. With the advent of more sensitive diagnostics, *P. vivax* infections have been increasingly reported. Concerted efforts targeting *P. falciparum* and *P. vivax* are urgently needed in co-endemic areas, such as Ethiopia, in order to achieve successful malaria elimination. The purpose of this study was to identify factors associated with fine-scale heterogeneities in antibody responses to serological markers of *P. falciparum* and *P. vivax* infections. We conducted a cross-sectional immuno-epidemiological study (N=746) in two areas of western Ethiopia with contrasting malaria transmission intensities: Arjo in the Oromia Region (low transmission area) and Gambella in the Gambella Peoples' Region (high transmission area). We measured participants' antibody reactivity to a panel of 6 *P. falciparum* (AMA-1, CSP, EBA175 RIII-V, MSP-1, MSP-3, RH2ab) and 4 *P. vivax* (DBPSal-1, EBP2, MSP-1, RBP2b) antigens to gain a better understanding of cumulative and recent malaria exposure in the study area. As expected, people residing in Gambella had higher prevalence and levels of antibodies to *P. falciparum* and *P. vivax* compared to people residing in Arjo. We used mixed effects logistic regressions to assess predictors of antibody response at the species-level and for each antibody. Age and male sex were strongly positively associated with odds of seropositivity in Arjo only. Distance to nearest perennial water source or irrigation scheme was not a strong predictor of antibody seropositivity in Arjo or Gambella. Concurrent *Plasmodium* PCR infection data will be added to the model when complete. Variations in individual- and community-level antibody prevalence and levels between the low and high transmission sites suggest the potential for adapting this serological panel to monitor spatiotemporal trends in transmission intensity following implementation of malaria control interventions.

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ANALYSIS OF PREDICTORS OF INSECTICIDE TREATED NET USE (ITN) IN GUINEA USING MACHINE LEARNING ALGORITHMS

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Malaria is the leading cause of outpatient visits (34%) and deaths (28%) among children in Guinea. The use of insecticide-treated nets (ITNs) reduces the burden of malaria. Despite very good household net ownership (61%), net use (23%) remains low in Guinea (2018 Demographic and Health Survey (DHS)). We used a machine learning model to identify predictors of ITN use to inform intervention planning. Data was obtained from the 2018 DHS. We first used a Rao Scott Chi-square test to select significant variables for inclusion in a Chi-square automatic interaction detector (CHAID) model. Region, marital status, household size, household wealth quintile, gender, place of residence, and educational level of the head of household were the significant variables. To identify the optimal predictors of ITN use, CHAID uses a multi-level successive fitting algorithm. In each level, the model identifies the predictor variable with the strongest association with the outcome. We obtained a model accuracy of 0.77 (0.767- 0.774). A logistic regression model was used to estimate odds ratios and 95% confidence intervals.Region was the best predictor of ITN use in the general population, with greatest use in the N'zerekore region, followed by the Faranah region. Using the Boke region as reference, the odds ratio of using an ITN was 1.90 times higher in the N'zerekore region (95% CI 1.35-2.67, p<0.001) and 0.51 times lower in Labe (95% CI 0.36-0.74, p<0.001). Place of residence, household size, and household wealth quintile were other significant predictors, depending on the region. In the region of N'zerekore, place of residence (urban or rural) was the most significant predictor of ITN use. In contrast, in Boke, the predictor most associated with ITN use was household size, with households of 8 or more members having lowest ITN use. The results of this study will allow the Guinea National Malaria Control Program to understand how to target ITN distribution in different regions of Guinea. Improved targeting of distribution and communications campaigns could have a huge impact on malaria burden and potentially allow Guinea to reach Global Technical Strategy targets for 2030.

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ANALYSIS OF MALARIA TRENDS WITH RESPECT TO THE IMPLEMENTATION OF CONTROL INTERVENTIONS BETWEEN 2014 AND 2020 IN DANGASSA, MALI

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Over the past decade, three strategies have reduced severe malaria cases and deaths in endemic regions of Africa, Asia, and the Americas, specifically: 1] artemisinin-based combination therapies (ACTs), 2] insecticide-treated nets (ITNs), and 3] intermittent preventive treatment with sulfadoxine-pyrimethamine in pregnancy (IPTp). Recent studies have shown significant declines in malaria incidence between 2010 and 2018. This study aims to analyze seven years of data for Dangassa, Mali in relation to implementation of control interventions from 2014 to 2020 in an effort to facilitate evidence-based and area-specific malaria interventions. This prospective study was based on a rolling longitudinal cohort of 1,401 subjects between 2014 and 2020. Participants were screened biannually at the start and the end of the malaria transmission season for the prevalence of asymptomatic *Plasmodium (P.) falciparum* infection. Incidence was assessed through passive case detection at community health centers. Electronic data capture and cloud-based databases were used for data collection, capture and management. Prevalence of infection ranged from 9.5% to 62.8% and from 15.1% to 66.7% at the start and the end of the transmission season, respectively. The incidence of uncomplicated malaria was found to remain high among children aged under five years. Starting 2015, we observed a shift in malaria prevalence, with more asymptomatic infection occurring among children aged 5 to 14 years. At baseline, prevalence of *P. falciparum* was over 60% followed by a significant year-to-year decline starting in 2015. Incidence of uncomplicated infected was greater among children aged under five years, while asymptomatic infection was more frequent among children aged five to 14 years. Efficient Implementation in 2015 of two main malaria prevention strategies in Dangassa substantially contribute to a reduction of both asymptomatic and symptomatic malaria in Dangassa from 2015 to 2020. The findings for this study are consistent with the progressive implementation of effective malaria control strategies in Dangassa.

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DECLINING MALARIA TRANSMISSION AND ANTIBODY PROFILE KINETICS TO PLASMODIUM FALCIPARUM: A LONGITUDINAL STUDY IN SOUTHERN ZAMBIA FROM 2008 TO 2015

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Limited information is available on the kinetics of the antibody responses to *Plasmodium falciparum* in low transmission settings. The catchment area of Macha Hospital in Southern Province, Zambia was mesoendemic, with malaria transmission peaking during the rainy season, but transmission declined dramatically over the past two decades and the area is now approaching elimination. We collected dried blood spots from 637 individuals (median age at baseline 13 years, interquartile range = 5.6, 23) repeatedly between 2008 and 2015 when malaria transmission declined. Parasite prevalence by rapid diagnostic test (RDT) among cohort participants was 4% in 2008 and 0% in 2015. Using a subset of samples (n=12), we previously tested the use of a multiplex bead assay comprised of 19 *P. falciparum* antigens and showed that higher antibody response was associated with the age at baseline, but not to the seasonality, suggesting that the antibody response acquired before the enrolment persisted instead of exposure to the parasite between 2008 and 2015. We are expanding this analysis to the complete longitudinal cohort of 637 individuals. Our aim is to obtain information on the baseline immunological profile as well as temporal variations in antibody responses in a setting of declining *P. falciparum* transmission. For every 637 participants, demographic information and malaria-related information (RDT, microscopy, bed net use) are available, and together, these data will provide insight into the kinetics of antibody responses to falciparum malaria in a near-elimination region.

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THE EMERGENCE OF A NEW MALARIA HOTSPOT: IMPORTED AND INDIGENOUS MALARIA IN THE BORDER STATE OF RORAIMA FROM 2016 TO 2020 AND CHALLENGES DURING THE COVID-19 PANDEMIC

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Since the political and economic crisis began in Venezuela in 2010, the country has become a hotspot for malaria in the region. The crisis has resulted in the mass migration of Venezuelans to other countries in Latin America, with over 260,000 Venezuelan migrants officially entering Brazil since 2010. Their primary entry point into Brazil is Pacaraima, in the northernmost state of Roraima. Little is known about how the epidemiology of imported and indigenous malaria in Roraima has changed since the surge in migration. Using surveillance data from the Malaria Epidemiological Surveillance and Case Notification System in Brazil (SIVEP), we describe seasonality, malaria parasite species composition, key populations, and temporal distribution of cases including imported and indigenous cases from 2016 to 2020. Using logistic regression methods, we identify risk factors associated with *Plasmodium falciparum* (Pf) and *P. vivax* (Pv) infection, and for imported versus indigenous cases. Between 2016 and 2020, there were 81,329 reported local cases compared to 15,089 imported cases. The proportion of reported cases in areas classified as indigenous increased in Roraima from 18.5% in 2017 to 63.8% in 2020. Pf cases declined from 7.4% in 2016 to 1.7% in 2017 but rose to 15.1% in 2020. The trend in imported versus autochthonous cases was highest in 2016 at 36.3%, decreasing to 3.9% in 2020. Overall, most imported cases were from Venezuela (81.8%), followed by Guyana (17.6%), but in 2020, cases from Venezuela dropped to 74.1%. During the study period, there was an increase in the total number of malaria cases in Roraima, likely generated from the influx of Venezuelan migrants arriving in the state. However, in 2020, when the border with Venezuela closed in response to the COVID-19 pandemic, there was a marked reduction in imported cases, but an increase in the total number of cases. Additionally, species composition shifted away from Pv to Pf. Establishment of strong cross-border surveillance systems and identification of the high-risk activities and demographic groups are needed to appropriately target and tailor interventions to maximize impact.

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PREDICTORS AND ASSOCIATIONS WITH MATERNAL AND BIRTH OUTCOMES OF PLASMODIUM FALCIPARUM INFECTION IN THE FIRST TRIMESTER AMONG NULLIPAROUS WOMEN FROM THE DEMOCRATIC REPUBLIC OF THE CONGO, KENYA, AND ZAMBIA

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Malaria infection in the first trimester of pregnancy could disrupt placentation and thus impair fetal growth. Despite the concern for adverse pregnancy outcomes associated with first-trimester malaria infection, malaria prevention measures are rarely initiated until the second trimester. We sought to (i) identify predictors of first-trimester malaria and (ii) estimate its associations with adverse maternal and birth outcomes. We studied a subset of participants from the ASPIRIN (Aspirin Supplementation for Pregnancy Indicated Risk reduction In Nulliparas) trial. Recruited from multiple sites in Democratic Republic of the Congo (DRC), Kenya, and Zambia, 1,513 nulliparous pregnant women were tested for first-trimester *Plasmodium falciparum* infection by qPCR and analyzed using parametric g-computation. First-trimester malaria prevalence among nulliparous pregnant women was 62.9% in DRC, 37.8% in Kenya, and 6.3% in Zambia. We first considered factors associated with first-trimester malaria in hopes of developing better prevention strategies. Predictors associated with higher crude first-trimester malaria prevalence included younger age (<20 years) among Kenyan women and lower education (no secondary education) among women from all three sites when pooled. We then considered associations of first-trimester malaria with pregnancy outcomes. After adjusting for confounders, first-trimester malaria was associated with higher prevalences of preterm birth (adjusted PD (aPD) = 0.06 [99% CI: -0.04, 0.16]) and low birth weight (aPD = 0.07 [99% CI: -0.03, 0.16]) among Congolese women only, and higher prevalence of anemia in late pregnancy among Kenyan (aPD = 0.05 [99% CI: -0.06, 0.17]), Zambian (aPD = 0.07 [99% CI: -0.12, 0.36]), and Congolese

women (aPD = 0.04 [99% CI: -0.09, 0.16]). In this first large multi-site study of first-trimester malaria, the high prevalence of infections in high-transmission areas along with associations of first-trimester malaria with higher prevalences of adverse maternal and birth outcomes suggest that screening women early in pregnancy may be needed to reduce the impact of first-trimester malaria.

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RESIDING AND TRAVELING AT THE ZIMBABWE-MOZAMBIQUE BORDER: A STUDY OF BORDER MALARIA IN MUTASA DISTRICT, ZIMBABWE

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International vector and human population movement are recognized as contributors to malaria transmission in border regions and impediments to national malaria elimination. However, their contribution in areas with seasonal malaria is not well characterized. In eastern Zimbabwe, border malaria with Mozambique is believed to play a key role in transmission. This study estimated parasite prevalence in Mutasa District, Zimbabwe, using community-based active surveillance data from 2012 through 2020. Changes in parasite prevalence relative to household distance to the border and self-reported travel to Mozambique were estimated using a Poisson regression model with robust variance estimation. The population attributable fraction arising from travel to Mozambique was estimated via bootstrapping. Living closer to the Zimbabwe-Mozambique border and recent overnight travel to Mozambique were significantly associated with increased parasite prevalence in Mutasa District. The relative risk of malaria declined 14% (95% confidence interval = 0.81, 0.91) with each additional kilometer from the border, while travel to Mozambique was associated with an 171% increase in risk (95% confidence interval = 1.42, 5.19). However, only roughly two percent of observed cases were estimated to be attributable to travel. Future control efforts may consider binational malaria control programming, including coordinated indoor residual spraying and long-lasting insecticide-treated net distribution, in addition to continued support for border health posts, clinics, and community health workers.

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DETERMINING ANTIGEN DIVERSITY AND IMMUNE SELECTION TARGETS OF LEADING PLASMODIUM VIVAX VACCINE CANDIDATES AND SEROSURVEILLANCE MARKERS

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Attention on *Plasmodium vivax (Pv)* infection and its impact on the global malaria control and elimination campaign is limited. One of the major gaps in *Pv* research is the lack of information on the diversity of surface antigens, which could provide relevant details on the parasite population structure and vaccine development. Population genetic data can not only assess transmission intensity but also provide insights in detecting genes under immune selection. We, therefore, aimed to determine the global distribution of antigen diversity and identify gene regions with signature of immune selection amongst leading *Plasmodium vivax* vaccine candidate antigens and sero-surveillance markers. Several signatures of diversity and balancing selection were measured based on published sequences from multiple geographic populations such as Thailand (n=111), Peru (n=40), and Papua New Guinea (n=21). Genetic relatedness of the sequences was visualized through haplotype network diagrams. Furthermore, sequences were mapped onto experimentally defined three-dimensional protein structures to analyze spatially derived nucleotide diversity (π) and immune selection. Initial results showed low nucleotide and haplotype (Hd) diversity in *P. vivax* antigen genes *rama* (π=0.68; Hd=0.71), $msp1_{19}$ (π=0.63; Hd=0.19), and *csp* (π=0.20; Hd=0.10); which is supported with limited immune selection hotspot. In contrast, high diversity was observed in antigen genes *rbp1a* (π=2.79; Hd=0.99), *rbp2b* (π=1.52; Hd=0.99), *dbp*RII (π=7.68; Hd=0.96), and *ama1* (π=6.89; Hd=0.99), which is consistent with the observed distantly related sequences in the haplotype network plots. Lastly, spatially-derived nucleotide diversity and Tajima's D were similar between different geographic populations suggesting that gene regions, which are the targets of protective antibodies, are potentially consistent across different populations. The data from this study will guide researchers in designing widely effective vaccines and serological tools against *P. vivax* parasites.

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CONTROLLED HUMAN MALARIA INFECTIONS WITH NF54 AND 7G8 STRAINS ELICIT DIFFERENTIAL ANTIBODY RESPONSES TO PLASMODIUM FALCIPARUM PEPTIDES

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Extensive genetic diversity of *Plasmodium falciparum* plays a role in immune system evasion. In endemic settings, past infection history confounds studies examining allele-specific versus cross-protective immune responses. Controlled human malaria infection (CHMI) allows the investigation of immune responses to diverse parasite proteins after a single infection with a known strain. Using a diversity-reflecting peptide microarray, we probed sera from 38 malaria naïve adults at baseline and 28 days after CHMI with *P. falciparum* (*Pf*) NF54 (n = 21) or *Pf* 7G8 (n = 17). The array contained 22,656 protein variants from 23 genome assemblies and field-derived genomic data from 411 samples for 227 *Pf* protein-coding loci, collected from 9 countries in 3 continents. Each variant was represented by 16 amino acid (aa) peptides with 15 aa overlap. Quantitative seroreactivity was based on average smoothed $\log_2$ fold changes relative to baseline. Serorecognition was defined as post-CHMI maximum smoothed $\log_2$ signal intensity >2.5 standard deviations above baseline of all subjects; a protein variant was considered serorecognized at the cohort level if signal for >15% of subjects exceeded the cutoff. All 227 proteins were assessed for target epitopes using ABCPred. The seroreactivity analysis identified 193 proteins with differential responses, of which 25 were also serorecognized. The 7G8 group had higher mean seroreactivity across all 25 proteins compared to the NF54 group. ABCPred predicted epitopes with a score of >0.8 in 52 proteins on the array; 27 overlapped with epitopes identified by our peptide array, five of which were highly cross-reactive with >95% of variants serorecognized. Overall, the peptide array discriminated strain-specific responses to peptide variants and identified cross-reactivity to predicted epitopes after a single malaria episode. Interestingly, despite earlier malaria diagnosis and antimalarial treatment (by 2.5 days), the 7G8 group had higher seroreactivity to 85% of proteins on the array. Further characterization of broadly serorecognized proteins may lead to a better understanding of acquired immunity to malaria.

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DEEP IMMUNOPROFILING OF FOLLICULAR HELPER (TFH) T CELLS ASSOCIATED WITH PLASMODIUM FALCIPARUM PFSEA-1A AND PFGARP RESPONSES FROM ADULTS AND CHILDREN IN KENYA

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Follicular Helper (TFH) T cells are essential to promote B cell class-switching and antibody production which make them a key player in the establishment of protective immunity against *Plasmodium falciparum* (Pf). However, because of their low frequency in peripheral blood, investigating human TFH cell subsets can be challenging. In this study we used OMIQ, an unbiased flow cytometry analysis platform to assess TFH cell subsets from adults and children living in a malaria holoendemic area of Kenya. Peripheral blood mononuclear cells from 15 adults and 14 children (7 years old) were stimulated *in vitro* with two malaria vaccine-candidate antigens, PfSEA-1A or PfGARP and stained with a 20-color TFH flow cytometry panel. PfSEA-1A and PfGARP specific IgG antibody levels and function were assessed by Luminex and C1q fixation, respectively. Using unbiased analysis methods instead of conventional gating for multiparameter flow cytometry data, we found a unique TFH cell subset (CXCR5intCD45RA+CCR7+CCR6neg CXCR3negPD1negICOSlow) only in children, yet it did not respond to malaria-antigen stimulation. Our analysis revealed graduated CXCR5 expression (classified as high, intermediate or low) for more activated TFH cell subsets that expressed less CCR7 and more ICOS and PD-1 within adults in contrast to children. After *in vitro* stimulation, PfGARP induced IL4 production from CXCR5intCCR6negCXCR3lowPD1intICOShigh TFH2-like cells (i.e. more efficient helpers) in adults but IL-21 from CXCR5intCCR6negCXCR3highPD1intICOSint TFH1-like cells in children. Interestingly, upon PfSEA-1A stimulation, IFNγ;; was produced by CXCR5highCCR6negCXCR3highPD1intICOSint TFH1-like cells only in adults, whereas we did not observe any response from children. Our findings are consistent with other studies of malaria and TFH cell subset phenotypes associated with age. However, our unbiased analysis methods revealed more granularity and antigen-specificity for TFH cell subsets. This has implications for evaluating the role of TFH cells in malaria vaccine-elicited antibody responses.

1114

PLASMODIUM LIVER STAGE-TARGETED ANTIBODIES CAN REDUCE PARASITE LIVER BURDEN AND SYNERGIZE WITH ANTI-CSP ANTIBODIES TO PROVIDE ENHANCED STERILE PROTECTION

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Antibodies (Abs) have long been known as important for protection against *Plasmodium* infection. However, neither traditional nor emerging Ab candidates appear capable of providing sufficient protection when targeted by vaccines or monoclonal Ab. The few efforts to combine antigens have confounded by mixing of vaccine platforms and issues of apparent immune interference. To date, nearly all Ab-based efforts have focused on preventing parasite cell entry at the extracellular sporozoite, merozoite or gametocyte stages with little consideration of the multi-day intracellular liver stages—likely because they reside within a parasitophorous vacuole membrane (PVM) within the hepatocyte cytoplasm. Here, we demonstrate that the PVM protein UIS3 is susceptible to Ab-mediated inhibition during these intracellular liver stages. Specifically, passive transfer of polyclonal anti-*Pf*UIS3 Abs can clear >90% of liver stage parasites in mice infected with *P. berghei* parasites carrying the *Pf*UIS3 gene as a replacement. This occurred when Abs were administered 4 hours *after* intravenous sporozoite injection when all parasites have left the circulation. Antibody efficacy was minimally affected by the absence of host FcRn, indicating the lack of receptor-mediated interaction with hepatocytes for parasite killing. On their own, anti-*Pf*UIS3 Abs did not provide any sterilizing protection in this model but increased the protective capacity of a suboptimal anti-*Pb*CSP monoclonal Ab from 0% to 60% when used in combination. Further mechanistic and monoclonal Ab studies are under way in wild type and humanized mice. These data confirm a single previous publication showing that the *Plasmodium* liver stage, and in particular PVM proteins, are susceptible to Ab-mediated inhibition. Moreover, they clearly demonstrate the potentiating power of a hereto unrealized class of Ab targets and add to a growing body of literature within and outside the malaria field indicating that combinations of Abs targeting different proteins and/or stages of infection will be needed to achieve high levels of protection.

1115

EVIDENCE OF ACTIVATION OF HOFBAUER CELLS IN ACTIVE PLACENTAL MALARIA

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Placental malaria (PM) is characterized by *Plasmodium falciparum*-infected red blood cells sequestering in the placenta. We previously showed that placental malaria is associated with increased levels of tumor necrosis factor alpha (TNF-α), gamma interferon (IFN-γ), CXCL9 and inflammatory cell infiltrate. Hofbauer cells (HBCs) are fetal macrophages residing in the stroma of placental villi, are generally described as M2-type macrophages. However, there have been limited studies on the HBC response to the pro-inflammatory environment during PM. Here, we assess whether there are changes in HBC polarization during inflammation associated with PM by comparing placentas from uninfected women, women infected during pregnancy and women infected at delivery. We conducted an immunohistochemical analysis of primigravid placental tissue samples using the following surface markers: IBA-1 (pan-macrophage marker), CD68 (M1, classically activated) and CD163 (M2, alternatively activated). Marker-positive areas were normalized to the total villous areas and the resulting percentage (%Area) logit transformed (log[%Area/(100-%Area)]) and compared. No significant differences ($p < 0.05$) were found in the expression of CD68 and CD163 between uninfected placentas (n=10) and those infected during pregnancy (n=8) or at delivery (n=12). IBA-1 adjusted signal was higher in placenta samples infected at delivery compared to uninfected placenta samples (6.5% vs 5.6% IBA-1 positive areas), but the differences did not achieve significance (p=0.08). IBA-1 expression was also higher in placental samples from women infected during pregnancy compared to uninfected, but the differences were not

significant (p = 0.1). The increase in IBA-1 observed in this pilot study suggests that a proinflammatory environment might modify HBCs during PM. Of note, this study did not examine whether IBA-1 signal may have changed due to an increase in HBCs numbers or IBA-1 expression. In future, we will isolate HBCs to explore changes in numbers and subtypes of HBCs using multiple surface markers.

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LONGITUDINAL MEASUREMENTS CHARACTERIZE ROLE OF INFLAMMATORY DYSREGULATION IN SEVERE MALARIAL ANEMIA

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We present a statistical analysis of the relationship between circulating inflammatory mediator production (25-plex) in children with acute malaria (day 0, pretreatment) and upon recovery (day 14, posttreatment). Children (n=70, age 3 to 36-months) were recruited at Siaya County Referral Hospital in western Kenya, a holoendemic *Plasmodium falciparum* transmission region. Fourteen of the children had severe malarial anemia (SMA), defined as presence of *P. falciparum* parasitemia (any density) and hemoglobin (Hb) levels below 5.0 g/dL, while 56 children had uncomplicated malaria (UM, *P. falciparum* parasitemia and Hb≥5.0 g/dL). Children with SMA had elevated levels of lymphocytes and monocytes and decreased production of type-1 cytokines (i.e., IFN-γ;, IL-12, and TNF-α;). SMA was also defined by a strong negative correlation between monocyte counts and blood glucose levels, an important metabolite for type 1 macrophages (M1) that is necessary for appropriate effector functions. Analysis of the inflammatory mediators on day 14 revealed that cytokines/chemokines associated with SMA on day 0 retained that association on day 14, with the exception of IL-12 and IFN-γ;. Between day 0 and 14, there was a significant change in 13 of the 25 inflammatory mediators. However, other than IFN-γ;, none of the inflammatory mediators that significantly changed across the timepoints were associated with the severity of malaria. Collectively, these results suggest that the pathogenesis of SMA is associated with an inefficient (suboptimal) type-1 immune response for macrophage polarization and that the observed imbalance in M1/M2 may be impacted by glucose levels.

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A PARASITE PROTEIN THAT LOCALIZES TO THE LIVER STAGE NUCLEUS IS ESSENTIAL FOR ITS COMPLETE MATURATION

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The biology of *Plasmodium* liver stage (LS) development remains largely unexplored. We used our curated LS transcriptome datasets to find LS-specific transcripts and identified an uncharacterized gene, PY17X_1465200 (*Py146*). We created an mCherry tagged *Py146* (Py146-mCherry) and detected mCherry expression only in the LS, starting at 36 hours post infection. Py146-mCherry localized to the nucleus and partially co-localized with histone markers. To further evaluate the function of Py146 in LS development, we generated a *Py146* gene-deficient parasite strain (*Py146*$^-$), which displayed normal blood stage and mosquito stage development. However, LS development was severely impaired and only 50% of BALB/c mice infected with 50,000 *Py146*$^-$ sporozoites transitioned from liver stage-to-blood stage. Mice that did become patent were severely delayed in their time to patency - wildtype infected mice became patent on day 3 whereas *Py146*$^-$-infected mice became patent between days 8 and 10. Late *Py146*$^-$ LS schizonts displayed abnormal plasma membrane invaginations and lacked well segregated exo-erythrocytic stage merozoites, indicating severe attenuation in completion of LS development. To investigate whether the observed defect in LS development was conserved across species, we generated a *Plasmodium falciparum* strain lacking the orthologous gene *Pf124 (Pf124*$^-$*).* LS development was evaluated by infecting FRG huHep mice with *Pf124*$^-$ sporozoites. *Pf124*$^-$ late LS schizonts also showed defects in the formation of exo-erythrocytic merozoites. Two of three mice infected with *Pf124*$^-$ sporozoites underwent liver stage-to-blood stage transition after infusion of human red blood cells, however, there was a 60,000-fold reduction in parasite load at the time of transition compared to wildtype. These results indicate that we have identified a novel candidate for the creation of a late liver stage arresting replication competent genetically attenuated parasite (LARC GAP) vaccine for pre-clinical study.

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ANTIBODIES TO PFEXP1 AND PFMSP1 ASSOCIATED WITH LACK OF INFECTIVITY IN CONTROLLED HUMAN MALARIA INFECTION IN EQUATOGUINEAN ADULTS

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Studies of mechanisms of protective immunity against *Plasmodium falciparum* (Pf) infection in the field are limited, because it is impossible to know if an individual was actually exposed to Pf during surveillance. Controlled human malaria infection (CHMI) circumvents this limitation. When $3.2x10^3$ PfSPZ of the PfNF54 strain (Sanaria® PfSPZ Challenge [NF54]) were administered by direct venous inoculation to 79 previously unexposed subjects in the US and EU, 100% developed Pf parasitemia. When the same dose was administered to 287 subjects in Tanzania, Kenya, Gabon, Mali, the Gambia and Equatorial Guinea (EG), the percent developing parasitemia ranged from 53.3% to 100%, likely reflecting different levels of naturally acquired immunity (NAI). For example, in a vaccine trial in EG, 13 of 19 (68%) of controls developed Pf parasitemia. A prior study in Tanzania had indicated that in subjects with low-level malaria exposure, antibodies to PfEXP1 and PfMSP1, proteins 1st expressed in the mid to late liver stages and in blood stages developed 4 weeks after first CHMI in 90.5% and 76.2% of subjects, respectively. We, therefore, assessed antibody levels (Abs) to PfEXP1 and PfMSP1 by ELISA in sera collected at time of CHMI in the EG trial to see if they were associated with protection against detectable parasitemia. Using the serum dilution at which the optical density was 1.0 (OD 1.0), the median PfEXP1 OD 1.0s were 2,960 vs. 456 (p=0.0125) and PfMSP1 OD 1.0s were 1,986

vs. 349 (p=0.0462) in non-infected and infected subjects, respectively. We interpret these findings to indicate that Abs to these antigens may be a correlate of functional NAI, but cannot determine if absence of detectable asexual blood stage parasitemia resulted from sporozoite and liver stage immunity, asexual blood stage immunity or a combination of both. Assessment of pre-CHMI sera from subjects in other PfSPZ Challenge in Tanzania, Kenya, Mali, Gabon, and EG for anti-PfEXP1 and PfMSP1 antibodies and by systems serology analysis will be done and presented. The goal is to use such data to increase our understanding of the mechanisms of protective immunity against Pf.

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THE IMPACT OF LOW MALARIA TRANSMISSION INTENSITY ON THE DEVELOPMENT OF ANTIBODY TO PLASMODIUM FALCIPARUM IN PREGNANCY

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Pregnant women have increased susceptibility to *Plasmodium falciparum* malaria, caused by the placental accumulation of infected erythrocytes (IEs) via a parasite ligand called VAR2CSA. Protective antibodies targeting the variant surface antigens (VSA) expressed on IEs are acquired in successive pregnancies; however, the development of these antibodies may depend on a combination of factors, including transmission intensity. Using plasma from 408 Brazilian pregnant women (207 women with *P. falciparum* infection and 201 without infection), we explored the dynamics of the antibody acquisition to pregnancy malaria in a low transmission setting. We measured IgG antibodies to pregnancy-specific and non-pregnancy-specific *P. falciparum* antigens using flow cytometry, ELISAs and Luminex assays. Only 7.8% of subjects had developed IgG that recognizes the pregnancy-specific VSAs on IES. The levels of these antibodies were higher in multigravidae than primigravidae (p=0.01), and malaria-infected women were more likely to have these antibodies (AOR (95% CI) = 4.1 (1.8, 9.1), p= 0.001) than uninfected women. A high proportion of women (24% to 63.7%) acquired antibodies that recognized recombinant VAR2CSA domains with the DBL5 being the most often recognized domain, however, levels of these antibodies did not differ between women with and without a history of malaria (p>.0.052). Moreover, these antibodies did not vary by gravidity (p>0.362). As expected, malaria-infected women had significantly higher antibody levels to non-pregnancy malaria-specific antigens, MSP1,19 (arbitrary unit median=28 vs 0.7, p<0.0001), and schizont extract (arbitrary unit median=6 vs 0.3, p<0.0001), compared to uninfected women. In correlation analyses, antibodies to VAR2CSA domains correlated with each other (r >0.55, p<0.0001) but not with the antibodies to VSAs on IEs (r<0.12, p>0.06). In summary, pregnant women living in a low transmission area in Brazil acquired low levels of antibodies to pregnancy-specific VSAs on IEs but more frequently recognized VAR2CSA domains and non-pregnancy associated *P. falciparum* antigens.

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LIVER STAGE PLASMODIUM INFECTION-INDUCED IFN-I SIGNALING REMODELS INTRAHEPATOCYTIC SIGNALING TO IMPAIR HEPATIC CD8 T CELL MEMORY

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Plasmodium liver stage infection is detected by host innate immune sensing pathways to initiate type I Interferon (IFN-I) signaling. This response has limited impact in controlling a primary infection, but we recently reported that IFN-I deficient mice immunized with live, whole sporozoite vaccines generate superior CD8 T cell responses compared to immunized wildtype mice. Importantly, a hallmark feature of this IFN-I-mediated, inferior CD8 T cell response is the increased expression of PD-1 and Lag-3, two markers of T cell dysfunction typically associated with chronic infections. We now show that T cell dysfunction is observed in hepatic memory CD8 T cells and not memory CD8 T cells in secondary lymphoid organs or in circulation, suggesting that hepatic T cell dysfunction is driven by immunological events in the liver. In support of this, we now show that immunized mice lacking the ability to propagate IFN-I signaling solely in hepatocytes do not generate significant frequencies of dysfunctional PD-1^{hi} hepatic memory CD8 T cells. Transcriptomic analyses of hepatocytes isolated from *Plasmodium yoelii*-infected mice, show that the induction of IFN-I signaling coincides with substantial remodeling of hepatocyte gene expression. We observed the upregulation of antigen presentation pathways, immunoregulatory cytokines, chemokine transcripts to facilitate immune cell recruitment into the liver and receptors implicated in the induction of T cell exhaustion signaling. Thus, IFN-I signaling likely reshapes signaling networks in hepatocytes to establish an immunosuppressive liver microenvironment around each infected hepatocyte that in turn promotes the development of dysfunctional hepatic CD8 T cell memory. This likely encumbers natural immunity to liver stage infection and lessens the efficacy of whole attenuated parasite vaccines. Our future studies will unearth the molecular mechanisms underpinning this dysregulated immune response and identify avenues for improving the quality of vaccine engendered pre-erythrocytic immunity.

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ESTABLISHING A RELIABLE PLASMODIUM KNOWLESI (PK) SPOROZOITE (SPZ)-BASED IMMUNIZATION AND CHALLENGE MODEL IN RHESUS MACAQUES

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We aim to improve *Plasmodium falciparum* (Pf) sporozoite (SPZ)-based vaccines against malaria, with parallel efforts focused on developing a reliable animal model to interrogate immunological mechanisms of SPZ-based vaccines in non-human primates (NHPs). To test the protective efficacy of irradiated (irr), purified, cryopreserved *P. knowlesi* (Pk) SPZ in rhesus macaques we based the immunization regimen on previous mouse studies with irr *P. yoelii* (Py) SPZ, and a recent clinical trial of PfSPZ Vaccine at the University of Tübingen in Germany in which 3 doses of $9.0x10^5$ PfSPZ Vaccine administered on days 1, 8, and 29 protected 83% of volunteers against a heterologous controlled human malaria infection (CHMI) 9.5 weeks after last dose of vaccine. We similarly immunized a total of six rhesus by DVI with $1.0x10^6$ irr PkSPZ (3 animals also received an adjuvant) on days 1, 8, and 29. As controls, 3 animals received adjuvant and 2 received diluent. Fifty days after the last dose of vaccine, the NHPs were injected with 1500 infectious PkSPZ. Of the 6 immunized animals, 3 remained parasitemia negative through the end of the study on day 21 post challenge, a highly significant 50% sterile protection by survival analysis (log rank). The mean pre-patent period for the 3 vaccinated NHPs that became infected was 13 days, compared to 8.6 days for 5 control NHPs ($p < 0.0001$, student's t test, unpaired). There are 8-10 merozoites in each Pk-infected erythrocyte and they rupture every 24 hours. If the replication rate every 24 hours was 5-fold, a 4.4 day increase in the prepatent period corresponds to a greater than 97.5% reduction in liver stage parasite burden in the three vaccinated NHPs that did not achieve sterile protection. In addition, this study constituted a third demonstration of the consistent infectivity of purified, cryopreserved, vialed PkSPZ. All control animals became parasitemic by day 8 or 9, with PkSPZ cryopreserved three years prior. We have now established a Pk model of immunization and challenge with Sanaria's purified, cryopreserved stocks of infectious and irradiated PkSPZ using an accelerated immunization regimen in non-human primates.

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THE ECONOMIC BURDEN OF MALARIA IN PREGNANCY IN ENDEMIC COUNTRIES: EVIDENCE FROM NIGERIA AND DEMOCRATIC REPUBLIC OF CONGO

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Malaria in pregnancy is a major public health problem in sub-Saharan Africa (SSA). Intervention strategies for malaria control during pregnancy are provided for free at the antenatal care (ANC) services of public health facilities. However, the coverages of such interventions are still low. Pregnant women face different barriers regarding malaria care, including remote access to health facilities, sub-optimal care management and limited availability of drugs. Households' costs associated with malaria health care may be an additional barrier. Estimating these costs is essential to informing policy decisions aimed at increasing access to malaria control interventions in pregnancy. We estimated malaria care-seeking and morbidity costs during pregnancy through a structured exit survey administered to 933 pregnant women when leaving the ANC visits in Nigeria and DRC. Women were asked to report direct and indirect costs incurred to attend the ANC services, as well as treatment costs associated to a malaria episode. Data collection, which is ongoing in Mozambique and Madagascar, is expected to finish in July 2021. The final analysis will include a pooled analysis across the 4 study countries. Average costs of attending an ANC visit, where malaria preventive treatment is provided for free, was 5.89 international dollars (I$). Of the 933 interviewed pregnant women, 64 sought care for severe malaria episodes (requiring hospital admission) and 304 for uncomplicated malaria episodes. The average cost associated with an episode of uncomplicated malaria was 46.97 I$. The cost incurred of an episode of malaria requiring hospitalization was 101.8 I$. For both complicated and uncomplicated malaria, women's indirect costs (value of the time lost in the main economic activity) constituted the largest share of total costs. Results of this study show that malaria infection imposes a high economic burden on pregnant women and their families and strengthen the importance of investing in malaria control strategies and thus reduce the burden of the disease during pregnancy.

1123

A DESCRIPTION OF MALARIA-RELATED KNOWLEDGE, PERCEPTIONS, PRACTICES, AND TREATMENT- SEEKING BEHAVIORS OF MOBILE MIGRANTS WORKERS IN JAZAN, SAUDI ARABIA

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Human movement along the Saudi/Yemen border is thought to play a strong role in malaria transmission in Saudi Arabia. Although mobile populations and migrant workers are a key population to containing malaria transmission, there is a limited representation of migrants in routine surveillance data. Understanding migrant's malaria-related knowledge, risk perceptions, treatment-seeking behavior, and use of prevention methods could increase the elimination program's success. A survey of migrant workers in the rural agricultural area took place in Abu-Arish region at the southern border of Saudi Arabia, using peer navigators. A questionnaire was administered, and the data were captured electronically.Bivariate and multivariate analysis was used to determine which factors are associated with preventive measurement and malaria infection. A total of 227 participants were included in the study. Ordinal logistic regression was used to identify factors associated with correct malaria knowledge and health perceptions.79% of migrant workers have been residents in Jazan region for more than six months. They have a 94% accuracy level of knowledge about malaria, which is considered high (214/227 = 94.27%). Overall, 65.2% of the participants had a positive attitude toward malaria. However, only 19% of the participants had received health messages or malaria information in the preceding six months. Bed net ownership was extremely low among participants, 5%, Interestingly more than two-thirds of the participants reported never visiting a health facility if they had a fever. Most of the migrant's agricultural border in Abu Arish do not receive health messages, nor do they benefit from the available malaria prevention tools in the area, which puts them at greater risk of malaria infection.

1124

A PATIENT FROM THE DEMOCRATIC REPUBLIC OF CONGO WHO TRAVELED TO CONNECTICUT THREE MONTHS AGO NOW PRESENTS WITH ARTHRALGIAS AND HEADACHE

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The patient was a 21-year-old male with no significant history originally from Kivu, Democratic Republic of Congo who reported 1-day history of joint pain. Four months prior, he traveled to Dar es Salaam, Tanzania, then Amsterdam, Netherlands. 2.5 months prior, he arrived to Connecticut, United States. One day prior, he noted 5/10 aching bilateral hip and knee pain. He reported a 1-day history of moderate headache, lower back pain, lower abdominal pain, and mild pain with urination. On exam, he was afebrile and hemodynamically stable aside from pulse of 102 bpm. He had bilateral conjunctival injection and mildly decreased range of motion with bilateral hip and knee flexion and extension. Laboratory data demonstrated low platelet count of 93,000 cells/μL, elevated total bilirubin of 3.1 mg/dL, hemoglobin level of 14.5 g/dL with MCV of 73 fL. Urinalysis demonstrated large ketones, trace blood. Quantitative buffy coat test was negative for parasites. Blood smear demonstrated malarial ring forms. Malaria Binax testing demonstrated <1% positive result for *Plasmodium vivax* or *Plasmodium malariae* or *Plasmodium ovale*. He was noted to be Duffy antigen Fy^{a+} and Fy^{b-}. He was treated with a course of atovaquone-proguanil and primaquine. In summary, malaria, caused by single-celled microorganisms, protozoans of the *Plasmodium* group, should be considered on the differential diagnosis in a traveler from a malaria-endemic area who may present with non-specific symptoms (i.e. arthralgias, headache). Classically, malaria is categorized into *P. falciparum* (more severe) and non-falciparum malaria, the latter of which includes *P. vivax*, *P. ovale, P. malariae,* and *P. knowlesi.* Signs and symptoms of malaria may include headache, fever, arthralgias, hemolytic anemia, jaundice, emesis. In terms of diagnostics, blood smear is the gold standard; qualitative immunochromatographic assay detecting *Plasmodium* antigens may support the diagnosis. Additionally, Duffy negativity offers protection against *P. vivax* blood-stage infection, however emerging evidence of *P. vivax* infection in Africa has been noted.

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COMMUNITY MALARIA RAPID DIAGNOSTIC TESTS (RDTS) BELIEFS IN WESTERN KENYA: HOW DOES EXPERIENCE WITH TESTING AFFECT CONFIDENCE IN, AND ADHERENCE, TO RDTS?

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The WHO recommends test-based management for malaria infections, a goal that has become more feasible with increasing access to malaria Rapid Diagnostic Tests (RDTs). However, community members' confidence in RDTs likely affects the success of such a policy, especially in places where many people seek treatment outside the formal health sector. We follow a cohort of 36 households (268) individuals in three villages in Western Kenya from June 2017, for a period of 30 months. We examined how experience with RDTs changes people's beliefs about RDT accuracy and how those beliefs affect behavior. Monthly surveys were conducted where questions about malaria-like illnesses experienced by household members in the past month, their treatment decisions, including whether the

individual was tested for malaria, and their beliefs about the accuracy of malaria RDT results were asked. Beliefs about RDT accuracy were elicited on a 5-point Likert scale from "very unlikely" to "very likely." Household members could request a free RDT from the study team any time they suspected a malaria illness. Over the study period, the proportion of survey respondents that said a hypothetical negative RDT result was "very likely" to be correct increased from approximately 55% to 75%. One year into the study period, we found that, controlling for initial beliefs, people who had been tested at least once with an RDT in the past year had 2.48 times higher odds (95% CI [0.99 6.22, P=0.054]) of saying a negative RDT was "very likely" to be correct, an effect that was largely coming from people who had received at least one negative test result in the past year (OR=2.30, 95% CI [1.13 4.69, P=0.022]. We also find evidence that confidence in testing is associated with behavior: those who believed a negative RDT was "very likely" to be correct had 1.91 times higher odds (95%CI [1.21 3.03], P=0.006) of adhering to a negative RDT result (by not taking ACTs) than those who were less certain about the accuracy of negative RDTs. Our results suggest that greater experience with RDTs can not only increase people's confidence in its accuracy but also improve adherence to the test result.

1126

NON-MONETARY STRATEGIES FOR MOTIVATION OF COMMUNITY HEALTH WORKERS IMPLEMENTING INTEGRATED COMMUNITY CASE MANAGEMENT OF MALARIA, PNEUMONIA AND DIARRHOEA IN UGANDA

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Integrated Community Case Management (iCCM) strategy aims to provide timely access to treatment for malaria, pneumonia and diarrhoea for under-fives in vulnerable communities. From 2010-2020, iCCM had rolled out to 120/135 districts in both public and private health facilities. iCCM is implemented by community health workers (CHWs) who receive basic training in major health programs to sensitize communities, offer treatment for malaria, pneumonia and diarrhoea to under-fives. The CHWs are given antimalarials, oral rehydration solution (ORS), Amoxicillin and sulfadoxine pyrimethamine. We aimed to assess the effectiveness of CHW motivation strategies. From January 2019 to December 2020, we interviewed 362 CHWs from 15 districts implementing the iCCM strategy in Uganda. The districts were purposively selected to represent the 15 participating regions, excluding Kampala. We used multi - stage cluster sampling to select CHWs. We administered a structured questionnaire to the CHWs and captured information about sociodemographic characteristics, current and proposed motivation strategies, knowledge about iCCM strategy and medical commodities. We analysed data using STATA version 15 Three hundred and sixty-two CHWs were included in the study. Over all, CHWs implementing the iCCM strategy in the private sector felt more motivated than those in the public sector. Of these, 243 (67%) received non-monetary motivation. VHTs felt motivated by; being trusted with medical supplies, provision of branded T-shirts, face masks and bags, and knowledge acquired during trainings. Other motivators were recognition as health workers at community and national level, recognition as high priority when seeking medical care at health facilities, provision of monthly facilitation and bicycles to aid mobility during routine work. CHWs proposed starting of savings' scheme, provision of monthly pay, and mobile phones and offering certificates after training as additional strategies. Although non-monetary incentive strategies motivated CHWs to implement iCCM strategy, regular financial compensation was suggested to achieve sufficient motivation.

1127

THE EFFECT OF THE INTEGRATED COMMUNITY CASE MANAGEMENT STRATEGY ON UNDER-FIVE MORBIDITY IN RURAL UGANDA

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Between 2010 and 2020, integrated community case management (iCCM) strategy was rolled out in 120/135 districts in both public and private health facilities in Uganda. The strategy aims to provide timely access to treatment for malaria, pneumonia and diarrhoea for children less than 5 years of age in rural Uganda. It is implemented at community level by trained community health workers (CHWs). We compared under-five morbidity between participating and non-participating districts in rural Uganda. Between January 2019 and December 2020, we interviewed CHWs and care takers of children under five years from 20 iCCM implementing and 5 non-implementing districts. The districts were selected through stratified sampling. We abstracted data from the district health information system (DHIS2) relating to reports submitted by CHWs. We determined the trends of out-patient attendance due to malaria, pneumonia and diarrhoea in under-fives and compared with the 2016 Uganda health demographic survey (UDHS). Data was analysed using STATA version 15. We interviewed 2888 care takers and 362 CHWs. About 73% (2394/2888) of caretakers were females and 25% stayed in the same location for at least 5 years. Fifty six percent (1606/2888; 95%CI: 53.8-57.4%) of care takers and 27.1% (98/362; 95%CI: 22.7-31.9%) of the CHWs had completed primary level education only. About 29% (95%CI: 25.4-32.4%), 18% (95%CI: 15.4-20.4%) and 14% (95%CI: 11.2-15.8%) of the under-fives had presented with fever, cough or diarrhoea respectively in the two weeks prior to the survey. Between 2012 and 2018, the number of sick children reviewed by CHWs tripled from 455,667 to 1,975,823. Outpatient attendance by under-fives due to malaria, cough and diarrhoea reduced in iCCM implementing districts. The number of acute diarrhea cases in non iCCM implementing districts increased by 14% compared to 0.2% in iCCM-implementing districts. No significant differences were seen in outpatient attendance due to pneumonia across the study districts. The iCCM strategy offers innovative ways of reducing childhood morbidity due to malaria, diarrhoea and pneumonia in rural resource limited settings

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STRENGTHENING POST TRAINING CONTINUOUS QUALITY IMPROVEMENT PROCESSES FOR BETTER CAPTURE AND REPORTING OF INPATIENT MALARIA DATA: A CASE FROM MIGORI COUNTY REFERRAL HOSPITAL IN KENYA

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Malaria is the second leading cause of pediatric morbidity and mortality in Migori County, Kenya with a 336 per 1,000 annual incidence. Migori County Referral Hospital admits an annual average of 3,000 pediatric patients, 19% with severe malaria. Impact Malaria's (IM) cross-sectional baseline survey of 186 health facilities in Migori and 7 other western Kenya counties identified 117 (63%) facilities with missing admission registers, incomplete inpatient summary tools, or incorrectly summarized case fatality data. To improve Migori County Referral Hospital pediatric inpatient case management (CM), data capture, and reporting, IM trained

four clinician mentors in severe malaria, who mentored 4 peer cohorts (15 total peers) in the pediatric inpatient department from December 2019-February 2020. Six follow-up visits were done at monthly intervals after training completion to assess mentor and mentee competencies, conduct root cause analysis of CM errors with a structured checklist, and implement pediatric ward processes improvements using the Kenya Quality Model for Health. Service delivery data were collected and summarized in routine data collection tools from January through December 2020. At baseline visit, 6/18 (33%) randomly sampled patient records identified delays of 30-60-minutes in second artesunate dose administration following treatment initiation, indicating a lack of treatment adherence. Root cause analysis associated the delays with patient handover process failures between shifts. Two checklist-based process improvements were implemented: a artesunate stock level monitoring communication loop and nurse station reminder cards, enabling 100% on-time second-dose administration at post-training record review (15/15). The 12-month service delivery data comparison showed intervention-associated median shifts in case reporting completeness from 168/174 (96%) to 402/408 (98%) and case fatality rates from 2/15 (13%) to 1/24 (4%). Continuous quality improvement training improved data reporting gap identification, root cause identification, and action point generation to address case management gaps.

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IMPROVING INPATIENT MALARIA DATA REPORTING AND OUTCOME MEASUREMENT ACCURACY THROUGH CLINICAL MENTORSHIP OF HEALTHCARE WORKERS IN KENYA, 2019-2020

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Impact-level malaria indicator monitoring in Kenya is hampered by under-reporting of inpatient data in Kenya Health Information System (KHIS). Data challenges include stock-outs of data capture tools, discrepancies between primary source tools and KHIS, and knowledge gaps in classification and coding severe malaria (SM). A 2019 baseline assessment conducted by Impact Malaria (IM) in 10% of health facilities in the 8 supported malaria endemic Counties indicated 93 (50%) of health facilities had a healthcare worker (HCW) trained in management of SM in the past 2 years, and none had complete inpatient morbidity and mortality data in KHIS. Between August 2019 and September 2020, IM designed and supported implementation of a 3-month SM clinical mentorship program for 84 HCWs from 72 admitting facilities. Targeted cadres were clinical, nursing, and medical officers stationed at pediatric and medical inpatient wards in 16 malaria-endemic sub-counties. Focus was on improvement of SM clinical skills, case notes recording, and capturing and reporting inpatient cases and deaths. Each month during the structured mentorship visits, targeted checklist-guided supervision was conducted. Discrepancies between inpatient data in KHIS and recorded admissions in Ministry of Health inpatient data tools served as a measure of data quality intra- and post-intervention. HCW pre- and post-testing assessed competencies. Post-intervention assessment was conducted between Jan - Sep 2020. In the 72 admitting facilities, the proportion of facilities with at least one HCW trained on management of SM increased from 50% to 72% (p-value <0.05). There was an increase from 31% to 67% (p-value 0.005) in data capture between baseline and December 2020. HCW competency levels in SM management improved from 64% to 77% (p-value <0.05. Data discrepancies between primary source documents and KHIS2 decreased from 67% to 35% (p-value <0.05). The inpatient malaria case fatality rate was 1.7% at the end of the intervention. SM mentorship in Kenya improved HCW competency, data capture, and is providing Kenya's National Malaria Program more accurate data for key decision making.

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A UNIQUE CHEMOPROPHYLAXIS APPROACH FOR MALARIA PREVENTION AMONG NON-IMMUNE OIL AND GAS WORKERS

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With international mobile workforces assigned or traveling to malaria-endemic countries, our company non-immune personnel are exposed to the disease with the risk of severe illness or death. To safeguard such workers from these malaria related risks, we have implemented the ABCD malaria program, which provides awareness, bite prevention, chemoprophylaxis, and early diagnosis and treatment. In addition, our malaria prevention includes a formal requirement for these non-immune travelers and assignees to take their chemoprophylaxis, with the ability for the company to confirm compliance by testing for the presence of these medications (i.e. Proguanil, Doxycycline, or Mefloquine) in the urine. Following the development of the laboratory based method for such verification, ExxonMobil (EM) formed a unique partnership with the French Army Biomedical Institute and two manufacturers in 2007. The intent was to develop a rapid detection test (RDT) using specific drug antibodies thereby replacing the more expensive and less practical laboratory method. The RDTs were validated in malaria-endemic areas with urine samples from personnel taking malaria preventive drugs, and laboratory procedures were used to confirm the findings after the initial proof of concept in the lab. The rapid detection tests were successively integrated into our workplace program with implementation completed by 2013 in all our sites located in malaria risk areas. As a result, our recent Malaria Chemoprophylaxis Compliance Program (MCCP) evaluation showed high employee compliance for the past five years (over 99% detect) and a decrease in non-immune malaria cases from over 200 in 2003 to only 1 in 2020. Overall, EM has averted over 2000 non-immune malaria cases since 2003 and has had zero deaths due to malaria among our workers since 2007. As Tafenoquine-a new chemoprophylaxis regimen is now becoming available, the challenge is to develop a test to integrate it into the panel of recommended malaria preventive medicines for our workers.

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GENOTYPING PLASMODIUM FALCIPARUM MATURE GAMETOCYTES USING AMPLICON DEEP SEQUENCING

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Understanding the dynamics of *Plasmodium falciparum* gametocytes *in vivo*, including parasitological factors that promote sexual commitment and gametocyte production, requires the ability to discern asexual parasites and gametocyte clones within polyclonal infections. Genotyping gametocytes requires a marker that is expressed exclusively in this life stage and is diverse enough to distinguish multiple clones. Identifying such marker is challenging owing to the limited diversity in gametocyte-specific antigens compared to most asexual antigens. In this study, we aimed to develop and test a gametocyte genotyping assay based on deep sequencing of short amplicons from polymorphic regions of gametocyte-specific antigens. Using publicly available single cell RNA-seq data to identify antigens expressed primarily in mature gametocytes, as well as a recently published list of diverse "microhaplotypes" from the *Plasmodium falciparum* genome, we identified two regions in the gene encoding P230 and one region in the gene encoding G377 as candidate gametocyte genotyping markers. We tested the specificity of these markers to detect gametocytes, assessed their ability to distinguish minor clones, and measured their diversity compared to pre-erythrocytic and asexual blood stage markers currently being used for genotyping based on deep amplicon sequencing. Results from RT-PCR of RNA from ring stages of a *P. falciparum* strain that does not produce gametocytes and mature gametocytes from NF54 targeting the three markers indicated that these

markers are specific to mature gametocytes. Based on 205 *P. falciparum* whole genome sequences from Malawi, we observed a similar or greater number of haplotypes at these markers (G377: 24, P230: 51 and 53) compared to polymorphic regions of *csp*, *sera2,* and *ama1*, which showed 3, 23, and 18 haplotypes in the same data set, respectively. We have developed a gametocyte genotyping assay based on sequencing of short amplicons from polymorphic regions of mature gametocyte genes. This assay will be an important tool for studies that are aimed at understanding the dynamics of gametocyte production in polyclonal *P. falciparum* infections.

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MAPPING HOUSE LOCATION WITH REMOTE SENSING AND MACHINE LEARNING METHOD IN WESTERN KENYA

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House location represents one of the most critical variables in the assessment of exposure to vector-borne pathogens. Accurate information on house location can improve modeling disease transmission risk and help planning interventions. However, obtaining precise house location in vast rural areas of Africa where there was no prior detailed mapping effort is labor-intensive, expensive, and time-consuming. High-resolution remote sensing images are helpful, but the traditional method to manually identify house location needs skilled technicians and prone to errors due to the morphological resemblance of ground objects to human dwellings. We conducted a case study by combining remote sensing and machine learning methods to detect human houses across the Homa peninsula in Homa Bay County, western Kenya. Using high resolution, multi-band pan-sharpened Pléiades satellite images with labeled dwellings from the ground survey, the Mask R-CNN, a deep convolutional neural network for object detection, was used to generate low-level features and high-level features. Bounded box regression and classification were conducted. The model was trained on a 0.1° x 0.1° satellite images with 22,319 labeled houses and validated on the other three adjacent 0.1° x 0.1° area. The average precision score for the potential model is 0.687, and the validation accuracy of the same images is around 99.3%. The overall accuracy of estimation is 88.2% on the adjacent area. We are currently testing several new machine learning algorithms to improve the modeling prediction precision and accuracy for different house types. A combination of remote sensing and machine learning methods is valuable to detect house location, estimate population density, and the population under risk in rural Africa.

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ESTABLISHMENT AND USE OF MALARIA E-TOOLKIT TO INCREASE ACCESS TO STRATEGIC MALARIA RESOURCES IN NIGERIA

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Available evidence suggests that an estimated 76% of Nigeria's population is at risk of contracting malaria because they live in high transmission areas. According to the World Malaria Report (2020), Nigeria accounted for 27% of the global malaria cases and 24% of global malaria deaths in 2019. Poor access to updated malaria strategic documents by frontline health workers and stakeholders affects the quality of service delivery which ultimately contributes to these statistics. In response to these staggering statistics, President Malaria Initiative for States (PMI-S) worked with the National Malaria Elimination Program (NMEP) to set up the NMEP e-toolkit. The e-toolkit is a platform where all updated policies, guidelines, standard operating procedures, and job aids for malaria prevention and control are stored and accessed by malaria stakeholders, program implementers and service providers in Nigeria. The process of setting up the NMEP e-toolkit was informed by a needs assessment. The e-toolkit is divided into the six key indicators of malaria interventions and pointers to improved malaria service delivery. The components are 1) surveillance and M&E (SME); 2) program management (PM); 3) procurement and supply chain management (PSM); 4) vector control (VC); 5) case management (CM); and 6) advocacy, communication, and social mobilization (ACSM). An analysis of the e-toolkit's page views per thematic area from February 2020 to March 2021 was conducted. The number of page views per thematic areas are 358 for CM, 209 for SME, 147 for PM, 136 for VC, 129 for ACSM and 104 for PSCM. Usage analysis of the e-toolkit during the same time frame indicates that 15,000 Nigeria-based users across the 36 states and Abuja have accessed it. Triangulation of findings from in-depth interviews and analytics suggest that the technology-enabled e-toolkit is a strategic tool which enables real time access to strategic malaria documents. These findings show that technology is a driver of structural change and standardization because the e-toolkit is a reference tool to access malaria prevention and control resources toward improved malaria service delivery in Nigeria.

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STRENGTHENED CAPACITY ENHANCES HEALTH WORKERS' ABILITY FOR MALARIA CASE MANAGEMENT IN CROSS RIVER STATE, NIGERIA

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WHO recommends testing all fever cases for malaria using microscopy or a rapid diagnostic test, and treating only positive cases with an Artemisinin-based Combination Therapy (ACT). However, Nigeria Demographic and Health Survey (2018) revealed that ACT was used in treatment of malaria for only 52% of children with fever in South-South, Nigeria. Only 12.4% of these under 5 years children were tested for malaria. The baseline analysis of the malaria program in Cross River showed that health workers were last trained on malaria case management in 2015, and that 85% of persons with fever were issued ACT without a malaria positive test. Consequently, there was a need to strengthen health care worker capacity to offer quality services. We used classroom training, onsite approaches, and routine mentoring of health care workers through phone calls and WhatsApp messaging to provide supportive supervision in 40% of PHCs and 75% of public secondary hospitals to build capacity of health workers on adherence to malaria case management guidelines. Data from the Nigeria Health Management Information System from January-December 2020 revealed that testing for suspected malaria cases increased from 90.7% in 2018 from the NDHS to 95% in January 2020 and further improved to 97.2% by December 2020. Similarly, treatment of confirmed cases with an ACT improved from 85% to 100% by December 2020, and clinical diagnosis dropped from 66% in January 2020 to 1.1% within the year under review. This data show improvement in malaria case management following skills transfer to healthcare workers. Trainings, mentoring and supervision visits and the mentoring outreach with the health care providers contributed to the improvement of malaria diagnosis and treatment. This approach built sustainability into the framework of our project interventions.

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STRENGTHENING COORDINATION SYSTEMS AND PROCESSES FOR BETTER FINANCING OF MALARIA IN NIGERIA THROUGH THE MALARIA ANNUAL OPERATIONAL PLAN DEVELOPMENT AND MONITORING PROCESS - CASE STUDIES OF FOUR STATES

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Nigeria's State Malaria Elimination Programs (SMEP) and their partners undertake Annual operational planning every fiscal year to identify high priority malaria control and elimination activities. The Annual Operational Plan (AOP) developed from the process is meant for integration in the annual health sector budget plan and formulation of the annual state budget. The planning should achieve three objectives: (1) link prioritized activities to strategic pillars of the State/National Strategic Health Development Plans which enables a connection between the strategic priorities and budgeting, (2) generate an implementation plan for the fiscal year, and (3) provide a monitoring framework comprising performance indicators against which implementation can be measured. SMEPs are chronically underfunded, making them dependent on donors to finance key functions and limiting their capacity to effectively steward malaria control efforts. AOP development workshops were held using a four step approach, which included: conducting situational analyses of the SMEP performance; prioritization/ranking of selected activities; cost estimation of the prioritized activities and the development of performance monitoring plans. The intervention states completed the operational planning early enough for its inclusion in the health sector plan and budget for 2021. Prioritized activities were successfully mapped to strategic priorities of the state health development plans, resources, funding sources were assigned to activities, and the resource gaps determined for each state. Findings from the cost estimation revealed cost of new activities accounted for 44% of the AOP, costs for procurement supply chain management activities accounted for upward of 75% of the cost of prioritized activities across all the states.

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RELIABILITY OF MALARIA INDICATORS IN HEALTH DISTRICTS IN BURKINA FASO: EFFECTS OF AN INTEGRATED QUALITY ASSURANCE PROGRAM

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High quality data is important for appropriate decision-making and to evaluate health interventions. For this purpose, a baseline data quality assurance (DQA) of malaria indicators was performed in 2019 in Orodara, Gaoua, and Banfora, three health districts in Burkina Faso, prior to the distribution of Interceptor® G2 (BASF) insecticide-treated nets (ITNs) as part of the New Nets Project. Based on results obtained in 2019, a DQA plan integrating trainings, supervision, and meetings was implemented immediately to enhance the capacity of health workers to provide better quality malaria data. The effect of these capacity strengthening activities on the reliability of malaria data transmitted to District Health Information Software 2 (DHIS 2) was then monitored. Health facilities were randomly selected for malaria DQA in 2019 and 2021. Source data were collected through source document review, such as health facility registers, while transmitted data were extracted from DHIS 2. The assessments focused on 10 key malaria indicators before and after the one-year continuous DQA plan. Reliability was assessed using the interclass correlation coefficient (ICC) between source and DHIS 2 data. Each ICC was compared between reviews through confidence intervals at 95%. Data were analyzed with SPSS software using parallel model, two-way mixed and absolute agreement type. A total of 45 health facilities were assessed. For seven indicators the ICC was significantly higher after than before the DQA plan. Most of the ICC were greater than 0.9 ($p<0.001$). However, there was no change for overall presumptive malaria cases (ICC=0.4 [95% CI: 0.2-0.6]) and severe malaria cases in pregnant women (ICC=0.7 [95% CI: 0.4-0.8]). The ICC for the malaria lethality indicator was 1 during the two surveys. DQA activities improved the reliability of seven malaria indicators. Thus, it is recommended to maintain these capacity strengthening activities in health facilities in Burkina Faso.

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A CASE STUDY OF TOXIC EPIDERMAL NECROLYSIS FOLLOWING ADMINISTRATION OF DIHYDROARTEMISIN-PIPERAQUINE (DPIN A MASS TREATMENT CAMPAIGN IN UGANDA

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We describe a case of a 2 year old female given a full course of Dihydroartemisin-piperaquine (DP) dosed at a tablet of 320/40mg per day for 3 days during a mass treatment campaign in Uganda. This child was not taking any other medications prior to DP ingestion. She developed a progressive skin rash and blisters which later formed painful raw areas associated with a low grade fever 7 days after DP ingestion. Blood work performed (CBC, RFTs/LFTs) was basically unremarkable apart from hypoproteinemia of 4.63(6.6-8.7 g/dl). Her constellation of symptoms and presentation were consistent with Toxic Epidermal Necrolysis (>30% TBSA). The causality was established as 'probable' to DP with both Naranjo scale and World Health Organization causality assessment scale. It could not be established whether the offending molecule was dihydroartemisinin or piperaquine but the child had previously taken artemether-lumefantrine for malaria with no reported complaints. The child was admitted to the burns unit, and managed basically with supportive treatment which included: steroids, anti-histamines, tetracycline ointment for eye care, prophylactic antibiotics, twice daily dressing with neomycin cream and plenty of fluids and high protein diet until she was discharged after 2 weeks. The child was monitored for re-appearance of lesions for up to 6 months but none were observed. The child and her caregivers were given a laminated card to be used at all health facilities, advising against any retreatment with DP.

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EFFECTIVENESS AND SAFETY OF INTERMITTENT PREVENTIVE TREATMENT FOR MALARIA USING EITHER DIHYDROARTEMISININ-PIPERAQUINE OR ARTESUNATE-AMODIAQUINE IN REDUCING MALARIA RELATED MORBIDITIES AND IMPROVING COGNITIVE ABILITY IN SCHOOL-AGED CHILDREN IN TANZANIA: A CONTROLLED RANDOMISED TRIAL

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In high transmission settings, majority of school-aged children harbour malaria parasites (*Plasmodium*) without showing symptoms (asymptomatic), thus, acting as a relevant reservoir for malaria transmission. Asymptomatic *Plasmodium* infections induce inflammation leading to anaemia that may impair cognitive levels. A clinical trial [NCT03640403] was conducted in Muheza, Tanzania (from March 2019 to Dec 2020), to determine the effectiveness and safety of two antimalarial drugs Dihydroartemisinin-piperaquine (DP) and Artesunate-amodiaquine (ASAQ) in preventing malaria-related morbidity in school-aged children (IPTsc) living in highly endemic areas. In this trial, 1563 schoolchildren aged 5-15 years were recruited from seven primary schools located in villages with high malaria prevalence. These children were individually randomised to receive either DP (n=526) or ASAQ (n=525), or control (standard of care, n=512), that was given three times a year at a 4-month interval. The primary endpoints were change from baseline in mean haemoglobin (Hb) concentration at month 12 and the number of malaria illness cases accrued from baseline to month 12 of follow up.Four months after one, two and three rounds of DP or ASAQ dosing; the respective mean Hb differences between DP and Control arms were 0.45g/dl (95% CI 0.26-0.65, P<0.0001), -0.74 g/dl (95%CI -0.94--0.54, P <0.0001) and 0.59g/dl (95% CI 0.23-0.94, P=0.0011), while for ASAQ and Control arms, the respective mean Hb differences were, -0.21g/dl (95% CI -0.40--0.02, P=0.029), 0.80g/dl (95%CI 0.59-1.02, P<0.0001) and 0.53g/dl (95% CI 0.17-0.89, P=0.0043). At month 12, malaria prevalence reduction was 16% (95% CI 9-23, P=0.002) and 8.8% (95% CI 1.3-16, P=0.0036) for DP and ASAQ arms respectively. Further analysis on impact of IPTsc on cognitive, malaria incidence and rebound effect is ongoing but will be available during the conference. In this study, both DP and ASAQ offered a safe protection against malaria parasitaemia, anaemia and clinical malaria, and were pragmatically feasible for scale up on malaria intervention in adoption to school health programmes when IPTsc becomes a policy.

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PRELIMINARY FINDINGS ON IMPACT OF SEASONAL MALARIA CHEMOPREVENTION ON MALARIA INCIDENCE AMONG CHILDREN 5 TO 10 YEARS OF AGE IN A HIGH MALARIA PREVALENCE REGION IN MALI (SIKASSO)

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In Mali, SMC campaigns target children less than 5 years once a month for four months to prevent malaria during the high transmission season. Because the burden of malaria appears to be shifting to older children (Touré et al., 2016), the National Malaria Control Program decided to expand SMC to children up to ten in two health districts in the high burden region of Sikasso in 2020, with the first cycles starting at the end of July. In June 2020, the NMCP also distributed second-generation (IG2) long-life insecticide-treated nets (LLIN) to one of the two health districts that expanded SMC to older children, while the other received standard LLINs. To assess the relative contributions of IG2 and the expansion of SMC up to ten years (SMC10), we extracted 2019 and 2020 data from the national health information system to compare the incidence of uncomplicated and severe malaria in children aged 5-14 years in four health districts. Selingue received IG2 and SMC10; Koutiala standard LLINs and SMC10; Kadiolo IG2 and SMC up to age 5 (SMC5); and Kolondieba standard LLINs and SMC5. All districts have been implementing SMC5 since at least 2015. In August 2020, Selingue (IG2 and SMC10) saw 6.6 confirmed uncomplicated malaria cases per 1000 vs 11.4 in August 2019; Koutiala (standard LLIN and SMC10) saw 4.6 per 1000 in 2020 vs 12.4 in 2019; Kadiolo (IG2 and SMC5) saw 10.2 in 2020 vs 19.8 in 2019; and Kolondieba (standard LLIN and SMC5) saw 4.8 in 2020 vs 10.9 in 2019. For severe malaria cases per 100,000, comparison of August 2020 to August 2019 were as follows: Selingue (IG2 and SMC10), 60.5 in 2020 vs 100.2 in 2019; Koutiala (standard LLIN and SMC10), 34.3 in 2020 vs 67.5 in 2019; Kadiolo (IG2 and SMC5), 77.5 in 2020 vs 107.2 in 2019; and Kolondieba (standard LLIN and SMC5), 89.7 in 2020 vs 120.4 in 2019. The incidence of confirmed uncomplicated and severe malaria in children aged 5-14 during August 2020 was lower than the same period in 2019 in all four districts, so it is not necessarily the case that it was due to the SMC treatment of older children. As SMC in children up to 10 years is expanded in 2021 further monitoring will be needed to determine the impact of this intervention.

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MOLECULAR MECHANISMS OF PLASMODIUM CANDIDATE PROTEINS INTERACTION DURING PARASITE INVASION OF MIDGUTS

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Malaria is a mosquito-born deadly infectious disease caused by *Plasmodium* parasites. Malaria claims nearly half a million lives every year. Most malaria eradication programs have been concentrated on eliminating the asexual stage of the parasite and controlling the mosquito population using insecticides. However, the increased parasite drug resistance and mosquito insecticide resistance are calling for alternative approaches. Hence, the research focus has shifted to transmission-blocking strategies. The sexual stages of the parasite are key for successful mosquito infection and malaria transmission. Ookinetes are the invasive form of the parasite that interacts with midgut proteins to penetrate the peritrophic matrix and midgut epithelial cells. Therefore, understanding the molecular mechanisms of interaction between the malaria parasite proteins and mosquito midgut proteins is crucial for developing an appropriate intervention. Our lab has identified six sexual stage parasite proteins that bound to the mosquito midgut and this research focuses on elucidating the molecular mechanisms of these proteins during parasite midgut invasion. We have cloned and expressed these genes in *E. coli* and insect cells. To study the mechanisms of interaction and identify the interacting partner, we will perform protein-protein interaction assays using pull-down, far-western blot, and western blot. We will also study the localization of these proteins using indirect immunofluorescence assay. Moreover, we will conduct a transmission-blocking assay to determine the role of these proteins in malaria transmission. These identified parasite proteins and their binding partners will be ideal candidates to block malaria transmission.

1141

TRENDS IN COVERAGE, OWNERSHIP AND USE OF LONG-LASTING INSECTICIDAL NETS WITH, AND WITHOUT, PIPERONYL BUTOXIDE AFTER A UNIVERSAL COVERAGE CAMPAIGN IN UGANDA: A SECONDARY ANALYSIS OF DATA FROM A PRAGMATIC CLUSTER-RANDOMIZED TRIAL USING OF LONG LASTING INSECTICIDAL NETS

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To evaluate ownership, coverage, and use of long lasting insecticidal nets (LLINs), we conducted surveys as part of a cluster-randomised trial embedded in Uganda's national LLIN distribution campaign in 2017-18. In total, 104 clusters (health sub-districts) were included. Proportionate randomisation was used to assign clusters to one of four arms; in total 52 clusters were assigned to the piperonyl butoxide (PBO) arm, and 52 to non-PBO. At baseline, 6, 12, and 18 months after LLIN distribution, 50 randomly selected households per cluster were surveyed (5,200 households and ~28,000 residents per survey) using two-staged cluster sampling. Multivariate logistic regression was used to evaluate associations between predictors of interest and LLIN coverage and use at 18 months. LLIN ownership and coverage rose from baseline levels of 65.0% and 17.9%, to 96.6% and 71.3%, respectively, after 6 months. LLIN ownership remained high (>80%) during follow-up, while coverage and use declined to 50.9% and 73.0% respectively. In the adjusted analysis, the strongest predictors of adequate LLIN coverage were smaller household size (2-4 vs ≥7: 66.4% vs 32.5%; aOR 5.30, 95% CI 4.46-6.29, p<0.001) and fewer residents (<3 per sleeping space) (aOR: 1.59, 95% CI 1.41-1.80, p<0.001). Compared to non-PBO households, those in the PBO arm had higher odds of adequate coverage (52.5% vs 49.2%; aOR 1.22, 95% CI 1.08-1.37, p=0.001) and residents were more likely to use LLINs (74.3% vs 71.8%; aOR: 1.13, 95% CI 1.03-1.25, p=0.01), although these differences were small. Heads of households were more likely to use LLINs than their 2nd degree relatives (81.9% vs 66.1%; aOR 1.94, 95% CI 1.72-2.18, p<0.001) while children aged 5-15 years were less likely to use LLINs (<5yrs vs 5-15yrs: 77.8% vs 67.1%; aOR 1.77, 95% CI 1.66-1.89, p<0.001). LLIN attrition following mass distribution is a major issue. LLINs may need to be distributed more frequently than every 3 years. Efforts are needed to ensure larger households are adequately covered, and school-aged children and extended family members should be targeted.

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FINDINGS FROM AN EFFECTIVENESS-IMPLEMENTATION HYBRID TYPE I EVALUATION OF THE FEASIBILITY, ACCEPTABILITY AND PROTECTIVE EFFICACY OF SEASONAL MALARIA CHEMOPREVENTION IN TWO DISTRICTS IN KARAMOJA, UGANDA

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To date, due to widespread prevalence of markers especially pfdhps540E, pfdhps581G, Pfmdr1 and Pfcrt mutants associated with sulfadoxine-pyrimethamine (SP) and amodiaquine (AQ) resistance, Seasonal Malaria Chemoprevention (SMC) has not been implemented at scale in East and Southern Africa. Although the effectiveness of SMC to reduce malaria incidence has been demonstrated in areas of low resistance to SP in the Sahel region, the feasibility, acceptability, and protective efficacy of SMC implemented in areas with high resistance to SP are unknown. Malaria Consortium in collaboration with Ministry of Health, Uganda, has implemented a five-months cycle effectiveness-implementation hybrid type 1 SMC project in 3 districts of Karamoja region, Uganda to assess the protective effectiveness of SMC as a complementary malaria control intervention. The evaluation components involved quasi-experimental non-randomized study in Kotido and Moroto as intervention districts and Nabilatuk as a control to understand: 1) the adaption of SMC to the Ugandan context; 2) acceptability and feasibility of SMC intervention, 3) coverage and quality of SP+AQ delivery using a cross sectional end-of-round survey; 4) baseline prevalence of SP and AQ resistance associated genotypes (including Pfdhps, Pfdhfr, Pfcrt and Pfmdr1 mutants); 5) monitor the safety of repeated use (for 5 months) of SP and AQ when used in SMC among children 3-59 months and 6) reduction in odds of clinically-significant malaria outcomes associated with receipt of SP+AQ based on a non-randomised controlled trial with eligible children in the two intervention districts and control district. Results on the following output and outcomes measures will be presented; malaria incidence, feasibility of SMC implementation in terms of coverage and quality of implementation, prevalence of resistance markers for SP and AQ among the children 3-59 months, malaria parasite prevalence, acceptability of SMC, adverse events related to SP and AQ.

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MALARIA CHEMOPREVENTION IN KENYAN CHILDREN WITH SICKLE CELL ANEMIA: A RANDOMIZED, OPEN-LABEL, TWELVE-MONTH TRIAL COMPARING DAILY PROGUANIL, MONTHLY SULFADOXINE-PYRIMETHAMINE/AMODIAQUINE, AND MONTHLY DIHYDROARTEMISININ-PIPERAQUINE

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Children with sickle cell anemia in areas of Africa with endemic malaria transmission are commonly prescribed malaria chemoprevention. The comparative efficacy of prevention regimens is largely unknown. We enrolled 246 Kenyan children aged 1-10 years with HbSS disease confirmed by electrophoresis in a single-site, randomized, open-label, 12-month trial. Patients were assigned in a 1:1:1 ratio to receive daily Proguanil (the standard of care), monthly sulfadoxine/pyrimethamine plus amodiaquine (SP-AQ), or monthly dihydroartemisinin-piperaquine (DP). The primary outcome was the incidence of clinical malaria, defined as fever with peripheral parasitization or as severe malaria, and the main secondary outcome was painful events, defined as pain lasting 2 hours or more that did not have an obvious cause. The incidence of these and other secondary tertiary outcomes in the SP-AQ and DP arms was compared to those in the Proguanil arm using generalized regression models to estimate incidence rate ratios. We also compared safety outcomes between prevention arms. The primary analysis set consisted of children in the As-Treated population. Planned subgroup analysis were also conducted based on enrollment values for age, sex, hydroxyurea use, sickle cell severity, and hemoglobin concentration. Clinical followup completed in December 2020, study closeout is in progress, and results will be available by mid-2021. The comparative effectiveness of alternate regimens on malaria and painful event prevention will help guide evidence-based care for children with sickle cell anemia in malaria-endemic areas.

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EFFECT OF LARGE-SCALE MASS DRUG ADMINISTRATION FOR MALARIA ON MORTALITY AND MORBIDTY IN ANGUMU HEALTH ZONE, ITURI, DEMOCRATIC REPUBLIC OF CONGO

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WHO recommends mass drug administration (MDA) for malaria in extreme complex emergencies. Angumu health zone in Ituri, DRC, a highly malaria-endemic area, has been hosting internally displaced persons (IDP) from lower-endemicity zones, with health system overburdened. Three MDA rounds (2 amodiaquine-artesunate + 1 artesunate-pyronaridine) were implemented with high coverage from September 2020 to January 2021 by Ministry of public health and Médecins sans Frontières for >2 months-old living in 4 health areas. We compare reported mortality and morbidity in locations where MDA has been performed and locations where it has not. A cross-sectional population-based retrospective mortality survey was conducted in March 2021, stratified by villages/IDP sites and MDA/non-MDA locations. For the villages, two-stage cluster sampling methodology was used; all IDP sites were surveyed with a systematic random sampling. The main outcome was crude and under-5 mortality in the different strata. Morbidity in the 2 weeks prior to the survey was also calculated. 2554 households were interviewed, and data collected for 15470 individuals, whom 721 died in the 18 month recall period. In the villages, the under-5 mortality decreased from 2.06 [1.22-2.9] deaths/10,000 people/day before the MDA to 0.91 [0.4-1.41] after the MDA in the MDA villages whereas it increased from 2.23 [1.33-3.12] to 2.83 [1.77-3.89] in the same time periods in the non-MDA villages. In IDP sites, the under-5 mortality decreased from 2.54 [0.4-4.68] deaths/10,000 people/day before MDA to 0.57 [0-1.36] after MDA in the MDA sites while it remains stable in the non-MDA sites. All-cause and malaria-specific morbidity in the 15 days before the survey appears lower in MDA villages and sites than in non-MDA ones: reported malaria-specific morbidity was 14.7% [11-18] and 30.4% [27-33] respectively in MDA and non-MDA villages, 25.0% [19-31] and 49.3% [45-54] respectively in MDA and non-MDA sites. The observed sharp decrease of under-5 mortality and morbidity after the MDA confirms that MDA has the potential to become an important malaria-control tool in emergency settings.

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ASSESSING COVERAGE OF SEASONAL MALARIA CHEMOPREVENTION IN GHANA IN 2020

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Seasonal Malaria Chemoprevention (SMC) was introduced in Ghana in 2015 in Upper West region, expanded to include Upper East region in 2016, and, in 2019, Northern region (now Northern, Savannah and North East regions), the same regions being included in 2020. Community Health Volunteers (CHVs) use Android phones to register eligible children in each household and record daily doses administered. In 2020, blister packs were given to the caregiver who was asked to administer the first daily doses observed by the CHV, and the remaining doses at the same time on each of the next two days. The date was written in the child's health record booklet. CHVs visited again on the second and third day, to check that doses were given by inspecting blister packs, and to give treatment for any eligible children who were not present on the first day. The Sicapp android app allows staff to monitor the number of treatments each day and compare with daily targets. Supervisors can then consider corrective actions if necessary. In 2020, a total of 4.1million treatment courses were administered over 4 months to a population of 1.08million children. A household survey was conducted to assess coverage with support from the OPT-SMC project. A total of 2100 households were visited in 150 clusters. Children aged 3-84 months at the last cycle were included, to assess coverage in the target age group and to assess whether older children were treated. Dates of SMC treatments were determined from the child's health record booklet, or from the Sicapp database if the booklet was not available, and caregivers were asked about adherence to daily doses. Focus groups with caregivers and interviews with CHVs were held to understand barriers to SMC uptake. A total of 87.6% of children eligible for four cycles, received four treatments. Very few (1%) of the older age group were treated. The percentage of day1 treatments that were directly observed, in cycle 4, was 95.8%. Limitations included lack of time for call-back visits to non-responders due to limited funds. Side effects of SMC drugs, taste of tablets, and charging costs for management of side effects, and over-tasking of volunteers, were identified as barriers to SMC uptake.

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A RECOMBINATION SYSTEM FOR THE CREATION OF STABLE ANTIPLASMODIAL PARATRANSGENIC ASAIA SP.

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Malaria, a disease caused by the parasite *Plasmodium sp*. and spread by the bite of infected female *Anopheles* mosquitoes, is responsible for hundreds of thousands of deaths each year. Reduction of global malaria cases has stagnated in recent years, highlighting the need for new control strategies. One such strategy, paratransgenesis, involves engineering mosquito symbionts to secrete antiplasmodial effectors within the midgut to kill parasites post infected blood meal. The bacterium *Asaia bogorensis* has been identified as an ideal candidate for antiplasmodial paratransgenesis, as it efficiently colonizes the midgut, ovaries, and salivary glands of *Anopheles* mosquitoes. Paratransgenic *A. bogorensis* strains have previously been developed which were shown to be effective at reducing oocyst prevalence within infected mosquitoes. However, the paratransgenic components of these strains are located on plasmids and rely on drug-selection for stability, which is unsuitable for field-release. One way to develop stable strains of paratransgenic *A. bogorensis* is to insert the antiplasmodial effectors into the chromosome. To this end, a site-specific recombination technique has been adapted for use in *A. bogorensis* in which I-SceI mediated recombination is linked to *sacB* counterselection. This technique was used to create several strains in which the general antimicrobial peptide scorpine driven by a previously identified blood meal induced promoter was inserted within neutral sites of the *A. bogorensis* chromosome. In this study, we analyze these strains in comparison to their plasmid counterparts.

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IDENTIFYING AN OPTIMAL DIHYDROARTEMISININ-PIPERAQUINE DOSING REGIMEN FOR MALARIA PREVENTION IN YOUNG UGANDAN CHILDREN

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Intermittent preventive treatment (IPT) with dihydroartemisinin-piperaquine (DP) is highly protective against malaria in children, but is not standard in malaria-endemic countries in Africa. Infants are at highest risk of malaria complications, but drug exposure-response relationships for piperaquine (PPQ), the drug responsible for the preventive efficacy of DP, are poorly characterized for this population. We identified for Ugandan infants optimal IPT-DP dosing regimens to maximize malaria preventive efficacy and reduce toxicity and antimalarial resistance selection. Among 280 infants, we analyzed PPQ concentrations (n=4,506), malaria incidence data (n=326), and *P. falciparum* drug resistance markers (n=143) from a trial of children 2-24 months of age randomized to IPT with DP every 12 weeks (n=184) or every 4 weeks (n=96). DP was administered daily for 3 days and the first dose was directly observed. Nonlinear mixed effects modeling was used to quantify malaria protective PPQ levels and risk factors for suboptimal protection. DP was associated with 95% protective efficacy (95% CI: 84-99%) when administered every 4, compared to every 12 weeks. A PPQ level of 15.4 ng/mL reduced the malaria hazard by 95%. Malnutrition reduced PPQ bioavailability by 10.2% for each standard deviation decrease in WHO height-for-age z-score. Adherence was lower than expected in the study, with 61% lower PPQ exposure when DP was taken without directly observed therapy. PPQ level was not associated with parasite genetic markers associated with decreased aminoquinoline sensitivity. In simulations, DP every 4 weeks was optimal for malaria prevention across a range of malaria transmission intensities. Age-based dosing, with dose increases at 6 and 18 months, improved malaria protection in young or malnourished infants. Age-based, rather than the currently recommended weight-band based dosing of DP improved PPQ exposure for malnourished Ugandan infants and may have operational benefits for community implementation of IPT in children.

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PERSPECTIVES OF HEALTHCARE PROVIDERS ON FACTORS ENABLING OR INHIBITING THE UPTAKE OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA FOR PREGNANT WOMEN IN OYO STATE, NIGERIA

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Malaria remains one of the highest contributors to the precarious maternal mortality figures in Sub-Saharan Africa. In Nigeria, 11% of maternal deaths are attributed to malaria. To mitigate the risk of malaria in pregnancy (MIP), intermittent preventive treatment of malaria in pregnancy (IPTp) with Sulphadoxine-Pyrimethamine (SP) is recommended. First dose is to be administered in the second trimester and monthly until delivery, with a minimum of three doses given. In Oyo State, despite these recommendations, only 64.7% of pregnant women received second dose and beyond (IPTp2+) and the drop-out rate between first (IPTp1) and third (IPTp3) doses was 86%. We surveyed health providers' perception of the barriers and enablers to IPTp uptake using a structured self-administered questionnaire. Data was collected from healthcare providers in selected primary health centers in the state electronically using Google forms. All 82 consenting healthcare providers rendering ante-natal care (ANC) services were enrolled and responded to the survey. Questions were structured to elicit responses on inhibitors and enablers to the administration of IPTp by healthcare providers. Analysis revealed factors such as routine ANC counselling, Directly Observed Treatment (DOT), capacity building, availability of MIP tools (SOPs, Job Aids, Algorithm) and guidelines coupled with a regular supply of SP were key enablers. All respondents claimed that routine ANC counselling is essential in ensuring improved IPTp uptake while 80% of respondents stated that regular supply of SP facilitated the uptake of IPTp in their facilities. Late booking of pregnant women at ANC was identified as the most common inhibiting factor (66%) alongside high patronage of traditional birth attendants (62%). Other inhibiting factors included stock out of SP, inadequate knowledge of pregnant women on MIP and non-availability of guidelines. Key enablers, such as counselling and supply of SP should be reinforced in health facilities. Factors inhibiting IPTp uptake could be addressed through community sensitization and dissemination of MIP guidelines to health facilities.

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IN HOSPITAL UTILIZATION OF LLINS IN THE NORTHWEST OF DEMOCRATIC REPUBLIC OF THE CONGO

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The promotion of LLIN utilization has become the main preventive measure against malaria in many LMICs. This has been expanded to specific households to improve coverage. We evaluated the utilization of LLINs in the hospital setting in the cities of Lisala and Bumba, in the northwest of the DRC. These facilities received donation of LLINs during the mass campaign in July 2016. Between October and November 2017, we conducted a cross-sectional study in 13 states, church-owned and private health facilities in the above setting. Institutional managers and 227 inpatients were interviewed. The available LLINs were directly assessed. Overall, the facilities surveyed received a total of 544 LLINs during the distribution campaign. 14 months later, 8 out of the 13 visited facilities had no remaining LLINs, representing an attrition rate of 74.4%. Lost LLINs were reportedly taken away by patients upon discharge from hospital. A total of 153 LLINs were used in the visited health facilities the night before the survey, of which only 30% were owned by the health facilities. The frequency of cleaning of LLINs in the visited health facilities was not regulated. It was done with mild soap either monthly, or when the LLIN looks dirty, or when the patient who had used it was discharged. Of 227 surveyed patients, 66.5% were female, 73.6% over 15 years old, and 67.4% (CI95%: 60.8-73.5) had spent the night before under LLINs. Inpatient use of LLINs was influenced by place of residence (AOR=17.1; 95% CI: 7.2-40.7); gender, men being more compliant than women (AOR=2.5; 95% CI: 1.1-5.7); previous use of LLINs at home (AOR=5.5; CI95%: 1.2-25.4); and by facility type, inpatients in private facilities being less likely to sleep under LLINs (AOR=0.3; CI95%: 0.1-0.9). The use of LLINs in hospital remains below 80%. The survival time of LLINs in health facilities seems to be lower than the survival time at the household level. Hence, there is a need to find mechanisms for more frequent LLIN renewal in order to maintain coverage. There is also a need to find an adequate means of cleaning that balances the need for effective vector control with minimizing the risk of spreading nosocomial infections.

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MALARIA PLASMODIUM CARRIAGE AFTER THE IMPLEMENTATION OF SEASONAL MALARIA CHEMOPREVENTION IN SENEGAL: CASE OF THE SARAYA HEALTH DISTRICT FROM 2013 TO 2015

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Seasonal malaria chemoprevention is a strategy adopted and implemented in Senegal since 2013 in the southern regions of the country in children aged between 3 months and 10 years old. The scaling up of this strategy requires its evaluation to assess its impact. This study was carried out to determine the dynamics of *Plasmodium falciparum* carriage after two years of implementation. Four household surveys were conducted in villages located in the Saraya health district which is a SMC implementation zone in Senegal. These villages were selected based of the proportional probability of the population. Each of the selected villages was divided into segments that had to contain at least 50 children. In the selected segment, a household questionary was administered to collect to parents or legal representative of children aged 3 to 120 months. Blood sample was collected to determine *Plasmodium falciparum* prevalence and heamoglobin level. A total of 2577 children were included in our study with an average age of 4.81 (+/-2.73) years. Children over 5 years of age represented 50.33% of our study population and male children 50.32%. The overall coverage of LLINs was 83.39%. Net ownership was 99.62%, 99.79%, 69.46% and 45.76% respectively in August 2013, January 2014, July and December 2015. Anemia prevalence was 79.08% and it was higher in children under 5 years of age. *Plasmodium falciparum* carriage was 9.42%. Parasite prevalence after SMC scaling had decreased from baseline (6.1% in January 2014 and 4.93% in July 2015 compared to 10.68% in 2013) before increasing in December 2015 (15.25%). Malaria prevalence was more important in children over 5 years old and boys. The overall prevalence of gametocytes carriage was 5.76% and it was more important in children under 5 years old. When the MSC was first scaled up in Saraya, there was a decrease in *Plasmodium falciparum* parasite carriage, which subsequently increased. The presence of gametocytes in children raises the question of whether primaquine should be added to this strategy for better.

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ENGAGING THE PRIVATE SECTOR IN ACHIEVING ZERO MALARIA, COUNTRY CASE STUDIES FROM COTE D'IVOIRE, DRC, LIBERIA AND UGANDA

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The ongoing fight against malaria has resulted in a 60% decrease in deaths from malaria and a 40% drop in malaria cases over the last two decades (2000-2020). However, malaria continues to remain a major health issue in many countries, with pregnant women and children under 5 being the most at risk. Thus, the journey towards elimination is far from over, and countries and development partners are increasingly looking at market-based approaches and investments from the private sector to help bridge gaps in both resource and expertise to sustain results to date and accelerate progress.The private sector is engaged in malaria control through various ways. For example, strengthened public-private partnerships (PPP) enable resource mobilization to scale up implementation of effective malaria interventions. However, many of their activities are not tracked by National Malaria Control Programs and this lack of information often prevents alignment with national goals and strategies. Given the important role of the private sector in the sustainable development of countries, and in line with USAID's Private Sector Engagement (PSE) Policy, the USAID-funded Local Health System Sustainability project is implementing a PMI-funded activity in four selected PMI priority countries, Cote d'Ivoire, DRC, Liberia, and Uganda, to: 1- do a landscape analysis of private sector contributions to malaria programming, and 2- Identify potential strategic opportunities to strengthen PSE. The methodology for this activity is a mix of literature review and in-country key informant interviews. The results of this activity are expected in August 2021. In each country we will develop a set of strategies to engage the private sector more efficiently and ultimately accelerate progress towards malaria elimination. This activity will also produce global learnings on PSE for malaria programing, especially the contextual factors to account for when designing collaborative activities with the private sector and advocating for more private sector funding for malaria in Low- and Middle-Income Countries.

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IMPROVING PREDICTION OF POPULATION LOCATION FOR HOUSE-HOLD BASED SERVICE DELIVERY: DESCRIPTIVE ANALYSIS AND MODELING USING GEOTEMPORAL DATA

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Mass campaigns consistently struggle to obtain good coverage for several reasons: poor planning and management of field teams, the lack of population knowledge of the intervention, and challenges in determining the location of eligible populations. These challenges could be addressed with a tool for locating eligible populations and predicting their movement: campaigns could be planned and deployed more efficiently, with sensitization efforts better targeted, and instances of missing subpopulations due to regular movement or permanent relocation reduced. The Reveal tool facilitates geospatial population estimation to reduce uncertainty in the volume and location of the population and has been applied in mass campaigns such as IRS, MDA, and vaccinations, but does not yet incorporate temporal dynamics. The Reveal Population Movement (RPM) project, funded by the Gates Foundation as a Grand Challenge Exploration (GCE) aims to address the challenge human movement poses to service delivery in two key ways. First, we are conducting a descriptive analysis of existing data, to understand temporal patterns in households being present and able to receive services. These data collected through implementations of house-to-house service delivery campaigns with the Reveal tool, contain geotags and timestamps on each household level data point, allowing granular exploration of temporal patterns. From this we will develop qualitative recommendations to inform deployment timing strategies. Second, we are constructing models of population movement based on cell phone provider data, to understand trends and build models that reveal spatio-temporal patterns in human movement. This model will provide a theoretical framework to make adjusted predictions in populations needing services in given areas at given times of year. We will present initial results from these two specific objectives and their proposed application to an implementation of the Reveal tool in a health service delivery campaign.

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PRIVATE SECTOR ENGAGEMENT TOWARDS MALARIA CONTROL: PERSPECTIVES FROM STATE ACTORS AND ASSOCIATIONS OF PRIVATE HEALTHCARE PROVIDERS IN AKWA IBOM STATE, NIGERIA

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Nigeria accounts for about 27% of global malaria cases and 23% of deaths. Private health facilities (PHF) play an important role in expanding access to malaria case management (MCM). In 2020, the USAID President's Malaria Initiative for States (PMI-S) project conducted a mapping study to identify opportunities to improve provision of high quality MCM, intermittent preventive treatment of malaria in pregnancy (IPTp), and data reporting among PHFs in Akwa Ibom State, Nigeria. The study included a review of relevant literature and in-depth interviews with seven state actors and four representatives of Associations of Private Healthcare Providers. The study found that a lack of awareness on guidelines on MCM and capacity deficits in the private sector have affected the delivery of high quality MCM and data reporting as seen in 13.9% of PHFs offering IPTp and 41.9% not using or not reporting on NHMIS. Not all private providers belong to these associations. There is a need to sensitize, train and retrain private health practitioners on MCM protocols. The associations expressed readiness to support MCM awareness creation within their memberships, as well as support efforts to increase compliance to national guidelines. Inclusion of MCM topics in continuing medical education (CME) based on the newly revised guidelines would be beneficial. Another recommendation is making CME programs on MCM mandatory for practitioners' license renewals. Given the importance of commodity supply and data management in ensuring delivery of quality-assured MCM and IPTp, access to state-sponsored commodity supply mechanisms at subsidized rates may incentivize private sector providers to report data. PMI-S is working closely with the state ministry of health to incorporate these recommendations into a private sector engagement strategy.

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UNLOCKING THE POTENTIAL OF PRIVATE SECTOR HEALTH FACILITIES IN MALARIA CONTROL: FINDINGS FROM A MAPPING STUDY IN OYO STATE, SOUTH WEST NIGERIA

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Nigeria accounts for 27% of global malaria cases and 23% of global malaria deaths. Private sector health facilities (PHF) play an important role in expanding access to malaria case management (MCM). In 2020, we conducted a mapping study to identify opportunities to improve provision of high quality MCM, intermittent preventive treatment of malaria in pregnancy (IPTp), and data reporting among PHFs in Oyo State, Nigeria. The study involved a desk review and a survey of 509 PHFs out of 733 identified in the state. Slightly over half (58%) of PHFs offered malaria rapid diagnostic testing (mRDT), while only 16.7% of PHFs offered microscopy, suggesting many PHFs still rely on clinical diagnosis to initiate malaria treatment. Findings revealed a low rate of provision of IPTp (30.6%) by the PHFs. Almost all PHFs (99%) offered Artemisinin-based Combination Therapy (ACT) for the treatment of uncomplicated malaria. Larger number of PHFs offered ACT than mRDT. Over half (54%) did not have the national guidelines on MCM available for use, raising quality concerns. Commodity stock out was highlighted as a prevalent problem by 60% of PHFs. Non-reporting of malaria data on the National Health Management Information System (NHMIS) was also quite common, as 35.5% of PHFs had never reported. The mapping team produced several recommendations for addressing these challenges. MCM and MIP guidelines should be made available to all PHFs to improve compliance and promote widespread use of mRDTs. Guidelines on management of malaria in pregnancy should also be made available to PHFs especially those offering maternity services. The state can consider extending subsidized commodity supply mechanisms to PHFs that regularly report health data as an incentive and motivation for others.

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PREVALENCE OF MALARIA INFECTION AND COVERAGE OF MALARIA CONTROL INTERVENTIONS AMONG PREGNANT WOMEN ATTENDING THEIR FIRST ANTENATAL CARE VISIT IN THREE HEALTH DISTRICTS OF BURKINA FASO: INTERIM RESULTS

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Health indicators are collected via household surveys and routine health surveillance activities. Data collected on malaria prevalence and coverage of malaria control interventions during antenatal care (ANC) clinic visits may provide an easily accessible, reliable, and representative source for monitoring population trends for select malaria prevelance and intervention coverage indicators. We assessed the potential to use pregnant women as a pragmatic sentinel population to collect health indicators in the health districts of Banfora, Gaoua, and Orodara, Burkina Faso. All pregnant women attending their first ANC visit in 21 randomly selected facilities in 3 health districts (7 per district) who gave informed consent were administered a standardized questionnaire and were tested for malaria infection using HRP-2-based rapid diagnostic tests. We analyzed preliminary data collected over 6 months (September 2020 to March 2021). In total, 4,587 pregnant women were enrolled. The proportion of pregnant women reporting that they owned an insecticide treated net (ITN) was 87.1% (95% CI, 85-89), 62.9% (60-65%), and 80.24% (78-82%) in Banfora, Gaoua, and Orodara, respectively. Among those possessing an ITN, the proportion of women reporting having slept under an ITN on the preceding night was 79.7% (77-82%) in Banfora, 58.8% (56-61%) in Gaoua, and 76.9% (75-79%) in Orodara. The prevalence of malaria infection among first ANC attendees was 27.5% (25-29%), 39.1% (36-41%), and 19.1% (17-21%) in Banfora, Gaoua, and Orodara, respectively. Comparison of data from ANC attendees with data from household surveys, to be collected in June 2021, will enable validation of the ANC platform for malaria surveillance. If validated to represent population-level trends, data from pregnant women attending ANC could provide a useful, routine data source to allow continuous monitoring of the coverage and impact of interventions.

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IMPACT OF TARGETED DELIVERY OF INTERVENTIONS TO MALARIA HIGH RISK POPULATIONS IN NAMIBIA

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Seasonal agricultural workers and cattle herders in northern Namibia have been identified as populations at higher risk of malaria, due to their

mobility, occupational exposures, and low access to effective malaria prevention. A quasi-experimental study using a matched pair pre-post-intervention design was used to evaluate the impact of targeted delivery of a package of interventions on coverage and malaria prevalence in these populations in the Ohangwena and Zambezi regions. A baseline cross-sectional survey was conducted between November 2019 and January 2020 in eight health facility catchment areas (HFCAs). A package of four interventions was rolled out at worksites in a random subset of four of the eight areas between January and March 2020, including: presumptive treatment with artemether lumefantrine, targeted IRS mop up with Actellic, a vector control pack (LLINs and topical repellents) and malaria education. Main outcomes (PCR-based *P. falciparum* prevalence and coverage of interventions) were assessed through an endline cross sectional survey conducted amongst 1,691 participants in May-June 2020. The secondary outcome was passively detected case incidence derived from health facility records. A generalized difference-in-difference approach using two-way fixed effects regression was used to compare change (baseline to endline) in intervention coverage, infection prevalence and HFCA-level incidence between intervention and control areas, adjusting for environmental covariates. Preliminary results suggest that the intervention resulted in an absolute increase of 57% (95% CI 51.7 - 63.5%) increase in coverage with an effective intervention ($p<0.0001$) in the target population, from an overall baseline of 26%, after controlling for age, gender and HFCA. There was 3.5% (95% CI 0.2 - 6.9) decrease in infection prevalence by RDT ($p=0.04$) in the target population, reducing it from 4% to 0.5%, and an overall decline in incidence of malaria in intervention areas ($p = 0.02$). Final analyses will be presented describing impact on PCR-based infection prevalence, and an assessment of heterogeneity of impact.

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THE IMPACT OF POPULATION-BASED INDOOR RESIDUAL SPRAYING, WITH AND WITHOUT A MASS TREATMENT CAMPAIGN, ON KEY MALARIA INDICATORS IN A HIGH TRANSMISSION SETTING IN NE UGANDA

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We describe results of a 32-month, prospective, controlled before and after trial investigating the impact of co-timed antimalarial mass drug administration (MDA) with population-based indoor residual spraying (IRS) on malaria burden in a high transmission region of NE Uganda. The study began in November 2016, and all arms had universal campaign coverage with non-PBO nets delivered by the Ministry of Health in April 2017. The study interventions were 4 rounds of IRS with pirimiphos methyl, alone or in combination with a simultaneous MDA with dihydroartemisinin-piperaquine. The third reference arm had nets alone. The study population was an open cohort of approximately 52,000 people in Katakwi District, Uganda. The three arms of ~16,000-18,0000 persons each were contiguous sub-counties with heavy entomological pressure. Prevalence in children under 5 measured by qPCR at baseline ranged from 48.5% to 58.4 %. All households in the study area were mapped and enumerated twice yearly. In the MDA arm, all medically screened persons over 6 months were eligible, and all households in intervention arms were eligible for IRS. MDA was directly observed day 1, and followed up after. Coverage of MDA was ~80%, and IRS coverage was ~98%. 6 cross-sectional prevalence surveys using rapid diagnostic tests, expert microscopy and qPCR were conducted during the study period, and a spatio-temporal geostatistical analysis was performed on the survey and household data for qPCR. Residence in the IRS arm was found to confer 64.7% reduced odds of infection with malaria compared with residence in the LLIN only arm, while residence in the MDA + IRS arm conferred an 80.1% reduction in the odds of being found infected. The additional protective impact of a single, co-timed round of MDA was detected via surveys performed (with one exception) more than 6 months after the MDA round. This shows that a single treatment round of antimalarials in a very high burden population, when combined with indoor spraying, can provide significant and prolonged protection from malaria infection. Limitations include non-random assignment to arm and the presence of only one cohort per arm.

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PERFORMANCE OF ZAMBIA'S NATIONAL MALARIA ELIMINATION PROGRAMME COMMUNITY HEALTH WORKERS

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As Zambia's National Malaria Elimination Programme (NMEP) continues to invest resources into community health workers (CHWs) as part of its strategy for achieving malaria elimination, understanding performance of these individuals and factors that influence outputs becomes increasingly important. Previous research has demonstrated competency of CHWs in conducting diagnostic tests and administering anti-malarials, though less work has been done to describe and assess CHW outputs. To provide further insight into NMEP CHWs performance, we first review performance in terms of both passive and active malaria testing rates between 2013 and 2019 across the entire population of CHWs. Using a subset of 404 CHWs drawn from a simple random sample for whom additional data were available regarding supervision, community support, incentive satisfaction, time demands, and other demographic characteristics, we assess relationships between these factors and individual CHW performance. The average number of passive tests conducted per month followed seasonal trends in malaria transmission, increasing as local malaria incidence increased. Active testing rates remained the same and the proportion of households followed up decreased during higher incidence periods, however. Zero-inflated negative binomial regression models showed that lower quality of supervision, lower incentive satisfaction, interest in monetary compensation, less time as a CHW, access to a bicycle, being single, and no access to electricity at home were individually associated with higher passive and/or active testing rates. After controlling for seasonal and geographic differences in local malaria incidence rates, greater satisfaction with current incentives was associated with lower (IRR: 0.69; p: 0.008) passive testing rates; active testing rates were higher (IRR: 1.47; p: 0.041) among CHWs specifically expressing interest in receiving monetary incentives. Understanding performance expectations and limitations of this largely voluntary workforce is important to optimize engagement and determine realistic assumptions for elimination timelines.

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FREEDOM FROM MALARIA INFECTION: A NOVEL FRAMEWORK FOR QUANTIFYING MALARIA ELIMINATION, AN EXAMPLE FROM INDONESIA

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The "freedom from infection" (FFI) framework is an approach, designed and used in veterinary epidemiology, to provide a quantitative estimate of the probability of detecting an infection in a population, if present above a predefined threshold. Here we show that the FFI method can be applied to the context of malaria to assess the sensitivity of the malaria surveillance system (SSe), a critical component in how likely it is an infection will be detected, and the corresponding probability of freedom (PFree). We collected monthly routinely reported malaria surveillance data from January 2017 to December 2019 and conducted semi-quantitative health systems surveys from 46 health facilities in Kulon Progo and Magalang Districts in Indonesia. The surveillance system sensitivity (SSe) was estimated, by conditionally modelling the flow of a potentially infected individual through the system (from the infection to being detected) and simultaneously modelling the underlying expected malaria cases in the population, using autoregressive spatio-temporal models in a joint inferential Bayesian framework based on the data collected. There was a high heterogeneity in the estimated probability of care seeking for malaria (PSeek; Mean=0.087, Min=0.012, Max=0.190), and the probability of being tested for malaria (PTest; Mean=0.17, Min=0.000, Max=1.000). This corresponded to the SSe per facility and PFree for detecting 1 or more cases, ranging between 0.00 and 0.14 (Mean=0.018) and 0.00 and 1.00 (Mean=0.277), respectively. Our results highlight that the FFI framework can be meaningfully applied to the context of malaria. Quantifying the SSe provides an approach to identify specific areas that can strengthen the malaria surveillance system and provide quantitative benchmarks for tracking progress, at all transmission intensities. Furthermore, the use of PFree can complement and guide malaria elimination decision-making by quantifying how likely it is that elimination has been achieved for better resource allocation.

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DOES REBOUND MALARIA EXPLAIN THE HETEROGENEITY IN RTS,S/AS01 MALARIA VACCINE EFFICACY?

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RTS,S/AS01 is the first vaccine against malaria to undergo pilot implementation. Trials reported lower vaccine efficacies in higher-incidence sites, potentially due to a "rebound" in malaria cases in vaccinated children. The rebound hypothesis posits that immune protection in the unvaccinated (acquired from natural infections) can temporarily rise above waning vaccine protection in the vaccinated, leading to a negative vaccine efficacy. Whether natural protection in the unvaccinated is superior to waning vaccine protection at certain time points is likely dependent on the level of malaria exposure. Using data from the 2009-2014 phase III trial (NCT00866619) in Lilongwe, Malawi; Kintampo, Ghana; and Lambaréné, Gabon, we evaluate the rebound malaria hypothesis by estimating malaria incidence over time by vaccination status and estimated malaria exposure. The phase III trial included two age groups: infants, who will not be vaccinated during pilot implementation, and children 5-17 months. Restricting to follow-up of unvaccinated infants after they reached 5 months of age, we fit a random forest model to estimate the relationship between malaria incidence and the following ecological predictors: rainfall, temperature, elevation, several vegetation indices, population density, nighttime lights, evapo-transpiration, aridity, household construction, and bed net use. Then, we apply our fitted model to estimate the malaria exposure for children in the phase III trial and fit a negative binomial model with a three-way interaction between vaccine group, time since vaccination, and estimated malaria exposure. We use the output from this model to calculate the incidence rate of malaria over time in each vaccine group and several malaria exposure settings. Malaria incidence in the unvaccinated decreased while vaccine efficacy waned, causing a rebound effect which increased with greater malaria exposure. For example, when exposure was set at 3 cases per-person-year (CPPY), modeled incidence in the three-dose vaccine group at 1 and 4 years post-vaccination was 2.01 and 3.19 CPPY, respectively, compared to 3.38 and 2.66 in the unvaccinated.

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HUMAN-CENTERED DESIGN PROCESS AND SOLUTIONS TO PROMOTE MALARIA TESTING AND TREATMENT-SEEKING BEHAVIOR IN GUYANA HINTERLANDS

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Malaria remains common among Guyana gold-mining populations. To address this public health challenge, an interdisciplinary team of public health professionals, graphic designers, and mining organizations collaborated on a human-centered design (HCD) process, including the i) Define and 2) Design & Test phases, facilitated by the USAID-funded Breakthrough ACTION Guyana and the MoH. In the Define phase, we conducted 108 qualitative interviews to understand experiences and challenges when seeking malaria testing and treatment services. These interviews were synthesized into 11 actionable insights on issues such as risk perception, malaria knowledge, preventive behaviors, traditional and self-treatment, testing, adherence to the correct treatment, and coordination and communication gaps. From these insights, during the Design & Test phase, we developed 33, "How might we…?" questions to stimulate creative thinking about potential solutions to the existing challenges. Five final prototypes included: (1) a "Little Mosquito, Big Problem" social behavior change campaign to address risk perception and other behavioral barriers; (2) rapid counseling cards to improve quality of malaria services; (3) branded malaria testing and treatment services located in mining areas; (4) innovations to improve treatment adherence; and (5) an integrative "participants, content, and logistics" strategy to ensure supply could meet demand. When applying HCD to public health issues, there are both opportunities and challenges to reconcile gaps that may exist between the two disciplines. HCD provides a new set of tools and mindsets that are not typically part of public health strategies. The synthesis produces innovative, feasible ways to organize work with migrant and mobile mining communities to encourage malaria testing and treatment services.

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DESIGN AND CHARACTERIZATION OF SELF-ASSEMBLING PROTEIN NANOPARTICLE PRESENTING PLASMODIUM FALCIPARUM PLASMODIUM FALCIPARUM THROMBOSPONDIN-RELATED ADHESION PROTEIN (TRAP) SUBDOMAINS IN CROSS-TERMINI DISPLAY

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Malaria remains a major health concern in tropical regions, causing an estimated 229 million clinical cases and 409,000 deaths in 2019 alone despite the development of novel prophylactic anti-malarial drugs and vector control efforts. Thus, a highly efficacious vaccine is critical to the control and eradication of malaria. Preclinical testing of novel malaria vaccine strategies achieved through rational antigen selection and particle-based delivery platforms is yielding encouraging results; however, to date, there is no licensed, efficacious vaccine for malaria. One promising particulate delivery system, the self-assembling protein nanoparticle (SAPN), has the potential to overcome limited vaccine efficacy through repetitive, high-density epitope display. Here, the von Willebrand factor A (VWA) and thrombospondin repeat (TSR) domains of *Plasmodium falciparum* thrombospondin-related adhesion protein (TRAP), an essential protein for sporozoite motility and liver-stage infection, were engineered onto the termini of a SAPN monomer, and particle formation was characterized. Contrasting previous SAPN requiring lengthy refolding processes in denaturing conditions, TRAP-SAPN monomers spontaneously formed into a particle after a one-step purification under non-denaturing conditions, likely due to the hydrophobic interactions between the continuous domains displayed across termini. Proper folding was confirmed by DLS, TEM, and epitope mapping by dot blot with conformation-specific monoclonal antibodies. Furthermore, stability testing indicated that TRAP-SAPN is stable at 4°C for one year, unlike most vaccine platforms requiring storage at -80°C. Current preclinical studies are exploring the functionality of this novel chimeric particle *in vivo* through immunization of BALB/c mice and *in vitro* through ELISA, ILSDA, and sporozoite gliding motility assays.

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INFLUENCE OF TRUST ON THE ACCEPTANCE OF THE MALARIA VACCINE IN THE KASSENA-NANKANA DISTRICTS OF GHANA

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The availability of a malaria vaccine for routine use will be a major milestone for the eradication of malaria mortality among children of under five years. Nonetheless, trust by the public for the vaccine could pose a major challenge for its comprehensive acceptance referencing documented evidence from the oral polio vaccine boycott in northern Nigeria and the failure of the Ebola vaccine trial in Ghana among others. A total of 390 structured questionnaires were administrated to mothers and caregivers of children of under five years and 15 in-depth interviews were conducted with mothers and health workers. The data was analyzed using STATA Version 16.0 and QSR NVivo 12 software. We performed a bivariate and multivariate regression analysis to determine the influence of trust on vaccine acceptance. The results were further explained and affirmed by the qualitative data. We found that knowledge about the vaccine was widespread (95.4%) and uptake of the vaccine was high (82.4%). In a bivariate analysis, health education about the vaccine and perceived ineffectiveness were found to be associated with acceptance of the vaccine. A multivariate regression of the factors found trust for the malaria vaccine as the main factor that positively influenced the acceptance of the vaccine among the mothers in the district (OR= 0.55[95% CI 0.30-0.84] P<0.001). This was confirmed by the qualitative data which similarly revealed trust for the vaccine as the main determinant of uptake. The results of this study suggest that trust for the malaria vaccine is critical for its uptake. Therefore, efforts towards improving comprehensive acceptance of the malaria vaccine and other vaccines should be focused on building and sustaining trust for the vaccine among mothers and community members.

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IMPACT OF A DELAYED AND FRACTIONATED THIRD DOSE OF R21/MATRIX-M ADJUVANT IN UK ADULTS

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Malaria remains a leading cause of childhood mortality, highlighting the need for an effective vaccine. R21 is a virus-like particle (VLP) vaccine displaying the immunodominant NANP repeat region and C-terminus of the circumsporozoite protein (CSP) of *Plasmodium falciparum*. R21 is novel in that CSP epitopes cover the particle surface of Hepatitis B surface antigen (HBsAg), whereas the current leading malaria vaccine candidate, RTS,S, has a much higher ratio of HBsAg to CSP. R21, combined with the saponin-based Matrix-M adjuvant (MM) has been assessed previously in Phase I and Phase II trials to determine safety, immunogenicity and efficacy with different vaccine doses but in this study the effect of a delayed and fractionated third dose is investigated. In a Phase I controlled human challenge model in Oxford, R21/MM, induced high levels of anti-CSP-specific IgG when administered to malaria naïve adults. Protection against clinical malaria after being immunized with the standard dose regimen of 3 low doses of R21/MM (10,10,10µg) administered one month apart was 63%. In the Phase I/IIa study to be presented here, dose and regimen timing are investigated. We compare immunogenicity and efficacy of the standard dose regimen (10,10,10µg – Group 3) with a delayed third dose (given at 6 months – Group 2). Additionally, a delayed fractional dose regimen with a lower final dose given at 6 months (10,10,2µg – Group 5) and a higher delayed fractional dose regimen (50,50,10µg – Group 4) were investigated. Data that will be presented support antigen dose-sparing, thereby impacting access and having economic implications of vaccine delivery. Regardless of dose, volunteers with an antibody response greater than 1100 ELISA units were significantly more likely to be protected when challenged with *P. falciparum*, making this a potential correlate of protection for R21/MM. Further work to assess antibody isotype and subclass, in addition to humoral and cellular responses to other vaccine constituents, will be presented. Lastly, a direct comparison of immunogenicity between R21/MM and another vaccine candidate, RTS,S/AS01, will be presented.

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A NOVEL PHYSIOLOGICAL MODEL TO STUDY PLASMODIUM FALCIPARUM INTERACTIONS IN PLACENTAL MALARIA

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Placental malaria poses a major health threat to women, resulting in fetal morbidity and low birth weight infants. It develops when infected red blood cells (iRBCs) adhere to chondroitin sulfate A (CSA) glycosylated syndecan-1 (SDC-1) on placental syncytiotrophoblasts (ST) via VAR2CSA, a parasite protein localized to the iRBC surface. Current vaccine efforts are directed at VAR2CSA to disrupt the interaction with CSA glycosylated SDC1 on the ST for protection against placental malaria. Current assays use CSA-coated plates; these are non-physiological and prone to high inter- and intra-assay variability. A more relevant tissue-based iRBC binding model will close the gap between *in vitro* assays, vaccine development and human clinical trials. We developed a new model using villous tissue

explants from human term placentas. We first confirmed that SDC-1 localized to the apical membrane of ST layer in villous explants, making it a suitable tissue for this model. iRBCs were stained with ethidium bromide to detect parasite DNA and uninfected RBCs were stained with DiD. Infected or uninfected RBCs were added to explants and rocked. After washing, explants were fixed, whole mounted and RBC binding was quantified manually in 5 fields of view using fluorescent microscopy. The iRBC n=3 binding between assays varied 19.7(%CV). ST area will be quantified since different levels could explain this high variability in binding. Controls include chondroitinase cleavage of CSA from explants and soluble CSA competition. Blocking iRBC binding with rabbit anti-VAR2CSA IgG validated the assay. Specific iRBC binding to SDC-1 on villous explants was reduced with chondroitinase treatment by 76.02±15.3%. Preincubation of iRBCs with soluble CSA or rabbit anti-VAR2CSA IgG reduced binding by 80.19±15.3% and 93.94±17.1%, respectively. Average background binding levels of uninfected RBCs was 28.89±19.4 compared to bound iRBCs 127.3±19.4 in 5 fields of view. This study presents a more physiological model to test the functional activity of antibodies from pre-clinical vaccine studies and human trials with candidate vaccines against placental malaria.

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POTENT HUMAN MONOCLONAL ANTIBODIES TARGET DIFFERENT DOMAINS OF MALARIA TRANSMISSION BLOCKING VACCINE CANDIDATE PFS48/45

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Malaria continues to be one of the most devastating infectious diseases worldwide. The spread of malaria parasites is highly efficient and occurs through bites of infected *Anopheles* mosquitoes. Malaria transmission blocking vaccines (TBVs) aim to interrupt malaria parasite development in the mosquito and thereby provide powerful tools for malaria elimination efforts. The sexual stage protein Pfs48/45 is a leading TBV candidate and will soon be evaluated in Phase I clinical trials. We previously demonstrated that naturally acquired polyclonal antibodies against Pfs48/45 reduce transmission *ex vivo*. However, little is known about the epitope specificity and potency of monoclonal antibodies (mAbs) that make up this functional polyclonal response in humans. Following single cell sorting and screening of B-cells from two donors with naturally acquired transmission reducing activity, we identified a panel of 100 Pfs48/45-specific human monoclonal antibodies. We characterized these antibodies in terms of affinity, epitope specificity and potency, and defined novel protective epitopes. Affinity was determined using surface plasmon resonance and native antigen recognition was confirmed in surface-immunofluorescence assays with live gametes. While western blot analyses demonstrated most mAbs were conformation dependent, some targeting the N-terminal domain of Pfs48/45 (domain I) were not. Competition experiments showed the mAbs can be grouped into four large clusters, targeting domains I, II or III, or both domains I and III. Interestingly, only domain I- and domain III-specific mAbs strongly reduced transmission in standard membrane feeding assays. MAbs against domain III, also known as the 6C domain, reduced transmission up to 95% at 2 µg/mL. This observation aligns with the activity of the known domain III mAb TB31F, and domain III targeting mAbs are the most potent human transmission blocking mAbs described to date. Altogether we provide unprecedented insight in protective and non-protective epitopes on Pfs48/45 which will inform the design of this important transmission blocking vaccine.

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FIRST LIVER AND BLOOD STAGE INFECTION OF AOTUS LEMURINUS GRISEIMEMBRA WITH ASEPTIC, PURIFIED, CRYOPRESERVED NF54 PLASMODIUM FALCIPARUM SPOROZOITES

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Preclinical studies of *Plasmodium falciparum* (Pf) pre-erythrocytic stage vaccines have been hindered by the limitations of existing models that require engineered parasites or hosts; there are no available nonhuman primate (NHP) models for assessing the PfNF54-based vaccines being clinically evaluated world-wide. Aotus monkeys are a well-established NHP model that supports Pf and *P. vivax* blood stage infections. However, success with Pf sporozoite (SPZ)-induced infections has been variable in previous studies. We examined *Aotus lemurinus griseimembra*, which are known to support PfSPZ infection with the Santa Lucia parasite but not the NF54 strain of Pf, as a model for pre-erythrocytic infection using aseptic, purified, cryopreserved PfSPZ. In an initial comparative pilot study, one *A. griseimembra* and one *A. nancymaae* monkey (both splenectomized) were each inoculated with 5.4x10e6 PfSPZ; two needle biopsies of liver were collected on day 6 and daily blood samples on days 6-10 post-challenge. Both liver biopsy samples from *A. griseimembra* were PCR-positive with comparable estimated parasite burdens per gram of tissue; blood samples from days 6-9 were PCR-positive for low-level parasitemia which cleared without treatment. All samples from the *A. nancymaae* monkey were negative. To our knowledge, this is the first demonstration of infection of any Aotus with PfNF54 SPZ. We will report further development of the *A. griseimembra*/PfSPZ NF54 model as a platform to assess pre-erythrocytic stage malaria vaccines.

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STRUCTURAL AND IMMUNOLOGICAL DIFFERENCES OBSERVED IN TWO RECOMBINANT PFS25 MALARIAL PROTEINS USED IN HUMAN CLINICAL TRIALS

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Malaria is a mosquito-borne disease caused by the intracellular growth of protozoan parasites in RBCs. Development of a vaccine that blocks transmission of malaria parasites to humans and mosquitoes is considered critical for elimination and eradication efforts. A vaccine against Pfs25, a glycosylphosphatidylinositol (GPI) anchored protein on the surface of zygotes and ookinetes, is being evaluated for development of a transmission-blocking vaccine that would interrupt mosquito transmission to humans. The most extensively studied Pfs25 transmission-blocking vaccine is comprised of recombinant forms of Pfs25 expressed as a secreted protein without the GPI anchor in *Pichia pastoris* that are chemically conjugated to a recombinant carrier protein, ExoProtein A. Pfs25 contains four epidermal growth-factor like domains. The first-generation recombinant Pfs25, identified as Pfs25H, contained 14

heterologous amino acids positioned at the amino- or carboxyl-termini. A second-generation recombinant protein has been produced to remove the 14 non-native amino acids and is identified as Pfs25M. The purified forms of both were characterized biochemically and biophysically for identity, purity, biological activity and integrity including protein structure. The biological activity of Pfs25H and Pfs25M generated by monomeric forms or conjugated nanoparticles appeared similar. However, fine mapping studies with two transmission-blocking monoclonal antibodies detected fine structural and immunological differences. An evaluation of nonhuman primate antisera generated against conjugated Pfs25H or Pfs25M also identified these unique structural differences. Small modifications in amino acid content may modify recombinant forms of vaccine immunogens and should be made with thoughtful consideration or empirical comparisons.

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IMMUNOGENICITY AND PROTECTIVE EFFICACY OF COMBINED PLASMODIUM FALCIPARUM PRE-ERYTHROCYTIC AND TRANSMISSION-BLOCKING DNA VACCINES DELIVERED BY IN VIVO ELECTROPORATION

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P. falciparum circumsporozoite protein (PfCSP) and P25 (Pfs25) are leading candidates for development of pre-erythrocytic and transmission-blocking vaccines (TBV), respectively. Although considerable progress has been made in developing PfCSP and Pfs25 vaccines, neither has elicited complete protection or transmission-blocking activity (TBA) in clinical trials. The combination of antigens from various life stages is an alternative strategy to develop an effective malaria vaccine targeting multiple stages. In this study, female and male Balb/c mice were immunized with DNA plasmids encoding PfCSP and Pfs25, administered alone or in combination via *in vivo* intramuscular electroporation. Antigen-specific antibodies were analyzed by ELISA, and splenic T cell populations by flow cytometry. Immune protection against sporozoite challenge, using transgenic *P. berghei* expressing both PfCSP and luciferase (Pb-PfCSP-Luc), was assessed by *in vivo* bioluminescence imaging and blood-stage parasite growth. TBA was evaluated in standard membrane feeding assays. High levels of PfCSP- and Pfs25-specific antibodies were induced in mice immunized with either antigen alone or in combination. No difference in antibody levels were observed for both PfCSP and Pfs25 between the single antigen and combined antigena immunization groups. When challenged by PbPfCSP-Luc parasites, mice immunized with PfCSP (alone or combined with Pfs25) revealed significantly reduced liver-stage parasite burden as compared to mice immunized with Pfs25 alone (PfCSP: $p<0.05$; Combined: $p<0.01$). Furthermore, parasite liver burdens were negatively correlated with PfCSP-specific antibody levels (Ab concentration: $p=0.0117$; Ab titer: $p<0.0001$). The evaluation of TBA of Pfs25-specific Abs is still in progress. Our studies reveal that the combination of PfCSP and Pfs25 DNA vaccines does not compromise the immunogenicity and protective efficacy when compared with mice immunized with each antigen individually. Our studies pave the way for further evaluation of other multi-stage and multi-antigen combined DNA vaccines against *P. falciparum*.

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IDENTIFICATION OF A NOVEL EPITOPE IN THE CIRCUMSPOROZOITE PROTEIN C-TERMINAL REGION BOUND BY RTS,S AS01 INDUCED HUMAN ANTIBODIES

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The mechanism of protection for RTS,S/AS01 remains an active area of research and is widely expected to be linked to antibody responses to the repeat region of the dircumsporozoite protein (CSP). Multiple reports have also indicated a possible role for C-terminal region antibody responses. These include association of higher levels of vaccine efficacy against strain matched parasites, as well as the induced antibody magnitude, avidity, and isotype of C-term antibodies. At present however, the evidence for a mechanistic link to protection is considered less definitive than for antibodies to the repeat region. The lone study to date of structure-function relationships for C-terminal binding monoclonal antibodies induced by PfSPZ vaccination with chloroquine prophylaxis indicated the two induced mAbs did not have infection blocking functional activity. In our studies of antibodies induced in humans by RTS,S/AS01, we describe a family of mAbs that bind to a similar epitope to that previously described. Additionally, we identify new mAbs that bind to a novel C-term epitope which is structurally distinct. The novel epitope primarily contains the sequence invariant β-sheets of the C-terminal region of CSP on the opposite side of the CSP molecule. The mAb binding residues of the epitope were also independent of the critical residues associated with vaccine escape identified by sieve-analysis of field isolates in RTS,S/AS01 clinical efficacy studies. We hypothesize this novel epitope could be the site of reactivity that contributes to the repertoire of RTS,S/AS01 induced functional activity against the C-terminal region of CSP. We propose these findings should be considered for structure-based vaccine designs of next-generation CSP-based vaccines.

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OPEN LABEL TRIAL TO ESTABLISH A BLOOD-STAGE CONTROLLED HUMAN MALARIA INFECTION MODEL AND DETERMINE ITS SAFETY IN HEALTHY TANZANIAN ADULTS WITH VARYING PRIOR EXPOSURE TO PLASMODIUM FALCIPARUM (VAC 083).

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Plasmodium falciparum malaria still remains a disease of public health significance. This has been caused by the waning effectiveness of currently registered antimalarial drugs due to fast emergence and spread of resistance and the absence of an effective vaccine hence need for new malaria disease fighting tools to be used in the eradication program. The Controlled Human Malaria infection (CHMI) model is one of latest tools for evaluation of candidate malaria vaccines and antimalarial drugs. It provides a cost-effective method to down-select potential candidate malaria interventions. CHMI studies can also facilitate assessment of immune correlates of protection in participants to inform development of effective malaria vaccines and monoclonal antibodies. However, this model has not been used in Africa, due to difficulty in accessing viable PfiRBC, lack of necessary technical expertise and infrastructure to support these kinds of studies. Therefore, the purpose of this study is to assess the safety and feasibility of controlled blood-stage *P. falciparum* human malaria infection in healthy Tanzanian adults with prior exposure to *P. Falciparum.* The design for this study is a single-centre, open label trial to determine the safety and feasibility of CHMI model using *Plasmodium falciparum*-infected cryopreserved erythrocytes administered to 12 healthy Tanzanian adults aged 18 to 35 with varying prior exposure to *P. falciparum.* Safety

analysis will consist of descriptive summaries for high and low exposure groups. The overall percentage of volunteers with at least one local adverse event and the percentage with at least one general adverse event (solicited and unsolicited) during the post-CHMI period will be tabulated. Comparisons between groups will be made based on a two-sided Fisher's Exact Tests. Haematological and biochemical laboratory parameters will be measured at specific time points. Clinically relevant abnormal values will be graded, tabulated and a trend analysis could be performed if deemed necessary. The monitored data of this study will be available in September 2021.

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EVALUATION OF IMMUNOGENICITY AND PROTECTIVE EFFICACY OF DNA VACCINES ENCODING FULL LENGTH AND TRUNCATED PLASMODIUM FALCIPARUM CIRCUMSPOROZOITE PROTEIN ADMINISTERED VIA IN VIVO ELECTROPORATION

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Plasmodium falciparum circumsporozoite protein (PfCSP) is the leading pre erythrocytic vaccine target, however PfCSP subunit vaccines have not reached success in eliciting complete protection. PfCSP contains three major domains: an N terminal domain (NTD), a central repeat domain, and a C terminal domain (CTD). Sequences in the repeat domain and in the upstream junctional region are the major targets of protective antibodies. The majority of T cell epitopes are located within the CTD, and as such may account for genetic restriction of immune responses. Also, antibodies targeted against this domain have been identified to be non-protective. We sought to evaluate immunogenicity and strain specificity of PfCSP in the form of DNA vaccines. Balb/c and C57Bl/6 mice were immunized with DNA plasmids encoding full length CSP (aa 1-397) and 3 different CTD truncated CSP: SS/N/R/C12 (aa 1-284, truncated 12 amino acids downstream of the repeat domain); SS/N/R/Th-Tc (aa 1-337, retaining Th-Tc and T* cell epitope); and SS/N/R/Th3R (aa 1-370, retaining CTD up to the Th3R T cell epitope) via *in vivo* intramuscular electroporation (EP). Immunogenicity of the DNA vaccines was evaluated by analyzing PfCSP specific antibody levels. Immune protection from sporozoite challenge using transgenic *P. berghei* expressing PfCSP and luciferase (Pb-PfCSP-Luc) was assessed by *in vivo* bioluminescence imaging and blood stage parasitemia. In C57Bl/6 mice, high levels of specific antibodies were induced by all four DNA vaccines. However, in Balb/c mice, full length, SS/N/R/Th-Tc, and SS/N/R/Th3R induced high antibody levels while SS/N/R/C12 induced significantly lower levels. When challenged by Pb-PfCSP-Luc parasites, all C57Bl/6 mice had reduced liver burdens compared to the control. In Balb/c mice, Full length, SS/N/R/Th-TC and SS/N/R/Th3R groups had reduced liver burdens compared to the control, while SS/N/R/C12 showed no statistical difference. Our study demonstrates markedly improved functional immunogenicity of PfCSP DNA vaccines by EP and also reaffirms the importance of T cell epitopes in the CTD for species transcending immunogenicity.

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PROLONGING MALARIA IMMUNITY WITH MOUSE CYTOMEGALOVIRUS VACCINATION

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Development of a long-lived vaccine against malaria remains a major priority. Immunity to *Plasmodium* infection and vaccination both decay. In mouse models, both B and T cells are required for immunity. However, decay of protection correlates with loss of malaria-specific T cells, not antibody. Whole parasite vaccines, like infection and drug cure, protect. However, sterile immunity from infection or vaccination last less than 200 days, while effector T cells (Teff) decay. Therefore, we have reverse-engineered a vaccine strategy to induce a long-lived antibody response with live malaria vaccination, followed by a boost to the Teff response to prolong protection. Cytomegalovirus (CMV) is a chronic vaccine vector that induces T cell-mediated immunity and shows promise against SIV, tuberculosis and liver-stage malaria. Therefore, we used MCMV to test our hypothesis that chronic stimulation of Teff activation would prolong protection by a live parasite vaccine. MCMV encoding *P. chabaudi* MSP-1 epitope B5 (MCMV-B5) and B5 TCR Transgenic T cells were used to follow the MCMV vector-induced malaria-specific T cell response. We observed that Chronic MCMV infection protected mice from parasite upon *P. chabaudi* infection. Consistent with this protection, vaccination with MCMV-B5 activated and maintained B5 T cells, and promoted generation of Teff, which we have previously shown to be protective, for 2.5 months. Importantly, boosting the live malaria vaccine with MCMV synergistically led to longer protection from *P. chabaudi* parasitemia. In investigating the mechanisms of protection, we found that IFNg maintained by MCMV is required for prolonged protection. Furthermore, blocking IFNg before challenge led to lower IL-12 production upon infection. As IL-12 is known to boost enhance antigen-presenting cell function and phagocytosis, we are currently investigating these pathways. Our findings suggest methods and mechanisms to effectively prolong immunity to *Plasmodium* infection with CMV vectored chronic vaccines to redress the problem of short-lived protection to malaria infection and vaccines which is known to be due to decay of T cell memory.

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ANTI-PFGARP IS ASSOCIATED WITH DECREASED PLASMODIUM FALCIPARUM PARASITEMIA IN KENYAN CHILDREN

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In prior studies, we identified PfGARP as a target of antibodies which induce parasite programmed cell death. Vaccination with PfGARP induces significant protection against *Plasmodium falciparum* challenge in non-human primates and anti-PfGARP antibody levels are associated with decreased parasitemia in adolescents and adults. In the current study, we measured anti-PfGARP IgG antibody levels in N=394 children, ages 2-7 yrs living in a holo-endemic region of western Kenya and related these levels to parasite prevalence in a cross-sectional analysis. When analyzed as a continuous variable, even after adjusting for potential confounders including age and sex, higher anti-PfGARP IgG levels were significantly related to lower parasitemia ($P = 0.04$). When analyzed as tertiles, children with low levels of anti-PfGARP IgG (n=129) had 7.6-fold higher parasitemia levels compared to children with high levels of anti-PfGARP IgG (n=134), $P = 0.002$. This result remained significant even after we restricted the analysis to those children who were parasitemic at the time of sample collection. These results demonstrate that PfGARP antibody

levels are associated with protection from parasitemia in young children and further support targeting PfGARP as a vaccine candidate for pediatric falciparum malaria.

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ROLE OF CD4 T CELLS IN PFS230 VACCINE ANTIBODY RESPONSE

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One of the most promising strategies to prevent *Plasmodium falciparum* in endemic areas is the implementation of malaria transmission-blocking vaccines (TBV). TBV act by preventing the development of *P. falciparum* in the mosquito vector and limit further malaria transmission. Pfs230 is a *P. falciparum* gamete antigen and the most advanced TBV candidate. The development of TBV malaria vaccines is challenging, due in part to the difficulty to induce sustained high-titer antibody responses. To address this problem, adjuvants and carrier proteins are employed to enhance immunogenicity of parasite targets. We investigated the role of CD4 T cells in vaccine-induced immune responses to Pfs230 domain 1 (Pfs230D1) conjugated with Exoprotein A carrier and formulated with ALFQ (an AS01B biosimilar) adjuvant in C57BL/6 mice. By depleting CD4 T cells at different times during the immunization, we demonstrated Th lymphocytes are required for optimal increase in antibody titers after the 2nd but not the 3rd vaccination. Moreover, injection of Pfs230D1 monomer at the time of the 3rd immunization was sufficient to induce peak titers and enhance antibody functional activity to the same levels observed when Pfs230D1 plus the carrier were injected. Taken together these findings suggest that the formulation of vaccine with the carrier may not be beneficial after 2nd immunization because although help provided by CD4 T cells play an essential role in enhancing humoral response after the first two doses vaccination, it is dispensable for the boost effect after the third. On-going experiments include mouse and monkey studies to extend these initial findings and to evaluate the role of different adjuvants at the time of 3rd immunization. This study highlights the ability to efficiently boost with monomer as opposed to conjugate; and describes the requirement for T cell help for vaccine boosting and maturation during Pfs230D1 vaccination. Defining the characteristics of T cells required for efficient boosting of the vaccine response in preclinical studies can inform the design of more potent combinations of vaccine and adjuvants to overcome malaria-induced immunoregulation.

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DEVELOPMENT OF AN INTRANASAL/ORAL SPIRULINA-BASED PFCSP MALARIA VACCINE

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Antibodies against the *Plasmodium falciparum* circumsporozoite protein (PfCSP) can block hepatocyte infection by sporozoites and protect against malaria. Most PfCSP-targeted vaccines like RTS,S require needle-based administration. Needle-free vaccination strategies are desirable. Here, we evaluated *Arthrospira* platensis algae (commonly called 'spirulina') as a malaria vaccine platform. Spirulina has a strong safety profile and is widely used as a human food source. Lumen Bioscience genetically engineered spirulina to express virus-like particles (VLPs) consisting of woodchuck variant (WHcAg) of the human hepatits B core (HBcAg) bearing a $(NANP)_{15}$ PfCSP antigen on its surface. Experimental vaccine biomass consisting of spirulina bearing WHcAg VLPs bearing externally-facing PfCSP was dried and remained stable for one year without refrigeration. PfCSP-spirulina biomass administered three times orally to BALB/cj mice was safe and moderately immunogenic, with low rates of protection (20%) against challenge with PfCSP-expressing *P. yoelii* sporozoites. To enhance priming, PfCSP-spirulina was administered intranasally, resulting in uniformly strong anti-PfCSP antibodies that were enhanced by a oral PfCSP-spirulina booster. Intranasal priming was stronger using an extract of the PfCSP-spirulina biomass than raw biomass. Upon challenge, female BALB/cj mice showed high titer PfCSP responses from the intranasal priming/oral boosting regimen and were highly protected against subcutaneous challenge with PfCSP-expressing *P. yoelii* two- or twelve-weeks post-vaccination (70%). The intranasal/oral PfCSP-spirulina vaccine was also safe and highly immunogenic in C57BL/6 mice. Thus, our data demonstrates that intranasal/oral spirulina vaccination induces protective PfCSP antibodies in rodent models of malaria and warrants further development as a promising candidate malaria vaccine platform.

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EVALUATING THE ENTOMOLOGICAL IMPACT OF THE 2019 PMI-SUPPORTED ITS CAMPAIGN IN ZAMBIA ON MALARIA TRANSMISSION PARAMETERS

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Entomological monitoring of vector control interventions is vital for impact evaluation. The Zambian National Malaria Elimination Program rotated clothianidin-based insecticide from Actellic CS. In October/November 2019, the U.S. President's Malaria Initiative (PMI) funded the VectorLink project to support clothianidin-based indoor residual spraying (IRS) in 17 districts in Zambia for the first time. Entomological monitoring was conducted monthly or bi-monthly 4 months before and 5 months after IRS at sentinel sites in 5 districts, with one unsprayed (control) site per district. Indoor density (ID), human biting rate (HBR), parity rate, and sporozoite rate (SR) were determined. The two main vector complexes found were *Anopheles funestus* s.l. (80.4%) and *An. gambiae* s.l. (19.3%). Seasonal increases in vector abundance were seen in both sprayed and control sites after IRS. The pre- and post-IRS mean ID of *An. funestus* s.l. increased at sprayed (2.9 to 3.6 vectors/house) and control (4.2 to 5.0) sites; *An. gambiae* s.l. ID also increased at sprayed (0.1 to 1.0) and control (0.02 to 0.6) sites. The pre- and post-IRS *An. funestus* s.l. HBR increased at sprayed (21.0 to 21.3 bites/night) and control (5.5 to 35.5) sites; *An. gambiae* s.l. HBR also increased at sprayed (0.7 to 12.0) and control (0.1 to 3.2) sites. Biting times and location did not shift post-IRS. *An. funestus* s.l. parity decreased (56% to 39%, $p<0.001$) at sprayed sites and increased (52% to 57%) at control sites. *An. gambiae* s.l. parity decreased (48% to 39%) at sprayed sites and increased (33% to 56%) at control sites. The SR for *An. funestus* s.l. was lower at sprayed vs control sites (2.9% vs 3.5%; $p=0.635$) but SR for *An. gambiae* s.l. was higher at sprayed vs control sites (0.9% vs 0.6%, $p=0.673$). Clothianidin IRS may not overcome the seasonal increases in vector abundance in sprayed areas. However, vector parity, a key IRS metric in entomological impact evaluation, was reduced.

Similar results have been observed using Actellic CS-based IRS over 4 years of past use. Alternative interventions such as ITNs and complementary interventions such as larval source management should be considered.

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OPTIMISATION OF SGRNA EXPRESSION WITH RNA POL III REGULATORY ELEMENTS FOR THE DEVELOPMENT OF A SPLIT DRIVE IN ANOPHELES STEPHENSI

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Anopheles stephensi is a competent vector for *Plasmodium falciparum* and *P. vivax*. It is the main vector of urban malaria in South-east Asia and since 2012 has invaded into several countries in East Africa. The invasion and spreading of this mosquito in Africa threatens all the current efforts for malaria control which rely mainly on extensive insecticide spraying and insecticide-treated bed nets. Although these methods have shown to be highly efficient and have helped to reduce malaria incidence, the occurrence and increase of insecticide resistance as well as environmental risks have weakened their use. Researchers have therefore turned to genetic approaches such as CRISPR/Cas9-based gene drives in recent years and were successful in developing such drives in *An. gambiae* and *An. stephensi.* We are interested in the development of a geographically confineable gene drive system that would allow a local and transient control of a mosquito population. In our study, we assessed two components of the split drive system: the A element, located in the cardinal (*cd*) locus, being an sgRNA expressed by an endogenous RNA pol III regulatory element (U6A, U6B, U6C, or 7SK), and the B element being a zero-population growth (*zpg*)-driven Cas9 which would drive the A element. Using a series of experimental crosses, we were able to determine factors which may affect the viability of the drive. Inheritance rates of the A element was found to be increased up to 71-100% and that the increment depended highly on the pol III promoters driving the sgRNA. Results also show lower homing rates to be associated with lower cutting rates and vice versa, indicating the significance of optimising sgRNA expression levels to improve homing efficiency. Interestingly, maternal deposition of Cas9 was also found to induce both cutting and/or homing of up to 100% in the germline of individuals inheriting only the A element. Taken together, the conclusions obtained from this study will be used to inform future designs and development of confineable gene drives in *An. stephensi*.

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ADVANCING EVIDENCE FOR THE GLOBAL IMPLEMENTATION OF SPATIAL REPELLENTS (AEGIS)

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Goals of malaria elimination and eradication and the expanding scope of *Aedes*-borne viruses (ABVs) like dengue require the development of new, vector control tools to complement available interventions in order to meet public health demands. Spatial Repellents (SR) are one such tool. SRs are products designed to release volatile chemicals into the air and prevent human-vector contact within the treated space thus reducing pathogen transmission. A number of clinical trials have demonstrated positive impact against malaria using SRs, however data is still lacking to confidently support use for public health. The AEGIS program will evaluate a novel, scalable SR vector control product with the potential to contribute to dramatic gains in malaria and ABV control in a variety of relevant contexts. This will be achieved through three cluster-randomized-controlled trials in Kenya, Mali, and Sri Lanka, one operational research study in Uganda, and the integration of social science at several study sites. The evidence generated is meant to provide data on SR impact, feasibility, and safety to fill the current gaps required for an endorsement by the WHO for a global SR policy recommendation. Such a recommendation will support disease endemic countries in the adoption and implementation of SR products and strategies in national vector control programs.

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FIVE-YEAR IMPACT OF TARGETED INDOOR RESIDUAL SPRAYING WITH PIRIMIPHOS-METHYL IN AN AREA OF HIGH MALARIA TRANSMISSION IN LUAPULA PROVINCE, ZAMBIA

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A strategy of targeted indoor residual spraying (IRS) was adopted in Nchelenge District, Zambia, when the program switched to a long-lasting form of pirimiphos-methyl (Actellic 300CS) in 2014 and conducted five rounds of annual spraying prior to the annual rainy season. Malaria transmission in Nchelenge District is holoendemic and the two primary vector species, *An. funestus* and *An. gambiae*, peak at different times of the year. Parasite prevalence by rapid diagnostic test (RDT) and vector abundance in households were measured in the community from April 2012 - November 2019 from monthly cross-sectional surveys. Weekly malaria cases from passive surveillance at the health centers were also collected. In an adjusted analysis of households within the targeted spray areas that compared the pre-Actellic years to the last year of Actellic use, there was a slight but insignificant reduction in the odds of being RDT positive in households that received IRS compared to households that did not (OR: 0.83, 95% CI: 0.63 - 1.07). IRS did not reduce the number of *An. funestus* caught within a household (IRR: 1.33 95% CI: 0.91 - 1.94) however there was a moderate but insignificant reduction in *An. gambiae* counts (IRR: 0.68, 95%: 0.41, - 1.11). Similar results were found when the analysis was expanded to include unsprayed households outside of the targeted IRS areas. In an adjusted model using serial cross-sectional data for the years of Actellic use (2014-2019), there was a protective effect of living in a targeted spray area on the odds of being RDT positive (OR: 0.74 95% CI: 0.59 - 0.91) but no reduction in the odds of being RDT positive in sprayed vs unsprayed households. There was a 25% reduction in the incidence of malaria at clinics located in the targeted areas compared to clinics outside of targeted areas after 4 years Actellic use (IRR: 0.75, 95% CI: 0.71 - 0.80), although this model does not account for other changes in malaria control interventions that occurred during this time. The targeted IRS strategy used in this high transmission area had limited impact on vector counts and parasite prevalence in the community, but overall malaria incidence in health centers declined.

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COMPARATIVE EFFICACY OF SIX DIFFERENT TYPES PYRETHROID PIPERONYL BUTOXIDE (PBO) AGAINST PYRETHROID RESISTANT MALARIA VECTORS IN WEST AND CENTRAL AFRICA

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Pyrethroid-PBO nets have become available for malaria vector control and are being deployed in Africa. Several brands of these nets with varying characteristics and technical specifications have been developed presenting extra choice to vector control programs. Studies investigating the comparative performance of these different types of pyrethroid-PBO nets in different settings are essential. We performed a series of experimental hut trials to compare the efficacy and wash resistance of six types of pyrethroid-PBO nets (Olyset Plus, PermaNet 3.0, Duranet Plus, Duranet Plus 2.0, Veeralin and Tsara Boost) against wild pyrethroid resistant malaria vectors (*Anopheles gambiae* sl and *Anopheles funestus*) in experimental huts in Cameroon, Cote D'Ivoire and Benin. Comparisons were also made with pyrethroid-only nets. Susceptibility bioassays showed that the vector populations in all three study sites were highly resistant to pyrethroids and resistance could be mitigated by pre-exposure to PBO. Mosquito mortality was higher in huts with pyrethroid-PBO nets compared to pyrethroid-only nets in all study sites (20-40% vs. 10-25%). The levels of improved effect observed varied between sites and between the different types of pyrethroid-PBO nets and were substantially low compared to other newly developed types of dual insecticide ITNs tested in these sites. Wash resistance depended on the type of pyrethroid and the initial dose of both active ingredients on the net. DuraNet Plus, DuraNet Plus 2.0, Veeralin and Tsara Boost showed non-inferiority in terms of mosquito mortality and/or blood-feeding inhibition to at least one of the two brands which have so far demonstrated an improved epidemiological impact in community randomised trials (Olyset Plus and PermaNet 3.0). The laboratory bioassays (cone bioassays and tunnel tests) and chemical analysis results corroborated the findings in the experimental huts. Considering the low levels of improved effect observed, it is not clear whether either of these nets would have the same epidemiological impact against malaria demonstrated with pyrethroid-PBO nets in trials in East Africa.

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UNIVERSAL LONG-LASTING INSECTICIDAL NETS COVERAGE ON BIOKO ISLAND: THE CHALLENGE OF THE NET USE GAP

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Despite the known community effects of long-lasting insecticidal nets (LLINs) against malaria, achieving and maintaining universal coverage remains a challenge in many settings. Particularly challenging is narrowing the net use gap, which in most cases appears linked to lack of access more than to behavioral factors. In 2015, vector control on Bioko Island shifted towards maximizing LLIN coverage through triennial, mass-distribution campaigns (MDC) to guarantee LLIN access accompanied by intensive behavior change and communication efforts to promote use. We assessed LLIN coverage and the net use gap using malaria indicator survey (MIS) and MDC data to estimate standard LLIN indicators at a 1x1 km area grid. Data from the 2018 MDC provided the number of LLINs distributed while the 2018 and 2019 MIS data allowed quantification of LLINs observed by surveyors and of population use. On average, the 2018 and 2019 MIS took place 4.2 [range: 1.2 to 7.4] and 16.3 [range: 13.2 to 20.0] months post MDC. Following the MDC, virtually 100% of the Bioko population had access to LLINs. Mean population access based on LLINs observed declined to 48.4% [IQR: 21.5-70.9] in 2018 and remained at similar levels a year later (52.8% [IQR: 30.8-80]). Median LLIN use amongst respondents was 51.1% [IQR: 33.3-68.3] in 2018 and 40.0% [IQR: 19.4-55.0] in 2019. Median LLIN use to access ratio based on observed LLINs was 1.0 and 0.8, respectively, but in many areas this ratio was higher than 1, suggesting that there was an over demand of nets among those using them. Despite universal LLIN coverage was guaranteed during MDC, substantial LLIN loss occurred soon after the campaign, leaving around half of the population without access to a LLIN. There was considerable spatial variation in LLIN coverage, with some communities better protected than others. These findings corroborate that the net use gap in some areas of Bioko Island cannot be explained by lack of access. This translates into sub-optimal impact of LLINs and calls for a change in current integrated vector control strategies on Bioko Island that focuses on other mainstream interventions.

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CHALLENGE OF ESTABLISHING AN EXPERIMENTAL MOSQUITO FEEDING MODEL OF PLASMODIUM FALCIPARUM IN AN URBAN CENTER IN MALAWI

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Characterizing the infectious reservoir of *Plasmodium* is an active area of research with potential to contribute to malaria elimination. A critical component of defining the infectious reservoir is identifying who is carrying gametocytes. Beyond that, mosquito membrane feeding assays (MFAs) are a commonly used technique to assess human-to-mosquito transmission. To validate and optimize MFAs in Malawi, we sought gametocyte donors in Southern Malawi during two rainy seasons (December 2019 to May 2020, and November 2020 to March 2021). Patients diagnosed with malaria by rapid diagnostic tests (RDTs) at health centers (HCs) within 20 km of our insectary facilities in urban Blantyre, were screened for gametocytes using blood smears performed at the time of RDT diagnosis and read by expert research microscopists. Out of the 1008 blood smears collected in three HCs, 35 (4%) were found positive for gametocytes. The gametocyte carriage rate among patients was high at the onset of the rainy season and gradually decreased over time (7%, 7%, 1%, 5%, 3%, 2% and 0%, for November, December, January, February, March, April and May, respectively). The median gametocyte density (interquartile range) was 128 (-432 to 848) gametocytes/µL. For the two HCs closer to the insectary facilities (within a radius of 5.4 km), participants were asked to provide venous blood for MFAs; only 6 out of 14 consented. The main reason of refusals was fears concerning SARS-CoV-2 as the venous blood draw would occur at the district referral hospital, the center of COVID-19 care, which is adjacent to the insectary. Overall, rates of gametocyte carriage and willingness to participate were lower than anticipated. The preliminary results of this study show the difficulty in establishing MFAs in an urban center with low transmission intensity.

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PILOTING A NOVEL GEOSPATIAL PLATFORM TO SUPPORT INDOOR RESIDUAL SPRAYING (IRS) FOR MALARIA CONTROL IN CUANDO CUBANGO, ANGOLA

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Reveal is an open-source platform and digital global good that puts geospatial technology in the hands of Indoor Residual Spray (IRS) teams to support campaign delivery and management. The platform uses satellite imagery and enumerated residential structures to guide the movement of spray teams and collect data while enabling real-time monitoring of IRS delivery. Here we discuss the coverage and user experience of piloting Reveal during the IRS campaign in Menongue Sede in Cuando Cubango province, Angola from December 2020 through March 2021. Field teams were trained to use the platform to identify residential structures for spray and record structure spray status. Reveal-generated data guided campaign decisions to improve coverage and efficiency. Using Reveal, teams visited 58,418 of 76,177 total enumerated structures, indicating a total found coverage of 76.3%. Of those visited, 1,223 structures were ineligible for spraying. Of the remaining 57,208 structures, 53,543 were sprayed yielding a spray success rate of 93.6%. Real-time spatial representation of implementation progress enabled field teams to deploy their resources with greater efficiency and precision. Supervisors used Reveal to identify areas with low coverage and subsequently prioritize areas to be revisited. Daily syncing of data into custom web dashboards strengthened communication between supervisors, field teams, and local authorities. Custom safeguarding mechanisms strengthened data quality by reducing opportunity to capture inaccurate data. Challenges included limited connectivity, which reduced the frequency with which field teams were able to sync their data and review spray progress. However, teams overcame this barrier through novel strategies such as sending individual team members to locations with connectivity to sync devices. Factors that were critical to the overall success of this pilot include careful selection and thorough training of data collectors, and meticulous logistical planning to ensure proper handling of the mobile client. The Reveal approach can be used in future campaigns to continue to strengthen precision and increase impact.

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INTERIM RESULTS FROM A MIXED-METHOD ANALYSIS OF INSECTICIDE-TREATED NET USE ACROSS SIXTEEN DISTRICTS IN BURKINA FASO, RWANDA, MOZAMBIQUE, AND NIGERIA

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Global progress in the fight against malaria has stalled due in part to increased resistance to pyrethroid insecticides in key vector populations. New, dual-active ingredient insecticide-treated nets (ITNs) that are effective against insecticide-resistant mosquitos have been developed. The New Nets Project, funded by a partnership between Unitaid and the Global Fund to Fight AIDS, Tuberculosis and Malaria and led by IVCC, is conducting observational analyses accompanying the pilot distribution of these new ITNs in Burkina Faso, Mozambique, Nigeria, and Rwanda to generate evidence on their effectiveness and cost-effectiveness. One study component uses quantitative and qualitative methods to observe and describe time spent in and out of ITNs and to explore the determinants of ITN use through key informant interviews and focus group discussions with community members. Results will be used to inform and refine transmission dynamics model and to augment epidemiological and entomological findings by providing additional context. Participants described getting ITNs primarily through government distribution campaigns. There was variability across and within countries on whether households felt they received enough ITNs for their household and at the desired frequency. There was also variability in whether participants felt they could access ITNs through other means, with some participants having bought ITNs at pharmacies, while others said that the campaigns were the only way they could get an ITN. The inability to replace old, torn ITNs was repeatedly noted as a barrier to using ITNs. Other barriers included excessive heat and the smell of the ITNs. Benefits to using ITNs included protection from malaria, protection from mosquitoes and other insects, and warmth. Heterogeneity in access, perception, and barriers to use could have substantial impact on ITN effectiveness. This interim analysis highlights the importance of tracking qualitative evidence over time in pilot areas. Interview and focus group discussion guides have been refined based on findings from this interim analysis and will be used in subsequent rounds of data collection.

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DEVELOPMENT OF A SUSTAINABLE ALTERNATIVE MATERIAL FOR LONG-LASTING INSECTICIDAL NETS: CELLULOSE-BASED INSECTICIDAL FIBER YARN FOR MALARIA CONTROL

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Long-lasting insecticidal nets (LLINs) are an essential vector control tool for the prevention of malaria. These nets exhibit prolonged insecticidal properties even after constant exposure to environmental factors and repeated washing. Using material processing techniques, long-lasting insecticidal activity has been achieved through migration of insecticide particles to the surface of the filaments over time. These nets have contributed to great successes in malaria control and will continue to be distributed as countries make progress towards universal coverage; however, existing LLINs are made from nonbiodegradable and nonrenewable petroleum-based polymers. Alternatively, cellulose, an abundant natural polymer, is evaluated in this study due to its favorable properties which allow it to perform similarly to common synthetic polymeric materials. Green chemistry approaches, including the use of deep-eutectic solvents and ionic liquids, were used to disperse the cellulose pulp, and various modern fiber-spinning methods, including wet-spinning, were employed to create a cellulose yarn. The pyrethroid insecticide deltamethrin and the synergist piperonyl butoxide (PBO) were evaluated for their stability in the solvents to ensure the effectiveness of the processing parameters. Techniques such as Fourier-transform infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (NMR) were used to characterize the resultant fiber yarn and fundamental interactions with pyrethroids and evaluate slow release time scale. This work describes the development and evaluation of a cellulose-based renewable and biodegradable fiber yarn. These findings can be considered for the production of novel LLINs for effective and sustainable global malaria control.

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LEVERAGING ROUTINE DATA FOR MALARIA VECTOR CONTROL EVALUATION: METHODOLOGICAL CONSIDERATIONS FOR CONDUCTING OBSERVATIONAL ANALYSES

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As new vector control tools become available, national malaria programs (NMPs) need to make data-driven decisions to inform their deployment. While randomized controlled trials provide high quality evidence on intervention efficacy, they are costly and sparse. The availability and quality of routine epidemiological and entomological surveillance data continues to improve and could provide a continual information source for decision-making. While broad guidance exists for evaluating malaria interventions using routine data, there is little guidance on best practices for vector control evaluations, which are particular in their entomological and ecologic scope and periodicity. We will present a framework for designing vector control evaluations using routine data sources. Through this framework, NMPs and partners can rapidly and consistently gain insights into the impact of vector control interventions in their countries. Analyses have been published or are underway by the Next Generation Indoor Residual Spray Project and the New Nets Project partnerships to document the impact of indoor residual spraying and various insecticide-treated net types in seven countries in sub-Saharan Africa. These evaluations have used a combination of programmatic epidemiological and entomological surveillance data to assess the impact of the interventions on vectors and disease burden. Based on these experiences, we will provide practical guidance on the design and implementation of vector control evaluations using routine data. Specific topics will include considerations for selecting and using interrupted time series (with or without a control group), difference-in-difference, counterfactual models, and other common designs; options for managing data quality issues; common covariates, their calculations, and data sources; and considerations for selecting follow-up periods for each intervention. These recommendations are designed to increase the frequency and rigor of vector control evaluations using available data sources, so that these evaluations can effectively support national and subnational vector control decisions.

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ABUNDANCE, BITING ACTIVITY AND PREFERENCE OF ANOPHELES SPECIES IN TWO IMPORTANT MALARIA ENDEMIC REGIONS OF COLOMBIA

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In Colombia, malaria is an important problem of public health and the Bajo Cauca-BC and Pacific region-PAC are currently the most malarious endemic areas in the country. Components of vector capacity are assessed to estimate the ability of mosquitoes to transmit pathogens. This work evaluated abundance, biting activity and preference of *Anopheles* mosquitoes collected in Bagre-BC, and in Buenaventura and Istmina-PAC, during 2018-202, between 18:00 and 24:00 hours, using 70% barrier screens and protected human bait placed indoors and outdoors. A census was performed on the animals present at the sampling site on a 1 Km radius. Specimens were identified using morphological keys and molecularly confirmed by PCR-RFLP-ITS2. Biting activity and human biting rate (HBR) were determined. In addition, blood-sources were identified by PCR-*Cytb* in DNA extracted from midgut of resting mosquitoes. A total of 1987 *Anopheles* were collected, 528 specimens from Bagre-BC, 167 from Istmina and 1292 from Buenaventura-PAC. *Anopheles darlingi* and *An. nuneztovari* were the most abundant species in BC and PAC, respectively. The HBR values were, for *An. nuneztovari*, 94 bites/person/night (b/p/n) in PAC, and 19.8 b/p/n for *An. darlingi* in BC; both had activity mainly indoors. The preferred blood-source for both mosquito species was humans; this predominantly anthropophilic preference supposes a high risk for humans. Other vertebrate sources included pigs, dogs and cows, which also indicates an opportunistic behavior. These findings should direct the implementation of control measures to minimize human-vector contact.

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OVERVIEW OF MALARIA TRANSMISSION FROM AN OBSERVATIONAL STUDY IN FOUR SUB-SAHARAN AFRICAN COUNTRIES

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Emergence of insecticide resistance in vector populations continues to threaten the effectiveness of malaria vector control tools, including insecticide-treated nets (ITNs) resulting in slowed progress toward malaria control and elimination. Through the New Nets Project, dual active ingredient ITNs (Interceptor® G2 [IG2] [BASF] and Royal Guard® [RG] [Disease Control Technologies]) have been piloted in several countries, alongside pyrethroid-piperonyl butoxide (PBO) and standard ITNs. Observational studies in Burkina Faso, Mozambique, Nigeria, and Rwanda aim to assess the effectiveness of these dual active ingredient ITNs. Sixteen districts from the four countries had baseline cross-sectional surveys to assess malaria prevalence, household ITN ownership from previous net campaign, and ITN use the previous night. In Rwanda, all household members were included, while other countries sampled only children under five. Two districts in Rwanda and one in Burkina Faso had surveys during and after net campaign, respectively. Malaria prevalence was categorized as high (>50%), moderate (>20-50%), or low (<20%). Random effects meta-analysis computed the overall odds of effectiveness of ITNs for all countries combined adjusted for district, household ITN ownership, and use at night. Prevalence was low in 4 of 16 districts in Rwanda (2) and Mozambique (2), moderate in 7 of 16 districts in Burkina Faso (2), Nigeria (2) and Mozambique (3), and high in 5 of 16 districts in Burkina Faso (1), Nigeria (2), Mozambique (2). Household ITN ownership varied from 8% to 100% and was above 80% in 5/16 districts in Rwanda and Burkina Faso, below <30% in 4 of 16 districts in Mozambique and Nigeria. The population who slept under an ITN the previous night varied from 3% to 79%. Malaria prevalence decreased as the proportion of ITN use the previous night increased (coefficient = 0.63, p=0.003). The diversity in baseline prevalence and net use is important in understanding the geographic diversity of this study and will be important in generating data on effectiveness of IG2, RG and PBO ITNs, which will inform decisions on malaria vector control tools.

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ESTIMATING THE MALARIA PREVENTION IMPACT OF NEW NETS: BASELINE RESULTS FROM OBSERVATIONAL ANALYSES TO EVALUATE THE EVIDENCE GENERATED DURING PILOTED DUAL ACTIVE INGREDIENT INSECTICIDE-TREATED NET DISTRIBUTION IN NIGERIA

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The Nigeria National Malaria Elimination Programme is leading an evaluation of the impact of dual active ingredient insecticide-treated nets (ITNs) and pyrethroid + piperonyl butoxide (PBO) ITNs in comparison to standard pyrethroid-only ITNs on malaria transmission as part of the New Nets Project. The project will occur over three years in four high-burden local government areas (LGAs) in Nigeria with insecticide resistance: Ejigbo (alpha-cypermethrin ITNs) and Ife North (alpha-cypermethrin + PBO ITNs) in Osun State, and Asa (chlorfenapyr + alpha-cypermethrin ITNs) and Moro (pyriproxyfen + alpha-cypermethrin ITNs) in Kwara State. Following ITN distributions in late 2020, epidemiological, entomological, and anthropological methods are collecting data on several indicators, including malaria prevalence in children under five and ITN ownership and use. The baseline cross-sectional survey was conducted in October 2020 in 420 households per LGA and the collection of entomological data began in September 2020. The baseline cross-sectional survey revealed that ITN ownership ranged from 8.0% in Asa to 43.1% in Ejigbo. ITN use during the previous night ranged from 3.0% in Asa to 24.2% in Ife North. Malaria prevalence ranged from 38.4% in Ejigbo to 63.1% in Asa. Baseline human-baited CDC light trap collections indicate that *Anopheles gambiae* s.l. is the most abundant vector species group in Ejigbo (88% of all Anopheles spp. collected), Asa (100%), and Moro (100%), while in Ife North An. funestus s.l. (82%) was the most abundant. Molecular analysis shows the An. gambiae s.l. collected were predominately An. gambiae s.s. (70%) and An. coluzzii (27%), with very few An. arabiensis collected. The pyrethroid susceptibility of An. gambiae s.l., varied across the districts, from 12%-38% in Asa to 73%-94% mortality in Ejigbo. Baseline results show there is high malaria burden in these LGAs and malaria vectors are resistant to pyrethroids. The results also indicate some epidemiological and entomological differences across study LGAs which should be considered in future analyses.

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BED NET INDICATORS AND MALARIA PREVALENCE AMONG CHILDREN AGED 6 TO 59 MONTHS ONE YEAR AFTER INTRODUCTION OF DUAL ACTIVE INGREDIENT INSECTICIDE-TREATED BED NETS IN THREE HEALTH DISTRICTS AREAS OF BURKINA FASO: PRELIMINARY RESULTS

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Using insecticide-treated nets (ITNs) has contributed significantly to reducing malaria burden worldwide since 2000. However, since 2015, resistance to pyrethroids in mosquito populations has occurred in many malaria-endemic countries including Burkina Faso. New, dual active ingredient ITNs, Interceptor G2®(BASF) (IG2) (pyrethroid+chlofenapyr) and pyrethroid+PBO ITNs were deployed in 2019 in the districts of Gaoua, Banfora, and Orodara through a pilot study as part of the New Nets Project (funded by Unitaid and the Global Fund). The effectiveness and cost-effectiveness of ITNs in Burkina Faso will be evaluated over three years. As part of the evaluation, a household questionnaire was administered to capture data on demographic, socioeconomic and malaria prevention practices from 190 households per district in 2019 and 2020. Both surveys occurred before the annual seasonal malaria chemoprevention campaign and during the high malaria transmission season. Malaria prevalence among children aged from 6 to 59 months, using HRP2 based rapid diagnostic tests, was assessed during the surveys. At baseline, household ITN use was 20.8% (18.6-23.1%), 67.7% (64.9-70.3%), and 78.8% (76.1-81.2%) respectively in Gaoua, Banfora, and Orodara. These proportions increased respectively to 44.2% (40.9-47.5%), 90.4% (88.5-92.1%) and 84.8% (82.3-87.0%) the following year. The highest malaria prevalence was in Gaoua, at 81.0% (74.9-86.0%) in 2019, which decreased to 49.0% (41.9-56.1%) in 2020. Similarly, significant decreases in malaria prevalence were also observed in Orodara (PBO ITNs) and Banfora (IG2 ITNs) from 28.4% (22.4-35.3%) to 3.6% (1.8-7.5%) and 39.6% (33.0-46.6%) to 18.4% (13.5-24.6%), respectively. The preliminary results showed an improvement in ITN use and a decrease of malaria prevalence in children one year after mass distribution of ITNs in the three districts.

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ENTOMOLOGICAL SURVEY IN THREE HEALTH DISTRICTS IN BURKINA FASO WITH PYRETHROID, PIPERONYL BUTOXIDE-PYRETHROID, AND INTERCEPTOR G2 ITNS DEPLOYED

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Insecticide resistance threatens the efficacy of standard, pyrethroid-only insecticide-treated nets (ITNs) in Burkina Faso. Experimental huts trial results suggest that dual active ingredient ITNs could increase mosquito mortality and reduce malaria transmission in areas with pyrethroid resistance. Two varieties of dual active ingredient ITNs (Interceptor® G2 [IG2] [BASF]: alphacpermethrin + chlofenapyr, and Olyset Duo® [Sumitomo Chemical Company Ltd.]: permethrin + piperonyl butoxide [PBO]) were distributed in select health districts during the 2019 distribution campaign while the rest of the country was provided with standard ITNs. The large-scale adoption and deployment of new ITN types requires additional evidence to prove their effectiveness in killing pyrethroid-resistant mosquitoes within real-life conditions of use. As part of a larger observational study being conducted by the New Nets Project to measure the impact of the various 2019 ITN distributions, mosquito collections began in nine villages (three villages in each of three study health districts) prior to the deployment of nets (June to July 2019), and are still ongoing. They consist of regular mosquito collections using human landing capture and CDC light traps, once a week, in each village for three years. A total of 24,360 malaria vectors were collected during the first five months (July to September 2019, during "baseline" and September to December 2019 for the initial "post-distribution" period). From these, the dominant species were *Anopheles gambiae* s.l. (80%) and An. funestus (11%). Peak vector abundance was observed from August to October. More than half (57.1%) of the vectors were collected host-seeking outdoors, a trend that was consistent across study districts. The overall human biting rate was ~2 bites/person/night in the pyrethroid-only area, ~1b/p/n in the PBO-pyrethroid area and ~4 b/p/n, in the IG2 area. Vector abundance and the human biting rates varied according to study

district, with a consistent preference for outdoor biting. The entomological inoculation rate is not yet available, and the sporozoite infection analysis is ongoing.

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OBSERVATIONAL ANALYSIS OF THE IMPACT OF INSECTICIDE ROTATION FOR AN INDOOR RESIDUAL SPRAYING PROGRAM IN THE UPPER WEST REGION OF GHANA 2016-2019

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Two indoor residual spraying products, Actellic® 300CS (Act.) and SumiShield® 50WG (SS), were recently used in Upper West Region (UWR), Ghana as part of the Next Generation IRS Project. In 2016 and 2017 all 11 districts in UWR sprayed Act., in 2018 4 districts switched to SS, and in 2019 all 11 districts sprayed SS. The observational, retrospective analysis presented here explores the effect of switching from Act. to SS on malaria cases as reported to the District Health Information Management System 2 from 2016 to 2019. Cases represent all patients who sought care at public health facilities and tested positive. Monthly malaria incidence rates were calculated for all 11 districts, and differences in monthly incidence rates for each 6-month post spray window were calculated. Districts were then stratified into Group 1 or 2 based on their differing 2018 insecticide designation (Act. or SS). A two-sample t test at α=0.05 was used to determine the mean change in malaria incidence rate between years for each group, and linear regression accounting for clustering was used to compare differences in the means between the two groups. Between 2016 and 2017, when both Group 1 and 2 sprayed Act. during both years, malaria incidence increased by around 18% in Group 1 and 6% in Group 2, a non-significant difference-in-differences effect of 11% (p=0.473). Between 2017 and 2018 when Group 1 switched from Act. to SS and Group 2 continued using Act., malaria incidence decreased by around 29% in Group 1 and 48% in Group 2, a significant difference-in-differences effect of 19% (p=0.001). Between 2018 and 2019 when Group 1 used SS both years and Group 2 switched from Act. to SS, malaria increased by around 27% in Group 1 compared to 64% in Group 2, a significant difference-in-differences effect of 36% (p<0.001). These results suggest that IRS with either product is likely efficacious but that SumiShield may have had an attenuated impact on malaria reduction compared to Act., results that should be interpreted with caution given that a comparable no-IRS control area was not available and the general impact of IRS in this area, relative to no intervention, could not be quantified.

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BASELINE ENTOMOLOGICAL LANDSCAPE ACROSS SIXTEEN NEW NETS PROJECT PILOT DISTRICTS: PRELIMINARY DATA ON VECTOR BIONOMICS

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Through the New Nets Project, dual active ingredient (AI) ITNs (Interceptor® G2 [BASF] and Royal Guard® [RG] [Disease Control Technologies]) have been piloted in several countries, alongside pyrethroid-piperonyl butoxide (PBO) and standard pyrethroid-only ITNs. To help generate evidence on the impact of these dual AI ITNs in real-world implementation settings, data on several indicators of malaria transmission is being collected in five pilot study areas composed of 16 districts across four countries: Burkina Faso, Mozambique, Nigeria, and Rwanda. Observational studies will evaluate the effect of the ITNs, relative to standard pyrethroid-only ITNs, through 2022. The ecological diversity in these geographies is critical in understanding the effectiveness of dual AI ITNs across a range of settings, and here results of baseline entomological surveillance across all study districts are presented. Malaria vector bionomics are varied both between and within each evaluation context, with *An. gambiae* s.s. the dominant vector in 9 of 16 districts overall and in at least one study district from each of the pilot countries. Other dominant vectors include *An. funestus* s.l. (4 districts, at least one in each country except Burkina Faso), and *An. coluzzii* (3 districts). Variation in indoor-to-outdoor biting ratios was also noted by district, ranging from 0.5 to 10.0, but in 10 of 16 districts the ratio was not significantly different from 1.0. Also highly variable was the baseline pyrethroid susceptibility status of primary vectors across districts: WHO tube test mortality rates ranged from 12% to 94% in Nigeria, from 86% to 100% in Rwanda, from 60% to 100% in Mozambique, and were less than 50% in all sites in Burkina Faso. Exposure to PBO completely restored susceptibility in Rwanda and Mozambique, but only partly so in Nigeria and Burkina Faso. The rich diversity and variation in vector bionomics present across the pilot study districts will provide important real-world context that accurately reflects the dynamic reality of malaria transmission in Africa and the complexities of estimating vector control impact.

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THE POPULATION INTERPLAY OF TWO MALARIA VECTORS IN NE UGANDA AND THEIR MEASURED DECLINES WITH VECTOR CONTROL

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Entomological surveillance has been a component of an ongoing, multi-year study of malaria transmission and its control in a region of high transmission of NE Uganda. The An. gambiae and An. funestus mosquito species complexes are the primary malaria vectors in the area and we report on their seasonal and locational trends, and associated patterns of malaria transmission. As part of the study, two of three connected subcounties received indoor residual spraying (IRS) with pirimiphos methyl, while all received bednets through a universal campaign from Uganda's Ministry of Health. Mosquitoes in the subcounties, with and without these interventions, are collected for a single night at monthly intervals with CDC light traps in 90 sentinel huts for nearly three years. Evident in these collections are two time-ordered, independent vector density peaks, and each is strongly dominated by a single species. These sequential, species-specific population trends are repeated annually, an apparent interspecific interaction. Despite this seasonal coordination, a spatially complex picture of the mosquito densities is also found, indicating both species are frequently sympatric, but not syntopic, in the study area. The impacts of IRS are strongly evident on the mosquito densities, with percentage reductions generally around 70-80% compared to neighboring regions with only the universal bednets, and it is somewhat more effective on the

An. funestus population. The high spatial and temporal resolution of the research setting allows a practical estimation of the number of observation huts sufficient to infer different vector control impacts, with implications for programmatic entomological surveillance.

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MALARIA PARASITES CAN BE KILLED NON THERMALLY WITH MICROWAVES

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Abstract textThe WHO has reported a shortfall in meeting the internationally agreed goals of curving malaria. To add to this, the SARS-CoV-2 pandemic fallout could worsen the situation in the coming years. This adds to the ever present concerns of resistance to current treatments in South Asia. Our group embraced the need to find an alternative way of fighting against the disease. Using microwave energy (MW), we generated conditions in which more than 90% of the parasites that cause malaria are killed *in vitro*. This was achieved not by a thermal effect but via a MW field-induced programmed cell death that did not affect mammalian cell lines. Transmission electron microscopy revealed that exposure to MW leads to destruction of the hemozoin-containing food vacuole, consistent with the observation of leakage of calcium from the vacuole and acidification of the cytoplasm. Furthermore, parasites were protected from the effects of MW by calcium channel blockers, calmodulin, and phosphoinositol. As previously reported by our group, direct current electric fields cause the opposite effect, i.e., proliferation of the parasites by means of the same calcium signaling molecules. We found early evidence that a yet unidentified protein in *P. falciparum* could be involved in the differential inhibitory effect of MW on the pathogen. Finally, we have designed a MW applicator for testing the technology *in vivo* and have obtained results that prove that the inhibition of growth obtained *in vitro* with this applicator is reproduced *in vivo* without affecting the animals. Our findings open the door to a possible new paradigm for the treatment of malaria,

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MICROBIOLOGICAL QUALITY CONTROL OF INTENSIVE CARE UNIT ENVIRONMENT AT THE EPIDEMIC TREATMENT CENTER OF COVID IN ARISTIDE LE DANTEC HOSPITAL

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Health care-associated infections (HCAIs) is a serious public health problem in the world. With the COVID, pandemic where severe cases are being treated in intensive care units, strict hygiene measures should be taken to prevent bacterial infections of patients with this disease. The objective of this study was to control microbiological quality environment of intensive care unit in Le Dantec hospital. Technical sedimentation was used and air environmental was collected in petri dish about twenty minutes before and after cleaning and antiseptic pulverization. Bacteria such as *Staphylococcus aureus* and *Klebsiella pneumoniae* were isolated before and after cleaning and and antiseptic pulverization. Antibiotic susceptibility testing showed a broad-spectrum beta-lactamase-producing for *K. pneumoniae*, which was resistant to aminoglycosides and fluoroquinolones but susceptible to carbapenem. For *S. aureus* strains, most antibiotics were active excepted penicillin. In conclusion, cleaning and disinfection protocols should be reinforced to limit secondary bacterial infections cases. Keys words: COVID, infection, Health care-associated infections.

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PILOT RETROSPECTIVE MOLECULAR DIAGNOSIS OF NEGATIVE GENEXPERT AND AFB SPUTA OF TB SYMPTOMATIC PATIENTS IN MANILA PHILIPPINES

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In 2019, the Philippines was amongst the seven countries accounting for the greatest burden (90%) of tuberculosis (TB) in the world. We sought to investigate the presence of other potentially pathogenic organisms and their clinical features in archived sputum samples from acutely unwell patients clinically diagnosed with TB and admitted to a TB ward at San Lazaro Hospital Manila and not on treatment. These patients however, had a negative Xpert® MTB/Rif assay and Acid-Fast Bacilli (AFB). A pilot retrospective molecular analytical cross-sectional study on 82 sputa samples was carried out. DNA was extracted using a QIAGEN kit. Positive controls prepared from blood culture isolates were extracted using the same kit above following manufacturers protocol. Three multiplex Polymerase chain reactions (PCR) were designed for *Hemophilus influenzae, Moraxella catarrhalis*), *Streptococcus pneumoniae, mycoplasma pneumoniae*, *Legionella pneumoniae, Chlamydia pneumoniae,* and *Burkholderia pseudomallei, thailandensis*. Shimadzu MultiNA Microchip Electrophoresis System was used for DNA analysis. Statistics analysis was done on STATA® 15. The prevalence of bacteria was 74/82(90.24%). *Burkholdera pseudomallei* 72/82(87.80%) and *Hemophilus influenza* 22/82(26.83%) were most prevalent. The prevalence of an over 2-week cough duration was 51/82(76.12%) while that for x-ray findings consistent with TB was 59/76(86.76%). The odds of a cough of over two weeks and weight loss were about six {OR 5.70(CI: 1.79, 18.09)}, p=0.0008 and four folds {OR 4.44(95%CI:1.56, 12.62)} p=0.0022 respectively greater in those with positive Xpert/AFB compared to those negative. There can be other bacterial infections in patients suspected and/or diagnosed of PTB. This however, warrants more research to identify types and prevalence of bacteria. Clinical manifestations like cough of over 2 weeks, fever, chest x-ray lesions, and weight loss can be present in other lung affections/ diseases. In bacteriologically confirmed TB cases, a cough of over two weeks and weight loss were the most determining factors.

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DOES FIBERSOL-2 EFFICACIOUS IN REDUCING DURATION OF WATERY DIARRHOEA AND STOOL OUTPUT IN CHILDREN 1-3 YEARS OLD? A RANDOMIZED, PARALLEL, DOUBLE-BLINDED, PLACEBO- CONTROLLED, TWO ARM CLINICAL TRIAL

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Fibersol-2 has innumerable beneficial effects on human health. It is a fermentable, non viscous, water-soluble, indigestible dextrin containing 90% dietary fiber produced from corn starch. We aimed to evaluate whether additional intake of Fibersol-2 along with oral rehydration solution treatment can reduce the duration of watery diarrhoea and stool output in children 1-3 years. This placebo-controlled double-blinded, randomized parallel two arm trial conducted in Kumudini Women's Medical College Hospital in rural Bangladesh between March and October, 2018 used 5 gm of either Fibersol-2 or placebo, dissolved in 50-ml drinking water which was given orally to 92 children with watery diarrhoea on enrolment twice daily for a period of 7 days. Randomization was done using a randomization table. We randomly allocated 45 (49%) and 47 (51%) children in Fibersol-2 and placebo groups, respectively. Primary outcome measures were duration of resolution of watery diarrhoea, the proportion of patients recovered within 72 hours, and daily stool output. Primary and safety analyses were by intention to treat. This trial is registered at ClinicalTrials.gov, number NCT03565393.The mean duration of resolution of watery diarrhoea, the proportion of children recovered from watery diarrhoea within 72 hours and total watery stool output between Fibersol-2 and placebo were (35.86±25.06 hrs vs. 44.99±36.24

hrs; p=0.335); (91% vs. 82%; p=0.312) and (1095.32±522.78 gram vs. 1322.93±797.58 gram; p=0.259); respectively. No beneficial role of Fibersol-2 was seen in reducing duration of watery diarrhoea and stool output, compared to placebo in children 1-3 years old.

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CHANGES IN THE GUT MICROBIOME IN ANGOLAN CHILDREN WITH SICKLE CELL DISEASE

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Sickle cell disease (SCD) is one of the most prevalent genetic diseases, affecting between 20 and 25 million people worldwide and with an incidence of approximately 300,000 births per year. It is particularly common in the African continent, with nearly 80% of the SCD births occurring in Sub-Saharan Africa, contributing to 50-80% of under-5 mortality. Clinical manifestations of SCD are very heterogeneous and the intestinal microbiome seems to be crucial in the modulation of inflammation, cell adhesion and induction of aged neutrophils, which are the main interveners of recurrent vaso-occlusive crisis (VOC). Recent studies have reported frequent microbial and pathophysiologic changes in the intestines of SCD patients, and these include enterocyte injury, altered microbial composition, increased permeability and bacterial overgrowth. Moreover, it has been suggested that Hydroxyurea (HU), the only FDA-approved drug for SCD, shows a multimodal action and may reduce microbiome dysbiosis and aged neutrophils, which could explain the decreasing of VOC occurrences. In this project, we use Next-Generation Sequencing to sequence bacterial 16SRNA in order to characterize the gut microbiome of SCD Angolan children and healthy siblings before and after HU administration. The aim is to understand how the disease and the HU treatment modulates the microbiome and if these changes are related with severity. Our preliminary results showed an increase in Actinobacteria phylum and a decrease of Proteobacteria in homozygous SCD patients. In the same group of patients, it was also observed a reduction of Gammaproteobacteria class and an increase of Coriobacteriales order. Understanding the epidemiology and host-microbe interactions can demonstrate the importance of specific bacteria and their function in this disease and provide new insights for attenuating symptoms. This works as been supported by FCT/Aga Khan (project nº330842553) and FCT/MCTES (UIDB/05608/2020 and UIDP/05608/2020) -H&TRC.

1201

TRENDS IN ANTIMICROBIAL RESISTANCE OF PATHOGENS CAUSING SURGICAL SITE INFECTIONS IN EGYPT

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Surgical site infections (SSI) accompanied with antimicrobial resistance (AMR) cause high morbidity and mortality rates. This study aimed to evaluate AMR in subjects presenting with SSI at two University Hospitals in Egypt. From March 2018 to March 2021, 119 subjects were enrolled. Identification and antibiograms of isolated wound pathogens were done by VITEK2. In total, 145 pathogens were isolated from 88 subjects. A single pathogen was identified in 48 (40.3%) subjects, whereas, up to four pathogens were co-identified in 40 (33.6%) patients. *E. coli* (34.4%), *K. pneumonia* (27.7%), *A. baumannii* (13.4%) and *P. aeruginosa* (11.7%) were the main pathogens isolated. Overall, high AMR rates were observed among the isolated pathogens with 10% pan-drug resistant (PDR), 32.9% extensively drug resistant (XDR) and 36.4% multi-drug resistant (MDR). Of the *Enterobacteriaceae* isolates, 14% were PDR, 24.7% XDR and 40.9% MDR. The lowest resistance rates of *Enterobacteriaceae* were against amikacin (25%) and meropenem (27.2%), whereas, >80% of the isolates were resistant to 3rd and 4th generation cephalosporins. AMR profiles of *E. coli* were XDR (29%), MDR (56.1%) and Extended Spectrum Beta-lactamase producer (ESBL) (59%). *K. pneumonia* displayed higher level of AMR with PDR (36.4%), XDR (24.2%), MDR (18.2%); ESBL producers (30.3%) and resistance against tested antibiotics ranged from 52% to 88%, in levofloxacin and ceftriaxone, respectively. All *A. baumannii* isolates were either PDR (6.3%), XDR (81.3%) or MDR (12.5%), with one colistin resistant strain. For *P. aeruginosa,* 64.3% were XDR and colistin was the effective antibiotic against all its isolates. Importantly, 117 subjects received surgical prophylaxis, of whom, 89% were administered cephalosporins and 5.8% amikacin, which matches antibiograms noticed in *Enterobacteriaceae*. In conclusion, the excessive use of cephalosporins in surgical prophylaxis in Egypt might have contributed to the remarkably high rates of AMR observed in *Enterobacteriaceae*. The reported high rates of antimicrobial resistance highlights the urgent need for multidisciplinary actions for successful SSI prophylaxis.

1202

IMMUNOMODULATORY EFFECT OF LEPIDIUM MEYENII IN THE INFLAMMATORY RESPONSE OF HUMAN MACROPHAGES TO MYCOBACTERIA

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Lepidium meyenii (L.m.), also known as maca, is an Andean crop used medicinally for multiple purposes. Studies have shown an immunomodulatory anti-inflammatory effect in murine macrophages. Bacillus Calmette-Guérin (BCG) is the vaccine used to prevent non-pulmonary forms of tuberculosis. We aimed to assess the effect of L.m. on the inflammatory response of human macrophages to mycobacteria. Human monocytic THP-1 cells bearing two plasmid reporter systems for NF-kB and IRF activation, were differentiated into macrophages and then treated with L.m. at concentrations of 1ug/ml, 5ug/ml and 10 ug/ml for 48 hours. Cells were then infected with *Mycobacterium tuberculosis* (Mtb) strain H37Rv, *Mycobacterium smegmatis*, and BCG for 24 hours. L.m.-treated cells showed a decrease in NF-kB activation in a dose-dependent manner. No effect was observed in IRF activation. Phagocytosis was increased in L.m.-treated cells at any concentration compared to untreated cells. A decrease in the number of internalized Mtb, but an increase in internalized *M.smegmatis* and BCG were observed in L.m.-treated cells. Our results indicate that L.m. exerts an immunomodulatory effect on the NF-kB activation of human macrophages upon mycobacteria challenge, while at the same time improving phagocytosis. Our findings suggest a beneficial effect in active TB where an exacerbated inflammatory response is observed.

1203

SUSCEPTIBILITY AND EXPOSURE TO LEPROSY: AN EXAMINATION OF NUTRIENT DEFICIENCIES, PARASITIC COINFECTION, AND WATER, SANITATION AND HYGIENE (WASH) CONDITIONS

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Despite control measures and a declining number of human reservoirs, continued incidence of leprosy in excess of 200,000 new infections each year suggests that alternative pathways may play a role in continued endemicity. Nutritional deficiencies, parasitic coinfection, and limited access to safe water, sanitation and hygiene (WASH) have been suggested to predispose individuals to *M. leprae* infection. To further explore this

hypothesis, leprosy cases and uninfected controls were recruited from areas around North Gondar, Ethiopia throughout 2019. Participants completed dietary and WASH surveys in addition to providing stool for Kato Katz, urine for Schisto POC-CCA™ rapid diagnostic testing, and blood for micronutrient biomarker testing. A total of 80 men (59%) and women (41%) participated in this study with an average age of 40 (SD 15.0 years). Most leprosy cases were multibacillary (93.3%). There was a high prevalence of undernutrition among cases and controls, with 32.1% of participants classified as underweight. Food shortage [OR 4.57, 95% CI (1.62, 12.89)] and fewer meals consumed per day [OR 3.85, 95% CI (1.17, 12.67)] were both significantly associated with leprosy in the univariate analysis. Additionally, 64.1% of the study population tested positive for a helminth and WASH insecurities were widespread. On multivariate analysis, lack of soap for handwashing [aOR 2.53, 95% CI (1.17, 5.47)] and lack of toilet facilities [aOR 2.32, 95% CI (1.05, 5.12)] were significantly associated with leprosy. Positive directionality was identified for a number of other inputs, including helminth infection [aOR 3.23, 95% CI (0.85, 12.35)]. Taken together, these findings strengthen previous research conducted in 2018 implicating WASH as a driver of leprosy infection. Subsequent micronutrient results will be integrated to further explore nutritional risks and build upon the significant macronutrient findings from this analysis. Given that leprosy remains the leading infectious cause of disability in the world, future research should explore all of the above susceptibilities in more depth in order to curtail the global burden of disease.

1204

CHARACTERIZATION OF MRSA AND ESBL PATHOGENS FROM PATIENTS WITH SURGICAL SITE INFECTIONS IN ACCRA, GHANA

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Among syndromes involving AMR/MDR, surgical site infections remain a significant clinical concern—posing challenges to patients who undergo surgery by causing delayed wound healing and increasing healthcare costs. Unfortunately, throughout Ghana, treatment of SSI is often empirical and not based on knowledge of the organisms infecting surgical sites and their susceptibility patterns. In this study, we sought to characterize *S. aureus*, *E.coli*, *K. pneumoniae* and *P. aeruginosa* recovered from patients diagnosed with SSIs in Ghana to provide antimicrobial susceptibility data. This is cross-sectional study is being conducted at the 37-Military Hospital and Korle-Bu Teaching Hospital in Accra, Ghana. All organisms were identified by MALDI-TOF-MS and susceptibilities interpreted according to the CLSI 2018 guidelines. From a total of 90 patients recruited, 69 bacteria species have been identified; *E.coli* (33%) *P. aeruginosa* (21%), *K. pneumoniae* (20%) and *S. aureus (*2%;*)*. *S. aureus* isolates were susceptible to clindamycin, erythromycin, gentamicin, linezolid, rifampicin and norfloxacin but resistant to penicillin. *E. coli* and *K. pneumoniae* were resistant to tetracycline (85.4%), trimethoprim/sulfamethoxazole (81%), ciprofloxacin (75%), cefuroxime (69%), cefotaxime (69%), gentamicin (63%) and chloramphenicol (56%) but sensitive to meropenem (92%) and amikacin (88%). *P. aeruginosa* showed sensitivity to amikacin (89%), meropenem (79%), ceftazidime (79%), piperacillin-tazobactam (89%), cefepime (74%), gentamicin (63%) and ciprofloxacin (63%). ESBL-phenotype was detected in 53% of *E. coli* and 83% of *K. pneumoniae* isolates. *CTX-M* was the dominant ESBL gene in *E.coli* isolates. Of the 19 *P. aeruginosa* isolates, 2 harbored the *TEM* gene. From the preliminary data, we found an over representation of Gram-negatives compared to Gram-positives in the SSI samples analyzed. These findings further demonstrate the need for effective surveillance to monitor the rise and spread of MDR pathogens and guide stewardship best practices.

1205

GENOMIC EPIDEMIOLOGY AND ANTIMICROBIAL RESISTANCE IN NEISSERIA GONORRHOEAE ISOLATES FROM GHANA

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Tracking *Neisseria gonorrhoeae* antibiotic resistance rates provides combatant commands with insights into antibiotics effectiveness in specific countries. Therefore, this study sought to perform whole genome characterization of *N. gonorrhoeae* collected in Ghana to identify lineages of circulating strains and their phenotypic and genotypic antimicrobial resistance profiles. Whole genome sequencing was performed on 56 isolates using both the Oxford Nanopore MinION and Illumina MiSeq sequencing platforms. A total of 22 STs were identified by MLST, with ST-14422 (n=10), ST-1927 (n=8) and ST-11210 (n=7) being the most prevalent. Six novel STs were also identified and submitted for assignment of new sequence types (ST-15634-115641). Seven clusters of isolates with distinct AMR genotypes were identified after the wgMLST analysis, highlighting the presence of genome wide genetic variation. All isolates harboured chromosomal AMR determinants that confer resistance to beta-lactam antimicrobials and tetracycline. A total of (49/56)87.5% and (13/56)23% isolates contained fluoroquinolone and macrolide resistance markers, respectively. NG-STAR AMR typing identified 29 unique sequence types, with ST-464 (n=8) and the novel ST-3366 (n=8) being the most prevalent. Notably, 20 of the 29 STs were novel, indicative of the unique nature of molecular AMR determinants in the Ghanaian isolates. The novel sequence types were submitted for assignment of new sequence types (ST-3352-3353). Plasmids were highly prevalent: pTetM and p*bla*TEM were found in 96% and 92% of isolates, respectively. The TEM-135 allele, identified in 28.5% isolates, is a cause for concern because further changes to specific amino-acids might lead to the emergence of a stable extended-spectrum β-lactamase that could result in complete cephalosporin resistance. This study highlights the need for constant genomic surveillance with the looming possible emergence of extended-spectrum cephalosporin resistant bacterial isolates.

1206

OVERCOMING THE UTILITY BARRIERS OF MICROSCOPIC AGGLUTINATION TEST FOR THE DIAGNOSIS OF LEPTOSPIROSIS IN LOW INCOME SETTINGS

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Microscopic Agglutination Test (MAT) is still the standard diagnostic test for the diagnosis of leptospirosis; a neglected infectious disease in tropical and sub-tropical regions. MAT involves a time consuming, laborious procedure and expensive consumables, making it challenging to use in low and middle-income settings where the disease burden is highest. This study aimed to improve the efficiency and reduce the cost of MAT procedure, sustaining the validity to minimize the burden of Leptospirosis. We tested an extensive collection of serum samples from suspected cases of Leptospirosis, using a mixed approach to optimize the MAT panel. First, the WHO recommended *Leptospira* reference panel was obtained from the Centres for Disease Control and Prevention (CDC). Four laboratory strains isolated from Sri Lanka were also included in the panel to test the available samples. The second step involves analyzing the most suitable *Leptospira* antigen panel without compromising the representativeness of serogroups and local *Leptospira* isolates. Statistical analysis was done to select the maximum number of *Leptospira* antigens to achieve the highest sensitivity. Four hundred four samples were then tested using the new antigen panel. The final scheme included 14 strains (of the 24 tested). Of the selected 14 antigens, only 11 were required to complete the MAT quantitation. MAT

screening was done according to the standard protocol followed by MAT quantitation using a single diagnostic titre of 1/400. Antigens were used in a serial manner rather than a parallel manner for MAT quantitation. In the novel approach, 404 serum samples were tested, and 126 (31.1%) were screened positive, while 49 (12.1%) had a diagnostic titre of 1/400. This method reduced >40% of the *Leptospira* culture maintenance and test consumable costs along with 37.5% of microscopic observation time. These findings suggest that the proposed method will improve the current practice of MAT in resource-limited laboratories by saving consumables and time, thus serving the clinical diagnostic procedures better to minimize the burden of Leptospirosis.

1207

STAPHYLOCOCCUS AUREUS BACTERIURIA IS MORE COMMON IN MALES. PERSISTENT AND RECURRENT INFECTIONS ARE UNCOMMON WITH A HIGHER INCIDENCE IN OLDER AGE GROUPS: A STUDY OF 633 CASES

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Urinary infections are more frequent in young women. Recurrences are common. In a study of 633 *Staphylococcus aureus* culture positive patients, we found more infections in males. Persistent or recurrent infections were uncommon. There were more older patients. Methods: We studied *S. aureus* urine cultures of 633 patients reported by our network laboratory for a period of 3 years beginning January 2016. There were 344 males and 290 females aged 1-118. Only 22/633 (3.47%) had more than 1 positive culture. Of these the second positive culture was reported after 24 hours in 11, 48 hours in 5, 96 hours in 2 and 1 each on day 7,14,19 and 28. Only the last 4 was likely to have a recurrence or persistent infection (0.63%). Nine urine culture positive patients had positive blood cultures (1.42%). Two had positive wound cultures and 1 had a positive joint fluid culture. Only 69/633 (10.9%) were below age 30 and 84 (13.7%) below age 50. Three hundred and ninety-nine were above age 60 (63%). The median age was 67. All except one had multiple co-morbidities: diabetes 11, chronic Foley catheter 7, urinary retention 4, stones 4, bladder cancer 2, benign prostatic hypertrophy 2, suprapubic catheter 2, hydronephrosis 2, epidural abscess/paraplegia 1, prostatic abscess 1 cerebrovascular accident 1. All 22 with more than 1 positive culture were methicillin susceptible. Nine had minimal inhibitory concentration (MIC) to ampicillin <=2 mcg/ml, 1 with MIC 4 mcg/ml and the remainder had MIC => 8 mcg/ml. All were susceptible to cefazolin, gentamicin, nitrofurantoin, tetracycline, trimethoprim/sulphamethoxazole and vancomycin. Susceptibilities were checked repeatedly for accuracy. In a 5-year Irish study out of 425,013 urine cultures 542 were *S. aureus* positive (0.13%) and 151 were methicillin resistant (.036%). Thus *S. aureus* bacteriuria is uncommon. In our study *S. aureus* bacteriuria was more prevalent in older patients with a male preponderance and persistent and recurrent infections were rare, differing from common uropathogens such as *E coli*. In the sub group of 22 with more than 1 positive culture, there was no methicillin resistance which could be due to small numbers.

1208

DEVELOPMENT OF A NANOPORE SEQUENCING ASSAY FOR THE QUANTIFICATION OF HOST-RESPONSE FEATURES THAT PREDICT MORTALITY IN SEPSIS SUBJECTS

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Early recognition and characterization of infectious disease threats and improved clinical care to mitigate the effects of infection constitute two key components of our defense-in-depth approach to biological threats. The Austere environments Consortium for Enhanced Sepsis Outcomes (ACESO) aims to improve survival of patients with severe infections by focusing on the generation of new knowledge that can rapidly translate into preventing and improving the outcome of sepsis in the austere setting. ACESO seeks to identify host-response biomarkers that are predictive of clinical severity of sepsis that can be deployed on fieldable devices. Towards this goal, recruitment was performed for an observational study of sepsis at sites in Cambodia (n=208), Ghana (n=202) and the USA (n=185). Host RNA sequencing data were generated. We used topological data analysis (TDA) to cluster patients in an unsupervised manner as a means of stratifying subjects, identifying differences between patient groups of interest (28-day mortality) and as input for regression models. Fifteen gene expression features were identified in a model termed Predict_d28 that were predictive of outcome in the subgroups. To develop a sequencing assay capable of profiling the host response in sepsis subjects in a rapid fashion we chose the Oxford Nanopore Technologies MinION that is amenable to field applications. An assay that uses amplicon preparation and nanopore sequencing of RNA isolated from sepsis subject peripheral blood to quantify 15 features for the purpose of predicting outcomes was pursued. Automated software was developed (SepsiProgDx) to analyze the resulting sequence data to yield a risk score from the Predict_d28 model. To gauge the correlation between the output of the Predict_d28 prognostic assay between nanopore and real-time RT-PCR quantification of the same genes, ten clinical samples were measured using the nanopore sequencing assay and with real-time RT-PCR. Model weights were similar for all ten samples tested. Next steps include continued assay validation prior to field testing.

1209

A CASE OF STREPTOCOCCUS VIRIDANS MULTIFOCAL ABSCESSES WITHOUT BACTEREMIA AND ENDOCARDITIS

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Streptococcus viridans is an α-hemolytic streptococci part of the normal oral flora and a common cause of subacute bacterial endocarditis from dental caries. *S. viridans* is responsible for 40-60% of endocarditis on normal valves, particularly in males over 45-years-old. *Streptococcus intermedius* is one of the most common microbes associated with brain and liver abscesses and pulmonary empyema. Mucosal disturbance and liver abscess may predispose patients to *S. intermedius* brain abscesses. We present a 47-year-old male patient with alcohol use disorder and diabetes with a pulmonary empyema and multiple liver abscesses with positive body fluid cultures showing *S. intermedius* in the lungs and *S. viridans* in the liver without any antibiotic use prior to admission. Exam demonstrated generalized poor dentition with multiple reconstructive dental implants placed five years prior and no acute odontogenic infection clinically or radiographically. Two sets of blood cultures were negative and TTE and two TEE procedures demonstrated no vegetations. Ophthalmology confirmed no Roth spots and exam showed no Osler nodes. On day nine, he felt dizzy and fell with subsequent MRI brain showing several ring-enhancing brain abscesses in the left thalamus measuring up to 3.1 cm, right parieto-occipital region measuring up to 3.4 cm and right frontal lobe associated with mass effect and hydrocephalus. Body fluid for *S. viridans* showed a MIC 0.5 for ceftriaxone. For treatment of abscesses and to ensure penetration into the central nervous system, patient was started on ceftriaxone 2g q12h, vancomycin 1250mg q12h and flagyl 500 mg q8h for 6 weeks. This case demonstrates the importance of considering hematogenous seeding of *S. viridans* when presented with multiorgan abscesses to liver, lungs and brain in the immunocompetent host without positive bacteremia or endocarditis. Patients with more than one organ

affected by *S. viridans* may benefit from full body CT or PET scan to further elucidate embolization or hematogenous seeding of other organs. This case supports European standards for PET scans in all patients with suspected endocarditis or multiple abscesses.

1210

CHANGE IN SEROLOGICAL METRICS FOR CHLAMYDIA TRACHOMATIS BEFORE AND AFTER MASS DRUG ADMINISTRATION WITH AZITHROMYCIN IN KIRIBATI

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Trachoma, caused by ocular infection with *Chlamydia trachomatis*, is the leading infectious cause of blindness and is targeted for elimination as public health problem by 2030. One elimination target is for each formerly endemic district (evaluation unit [EU]) to achieve a prevalence of the clinical sign trachomatous inflammation—follicular (TF) of <5% in children 1-9 years old. A key intervention to help reach this target is district-wide mass drug administration (MDA) with azithromycin to clear infection. Serology may be a useful alternative indicator to TF for assessing ongoing transmission in children and monitoring recrudescence (TF >=5%) after elimination, when resources for implementation may be more scarce. Here, we measured antibodies to *C. trachomatis* in children before and after two rounds of MDA in two EUs in Kiribati (Tarawa and Kiritimati Island). Baseline surveys to determine the need for MDA were conducted in 2015, resulting in two MDA rounds, and trachoma impact surveys (TIS) to determine if MDA could be stopped were done in 2019. Dried blood spots were analyzed by multiplex bead assay (MBA), ELISA, and lateral flow assay (LFA). Age-adjusted seroprevalence and seroconversion rates (SCR, number of seroconversions/100 children/year) were reported. In Tarawa, seroprevalence declined from 52.7% (95% CI: 43.3-61.9) at baseline to 31.4% (95% CI: 23.4-40.3) at TIS. SCR declined from 18.8% (95% CI: 16.7-20.8) to 8.8% (95% CI: 7.6-10.2) at TIS. In Kiritimati Island, seroprevalence was 46.8% (95% CI: 33.9-60.0) at baseline and 30.2% (95% CI: 22.1-40.1) at TIS; SCR was 16.9 (95% CI 13.7-23.1) at baseline and 8.9 (95% CI 7.5-10.7) at TIS. Among positive samples, the intensity of responses (represented by median fluorescence intensity, MFI) was lower at TIS compared with baseline in both sites (p<0.0001). Seroprevalence and SCR estimates derived from ELISA and LFA data were similar to those derived from MBA. Overall, reduction in antibody metrics after MDA was seen across assays in both EUs. Longitudinal data are critical to understanding antibody dynamics following interventions.

1211

SEROLOGICAL RESPONSES TO TRACHOMA ANTIGENS PRIOR TO THE START OF MASS DRUG ADMINISTRATION: RESULTS FROM POPULATION-BASED BASELINE SURVEYS, NORTH DARFUR STATE, SUDAN

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After years of programmatic inaccessibility to the Darfur region, in 2019-2020 the Sudan Federal Ministry of Health Trachoma Control Program conducted population-based trachoma surveys in 3 localities (districts) in North Darfur state, Sudan. The goals of the baseline surveys were to determine the current prevalence of trachomatous inflammation-follicular (TF) among children ages 1-9 years and to further use serological markers to understand the historical trachoma burden within this mass drug administration (MDA)-naïve area. Multi-stage cluster random sampling was used to select 30 villages (clusters), with 25 households per locality. In addition to the collection of trachoma clinical data by trained and certified graders, trained nurses collected dried blood spot (DBS) samples from individuals of all ages within the selected households. DBS were assayed on a multiplex bead array for antibody responses to the *Chlamydia trachomatis* antigen Pgp3. Across the three localities, 8,325 individuals aged 1-99 years in 2,189 households were examined for trachoma clinical signs and 8,324 DBS were collected, including 3,674 DBS samples from children ages 1-9 years. The prevalence of TF among children 1-9 years was endemic (>10%) in 2 localities (El Seraif, and Saraf Omrah) and below the elimination as a public health problem threshold (<5%) in the third (Kotom). The Pgp3 seroprevalence among children ages 1-9 years was 35.9% in El Seraif, 32.2% in Saraf Omrah, and 13.8% in Kotom. Within this age group, by age 9 years seroprevalence reached as high as 48% and 59% in Sarah Omrah and El Seraif, respectively, and reached 29% in Kotom. Across the whole age-range sampled, the seroprevalence was 40.4% in El Seraif, 41.9% in Saraf Omrah, and 36.8% in Kotom. Starting at age 20-29 years, the age specific seroprevalence was ≥40% among all age groups in all localities. Serological data collected within these trachoma surveys has demonstrated that all three localities have had a long history of exposure to *Chlamydia trachomatis.* Research is needed in Kotom to understand the potential epidemiological shift that may have taken place in the last 10 years.

1212

ENDOTHELIAL ACTIVATION IS REDUCED BY HYDROXYUREA THERAPY AND PREDICTS TRANSCRANIAL DOPPLER VELOCITY IN THE NOHARM STUDY: A PROSPECTIVE CLINICAL TRIAL OF HYDROXYUREA IN UGANDAN CHILDREN WITH SICKLE CELL ANEMIA

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Inflammation and endothelial activation play a key role in sickle cell anemia (SCA) pathogenesis. However, there are limited data on endothelial activation in children with SCA in low-and-middle-income-countries (LMIC) where SCA disease-burden is highest. We evaluated longitudinal changes in biomarkers of endothelial activation and inflammation in children enrolled in a randomized double-blind placebo-controlled trial of hydroxyurea (HU) as a disease modifying therapy for children with SCA (NOHARM study). A total of 205 Ugandan children aged 1.00 to 3.99 years were enrolled with routine hematologic monitoring and a transcranial doppler (TCD) exam at one-year follow-up to assess cerebral blood flow velocity. Increased TCD velocity is associated with stroke risk in children with SCA. We measured plasma levels of C-reactive protein, angiopoietin-2 (Angpt-2), soluble E-Selectin (sE-Selectin), P-Selectin, soluble ICAM-1, and soluble VCAM-1by ELISA at enrollment, 2, 4, and 12 months follow-up. At enrollment, Angpt-2 levels were elevated in children with a history of blood transfusion, as well as ischemic vascular complications of SCA including vaso-occlusive crises, acute chest syndrome, and dactylitis (p<0.05 for all). Using linear mixed effects models to evaluate longitudinal changes in biomarkers, there was a significant reduction in Angpt-2, sE-Selectin and P-Selectin with HU therapy with differences evident by four months follow-up (p<0.01 for all). At both

enrollment and 12 months follow-up, Angpt-2 and sE-Selectin were independently associated with TCD velocity. We conducted longitudinal correlation analyses and generated a heat map between biomarkers of endothelial activation and hematologic parameters modified by HU over time. There was evidence that P-Selectin, Angpt-2 and sE-Selectin concentrations are associated with changes in fetal hemoglobin in children treated with HU. Additional studies are needed to understand the mechanisms of reduced endothelial activation and lower TCD velocity in children treated with HU.

1213

BIOLOGICAL AND CLINICAL CHARACTERISTICS OF SUBJECTS TESTED POSITIVE FOR LEPTOSPIROSIS AMONG ACUTELY FEBRILE OUTPATIENTS ATTENDING PRIMARY CARE CLINICS DURING THE RAINFALL SEASON IN BURKINA FASO, WEST AFRICA

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Leptospirosis has a significant public health impact worldwide and is suspected to be endemic in Burkina Faso. However, in the absence of systematically collected data on fever etiology, biological and clinical data are scarce. The present study aimed to explore the biological and clinical characteristics of subjects tested positive for leptospirosis IgM and DNA among acutely febrile outpatients attending primary care clinics during the rainfall season in Burkina Faso. Patients were recruited from primary health care centers in Bobo-Dioulasso. Patients tested negative for malaria using a rapid diagnostic test (SD Bioline malaria®, Standard Diagnostics Inc, South Korea) were considered as eligible and further explored for leptospirosis. Subjects tested positive for leptospira IgM using a rapid diagnostic test (Leptocheck®, Zephyr Biomedicals, India) or an ELISA kit (Serion®, Serion Diagnostics, Germany), or for leptospira DNA by PCR, were considered as probable leptospirosis cases. Of 350 patients screened, 170 met eligibility criteria and were enrolled. Among 170 participants, 48 (28.23%) at least one leptospirosis positive test and were considered as probable leptospirosis cases. A flu-like syndrome was observed in most cases, including fever, headache, cough, muscle pain, vomiting and diarrhea. Most of the patients had a mild form of the disease, generally not requiring hospitalization. The biological profiles of leptospirosis IgM positive people were variable and not significantly different from people tested negative for leptospirosis. On bivariable regression, variables associated with leptospirosis included water = (OR 4.70; 95% confidence intervals (CI): 2.39-9.24; $p \leq 0.001$) and animal exposure (OR 2.63; CI: 1.36 - 4.09; $p \leq 0.01$), and the presence of a skin wound (OR 2.81; CI: 1.27 - 6.23; $p \leq 0.01$). Diagnostic testing of leptospirosis may be requested when there is a suspicion for leptospirosis based on symptoms and exposure to animal and water environments. Other epidemiological aspects of the disease such as the carriage of leptospira in domestic animals, slaughterhouse workers, muddy soils and surface water.

1214

DOOR-TO-DOOR CHILD IMMUNIZATION: AN ACCEPTABLE ALTERNATE WAY TO CONTINUE CHILD IMMUNIZATION SERVICES FOR VULNERABLE POPULATION OF INFORMAL SETTLEMENTS IN NAIROBI, KENYA

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In the first quarter of 2020, due to COVID-19 pandemic, there was a considerable disruption of immunization services in the informal settlements of Kibra and Langata in Nairobi county along with a raising concern for outbreaks of vaccine-preventable diseases. Given the high population density within informal settlement areas in Nairobi county, we facilitated and supported Nairobi County's Ministry of Health (MOH) to conduct door-to-door immunization outreach services to vulnerable children as well as to locate, trace and vaccinate missed children. Reportedly, 710 children under five received immunization services, 277 children were dewormed, and 211 children received vitamin A supplementation during these door to door campaigns. A total of 403 missed children were all successfully traced and received vaccines too. Child immunization session data from three counties within project area revealed minimal observed disruption to outreach immunization activities, with a considerable increase in both planned and delivered outreach sessions during March to October 2020, corresponding time period when we facilitated the door to door campaigns. However, there was a slight decline in numbers of children vaccinated in 2020 compared to 2019. The physical distancing requirements as well as community reluctance affected immunization sessions and given the challenges during COVID-19 pandemic days, these achievements are potentially attributable to the adopted strategy for organizing door-to-door immunization outreach immunization session as well as the uninterrupted support offered to Nairobi County MOH and local partners within the project area. Community members in informal settlements of Kibra and Langata sub-counties were also found extremely supportive to these door-to-door immunization campaigns. While collaboration with MOH and local partners are mandatory for continuing such community focused alternate business model to keep the essential services available to vulnerable population, it is also important to ensure community support and community participation in those adopted strategy implementation steps.

1215

ESTIMATING THE PROPORTION OF CHILDREN LESS THAN FIVE YEARS OLD WHO ARE FULLY IMMUNIZED ACCORDING TO EXPANDED PROGRAMME ON IMMUNIZATION IN THIRTEEN HIGH MALARIA TRANSMISSION COMMUNITIES IN MALABO DISTRICT, BIOKO ISLAND

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According to the United Nations International Children's Emergency Fund (UNICEF), between 2009 and 2018 the coverage of routine immunization has been declining at an estimated rate of 10%-20% in Equatorial Guinea (EG). Although the national Expanded Programme of Immunization (EPI) with UNICEF has made progress in the elimination of polio in 2019, there is still a lack of quality immunization data in the country. The purpose of this study is to improve the quality of the data estimating routine coverage, which then will help to identify the risk factors that contribute to children not being vaccinated at the community level. The objective is to describe socio-demographic status and determine the actual EPI coverage rate among participants using both estimated national census data and Bioko Island Malaria Elimination Project (BIMEP) census data. In addition we will determine the associated factors for routine immunization rate among participants of a pilot study (EG-RESPAR) that aims to optimize recruitment and screening procedures to create a registry of ~3000 potential research participants for a future Phase III clinical trial of PfSPZ Vaccine in Malabo district. A descriptive cross-sectional assessment of routine immunization data of children <5 years old that reside in both urban and rural high malaria transmission communities will be undertaken. Caregivers will provide immunization history of children

and data verification will be done by vaccination card or by a recall, including vaccination health clinics used for the children. The proportion of children <5 years old who are fully immunized will be evaluated according to the national routine vaccination schedule. These results will address the knowledge gap and help to identify potential factors that lead to low immunization coverage among children <5 years old. The study will provide information for decision-makers to identify policy gaps and develop public health strategies to improve immunization adoption among those most vulnerable to vaccine-preventable diseases.

1216

INTEGRATED MANAGEMENT OF CHILDHOOD ILLNESS: DANGER SIGNS AND PAEDIATRIC SEVERITY SCORES IN INPATIENT AND OUTPATIENT FEBRILE CHILDREN FROM FOUR COUNTRIES IN AFRICA AND ASIA

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Identifying which febrile children can be safely sent home, and which require prioritized care can be challenging, especially in resource-constrained settings. Along with IMCI guidelines 'danger signs,' several paediatric severity scores have been developed to guide acute management decisions. However, most scores have only been validated among inpatients, where the decision to admit has already been made. Using prospectively collected data from the Febrile Illness Evaluation in a Broad Range of Endemicities (FIEBRE) study, we evaluated IMCI danger signs, and existing severity scores among both in- and outpatient children <16 years presenting with fever in Lao PDR, Malawi, Mozambique and Zimbabwe. We estimated discriminative performance using areas under the receiver-operative curve (AUC) and the positive and negative likelihood ratios for each of the following scores: LODS, LqSOFA, ED-PEWS, qPELOD-2 and modified FEAST-PET; at previously published cut-off-points. Of 3,425 children (1,412 inpatients; 2,013 outpatients) 725 were enrolled in Lao PDR, 876 in Malawi, 937 in Mozambique and 887 in Zimbabwe. Median (IQR) age was 4.3 (2.0-7.9) years. Treatment with antimicrobials varied by site, from 46 to 90% of first encounters. Of 2,946 children with complete follow-up data at 28 days, there were 22 (1.6%) and 3 (0.2%) deaths among inpatients and outpatients, respectively. At least one IMCI danger sign was present in 669 (33%) outpatients and 861 (61%) inpatients. Yet, 6 (24%) of the 25 children who died had no documented danger signs. Most severity scores demonstrated moderate-to-low discriminative performance, and were especially limited in ruling out mortality risk. The ED-PEWS and LODS scores had the highest AUCs; 0.81 (95% CI 0.71 - 0.90) and 0.80 (95% CI 0.71 - 0.90), respectively. The AUC was 0.74 (95% CI 0.62 - 0.86) for the modified FEAST-PET score. The LqSOFA and qPELOD-2 scores performed relatively poorly in this cohort (AUCs 0.66 (95% CI 0.55 - 0.76) and 0.57 (95% CI 0.48 - 0.67)). As continued progress is made to reduce child mortality, better risk-stratification tools are urgently required to guide management decisions.

1217

DIAGNOSIS AND MANAGEMENT OF ENTEROBIASIS IN MILITARY TREATMENT FACILITIES

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Enterobius vermicularis is one of the most common nematode infections in the world, as well as in individuals within the Military Health System (MHS). An average of over 4300 pinworm infections are diagnosed in the MHS per year. Despite being a common disease that is frequently encountered in clinical practice, few studies have evaluated current clinical practice. To determine the practices being used in the diagnosis and treatment of individuals with pinworm infection, we conducted a retrospective analysis of 112 patients diagnosed with enterobiasis from 2011 to 2018. The patient population was approximately 58% female with a median age of 8 years old (range: less than 1 year to 59 years). Signs or symptoms of infection were present in 82% (92/112) of cases, while 17.8% (20/112) of people presented for empiric treatment due to proximity to an individual with symptomatic enterobiasis. Of the symptomatic cases, a clinical diagnosis was made in 89.1% (82/92) of cases. Diagnostic studies were ordered in 25.0% (23/92) of symptomatic cases. Diagnostics included pinworm paddles, or similar adhesive preparations (52.1%, 12/23), as well as studies not geared towards the diagnosis of pinworm, such as ova and parasite exams (34.8%, 8/23), stool cultures (13.0%, 3/23), and enteric parasite PCR panels (8.7%, 2/23). Initial treatment consisted primarily of albendazole (67%), mebendazole (17.9%), and pyrantel (9.8%). Empiric retreatment 2 weeks after the initial dose was prescribed or recommended in 85.5% of cases, and empiric treatment of household members was prescribed or recommended in 45.5% of cases. Counseling on hygienic measures was documented in approximately a quarter of cases: washing sheets or bedclothes (26.1%), washing pajamas or underclothes (16.2%), hand hygiene (21.6%), clipping fingernails (12.6%).These results are preliminary, with plans to review a total of 200 charts. Thus far, our findings suggest there is an opportunity for education efforts to be made in the future with regard to best diagnostic and treatment options for pinworm, as well as highlighting pyrantel as a cost-effective therapeutic option.

1218

ADDRESSING THE MENTAL HEALTH OF PERSONS LIVING WITH LYMPHATIC FILARIASIS IN LEOGANE, HAITI: EVALUATION OF A CHRONIC DISEASE SELF-MANAGEMENT PILOT PROGRAM

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There is mounting evidence that persons living with chronic illness resulting from neglected tropical diseases (NTDs) are at risk of mental illness, and many are living with the comorbidity of depression. There is also growing recognition of the importance of mental well-being in NTD prevention, elimination, and eradication efforts. Yet, there are few validated interventions for addressing the burden of chronic NTDs and co-morbid common mental disorders, especially in contexts affected by poverty, insecurity, and fragility. This study aims to fill this gap by determining the prevalence of depressive symptoms among persons with lymphatic filariasis in Leogane, Haiti and assessing the effectiveness of a chronic disease self-management (CDSM) intervention in improving mental health outcomes. CDSM is a six-session intervention delivered by peer facilitators within Hope Clubs. The study, carried out between July 2019 and April 2021, employed a randomized, waitlist-controlled design to assess the effect of CDSM on the treatment arm 1 (n=118) and the waitlisted treatment arm 2 (n=82) on symptoms of depressive illness, using the Zanmi Lasante Depression Symptom Inventory (ZLDSI) tool, which was created and validated in Haiti. Baseline data revealed half of respondents in Arm 1 (48%) and Arm 2 (52%) screened positive for symptoms of depressive illness, defined as a mean ZLDSI score >13 (Arm 1, 14.2, SD. 9.0; Arm 2, 13.8, SD. 8.2). At midpoint, following CDSM intervention

in Arm 1, prior to Arm 2, mean ZLDSI scores decreased in both Arm 1 (10.8, SD. 7.2) and Arm 2 (11.7, SD. 7.2). At endpoint, following CDSM intervention in Arm 2, mean ZLDSI scores again decreased in both Arm 1 (10.2, SD. 8.4) and Arm 2 (10.7, SD. 6.5). Amid limitations to the study, including delays imposed by COVID-19, these pilot data suggest overall trends that the CDSM intervention in the Hope Club setting promotes a reduction in symptoms of depressive illness among persons living with lymphatic filariasis. These findings should be considered observational, hypothesis-generating and encourage further research into the integration of mental health care into NTD care management.

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ROUTINE IMMUNIZATION STATUS AND FACTORS ASSOCIATED WITH TIMELY IMMUNIZATION AMONG CHILDREN AGED 10 -17 MONTHS OLD IN BAGAMOYO, TANZANIA

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Universal immunization of children against vaccine-preventable diseases (VPDs) is the most cost-effective public health intervention to reduce childhood mortality and morbidity across the world. The challenges remain in ensuring wide vaccine coverage. World Health Organization (WHO) targeted coverage for the routine immunization program is at least 90%. Also, WHO recommends that every child should receive every dose of vaccines at recommended ages and intervals to get the best protection possible. However, according to TDHS 2014-2015, the immunization coverage in Tanzania is 75%. Little data (if any) is available to explain local factors for this low coverage below the target. This information is vital for the development and implementation of appropriate solutions. Therefore, this study is designed to fill this lacuna in knowledge by investigating the current status of routine immunization and factors associated with timely immunization among Children aged 10 –17 Months Old residing in Bagamoyo district, Tanzania. This study is based on a secondary analysis of cross-sectional data from the dataset of the study entitled "A Phase Ib age de-escalation dose-escalation open-label study of the safety and immunogenicity of RH5.1/Matrix-M, administered intramuscularly in healthy adults and infants in Tanzania (NCT04318002)". The population of this study is healthy adults (18-45 years) and infants (5-17 months) residing in Bagamoyo district, Tanzania. All data will be analysed by using SPSS computer software. Information on immunization coverage will be obtained from the child's health card and will be expressed in terms of percentage. Descriptive and analytical statistics including bivariate and multivariable analysis will be performed. Bivariate and multivariate analyses will be used to examine the association between dependent (timely immunization) and independent variables (sociodemographic variables). A corresponding P-values of &lt0.05 will be considered significant. The study started in January 2021 and screening visit monitored data is expected to be available in September 2021.

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AN INVESTIGATION OF PREGNANCY OUTCOMES AMONG LASSA FEVER PATIENTS IN SIERRA LEONE

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Lassa fever (LF) is a rodent-borne zoonotic disease endemic to West Africa. LF infected pregnant women are more likely to die than non-pregnant women. However, the cause of this increased mortality is not understood. In this study we characterize the relationship between pregnancy and severe outcomes of LF and present two case studies of LF in pregnancy. The study population consisted of 53 pregnant and 250 non-pregnant women of reproductive age who were admitted to the Lassa Fever Ward at Kenema Government Hospital (KGH) in Sierra Leone between 2010 and 2019. Two cases from this study population are presented in depth. Statistical analysis was conducted in SAS, using Chi square tests to compare groups. In all, 58.49% of pregnant patients died compared to 24.20% of non-pregnant patients ($p<0.001$). Based on an ELISA test, 53.85% of pregnant patients tested positive for LASV antigen compared to 27.35% of non-pregnant patients ($p<0.001$). Among the cohort of pregnant women with accessible patient charts (n=24), absent uterus movement ($p=0.001$), malaise ($p=0.01$), and abdominal tenderness ($p=0.02$) were associated with increased rates of maternal and fetal death. Patient A was a 38-year-old woman from Pujehun District admitted to KGH after transfer from a local clinic. Upon exam, the gestational age was 38 weeks and no fetal heartbeat was detected. On hospital day 2 (HD2) Patient A went into labor and experienced convulsions, hemorrhaging, and expelled a macerated fetus and placenta. Patient A received a blood transfusion but died before receiving a full course of ribavirin. Patient B was a 22-year-old woman from Kenema District admitted to KGH presenting with anemia and swelling. The fundal height indicated the fetus was 28 weeks and the fetal heart rate was strong. Patient B went into labor on HD 7 and the neonate received a 9/10 Apgar score. The mother was discharged on HD 11 after receiving a full course of ribavirin. The findings of this study underscore the risks pregnant women with LF experience. The disparate outcomes between Patients A and B highlight the importance of treatment for successful maternal and fetal outcomes in the context of Lassa fever.

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UPDATE ON ENVIRONMENTAL TRAITS, FREQUENCY AND LIVING CONDITIONS ASSOCIATED WITH RICKETTSIA AND LEPTOSPIRA INFECTIONS IN YUCATAN, MEXICO

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Many houses located in rural areas of Yucatan still have no access to running water, and thus, cenotes are one of their sources for freshwater. Nevertheless, their underwater connection makes them vulnerable to contamination by human activities. Domestic dogs in rural Yucatan spend most of the time in public areas and defecate freely in any place since no measures for recollecting feces are implemented. During the rainy season, water is not easily absorbed by the rough soil, generating puddles where animals find their drinking source. In 2011, in a reservoir seroprevalence study, the majority of dogs and cats slept outdoors of which 45.2% and 15.2% respectively were found positive for Leptospira, an anaerobic spirochete. Leptospirosis prevalence in humans in the region was reported to be 14.2% in 2002, with more prevalence in rural areas and 74.3% cases during the rainy season. However, as dogs are in contact with other dogs, wild animals, cattle and their owners, it's not uncommon to find them carrying ectoparasites such as ticks which may transmit Rickettsia. Rickettsial and Leptospiral infections have been continuously increasing during the last decade in Yucatan, Mexico, and therefore, the objective of this case report is to update the frequency, seasonality and trends of both diseases in the context of rural Yucatan, Mexico. This is an observational epidemiological surveillance-based study that includes all official epidemiological registries reported in 2020 in Yucatan, Mexico. A total of 187 confirmed cases of Rickettsia infections were reported in Mexico, two (1.06%) of them occurred in Yucatan during the dry and rainy season. Additionally, 104 new cases of leptospirosis were confirmed nationwide of which four (4.16%) were from Yucatan in the rainy season. Coinfections between Rickettsia and Leptospira are uncommon, but may be included in differential diagnosis when poor sanitation and cohabitation with infested animals converge. Both conditions may or may not coexist, but when

clinical manifestations in patients with environmental determinants are present, due to their incidence in the region, should both be considered and tested.

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A COMPARISON OF THREE NUTRITIONAL ASSESSMENT TOOLS AMONG RENAL PATIENTS AT THE KOMFO ANOKYE TEACHING HOSPITAL, GHANA

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The study aimed at comparing three nutritional assessment tools; Minimal Nutritional Assessment (MNA), Subjective Global Assessment (SGA), and Malnutrition Universal Screening Tool (MUST) among renal patients at the Komfo Anokye Teaching hospital (KATH), Ghana. In a cross-sectional study, 110 renal patients aged 18 years and above and diagnosed with renal impairment were recruited from the Nephrology Unit of KATH. The MNA, SGA and MUST were used to obtain nutritional status data of the participants. According to the MNA, SGA and MUST, 49.1%, 12.7% and 16.4% of the renal patients were malnourished respectively. The MNA showed significant potential for predicting clinical outcomes such as decline in food intake ($k = 0.972$, $p = 0.015$), weight change ($k = 0.827$, $p = 0.024$) and change in body mass index (BMI) ($k = 1.00$, $p = 0.048$) in patients. The MUST showed a significant difference in change in BMI of patients ($p = 0.011$), while no significant clinical outcome was observed in patients using the SGA tool. Thus, the MNA tool may be a better alternative to SGA and MUST for the assessment of the nutritional status of renal impaired patients. Additionally, the Ghana Health Service should provide regular training of health workers on the nutritional assessments of renal patients to enable effective identification and management of individuals who may be at risk of malnutrition.

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OTORHINOLARYNGOLOGICAL SERVICE ON SPECIAL NEEDS INDIVIDUALS: OTOSCOPIC APPROACH

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The aim was to identify common Otological conditions in special needs and to profer solutions to them. Special needs are neglected group in the society dependent on others for their day to day activities. They are usually stigmatized and denied medical care. This study availed them the opportunity to have medical attention through Otorhinolaryngological service to improve their hearing. This was a 4 year prospective case-control study from May 2014 to August 2018. The study centre was at Project Chari-love. The study population was special needs whose parents gave written informed consents. sampling technique was total population technique. Stratified technique was used for the control.The control was Sunday-school children attending St, Albert Catholic Church, University of Benin, Benin city. Ethical clearance and permissions and consents were duly obtained. Health talks on the aims of the study, the ear, care of the ear and hearing hygiene and possible interventions were given. Questionnaires were filled by the researchers and their trained research assistants. Otoscopy was done. Each ear was regarded as a separate entity. Speculum used for each participant was disinfected before re-use on another participant. Wax and foreign body seen were removed. Those that had ear pathologies were referred to centres with otorhinolaryngological facilities for further evaluation and management. There were 60 cases and controls, aged 1 to 18 years for the cases and the control. 16 males and 44 females in the cases while the control was 23 males, 37 female. They were pupils from pre- KG class to senior secondary 3, with consent. Mean ages were 4.80 +/- 4.50 years for the cases and 2.00 +/- 3.60 for the control. Common ear conditions identified in the cases were impacted cerumen auris, Otitis Media with effusion, Eustachian tube dysfunction and chronic Suppurative otitis media. In contrast, the control recorded both Otomycosis and foreign body in the ears.From this study, Otological conditions are common in special needs. It demonstrated that much can be achieved with Otoscopy.

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COVID-19 SEROPREVALENCE IN TWO MALARIA ENDEMIC RURAL COMMUNITIES IN MALI, WEST AFRICA

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In collaboration with the Mali Ministry of Health, the Malaria Research and Training Center and the Laboratory of Malaria Immunology and Vaccinology conducted a longitudinal seroprevalence study COVID-19 epidemiology in two malaria endemic rural communities, Bancoumana and Doneguebougou, located 60 km and 30km from Bamako respectively. Participants aged ≥6 months of age completed two study visits between July and September 2020, and December 2020 and January 2021. At each visit a serum sample for SARS-CoV-2 serology testing was collected. In Doneguebougou, the seropositivity rate was 4.1% (95% CI: 2.9-5.2) at Survey 1 (n=1109) and 25.8% (95% CI: 23.2-28.4) at Survey 2 (n=1088). After adjustment, it was 4.7% (95% CI: 2.6-6.8) at Survey 1 and 34.4% (95% CI: 27.4-41.4) at Survey 2. In Bancoumana, at survey 1 (n=963) and survey 2 (n=904), the seropositivity rates were 5.3% (95% CI: 3.9-6.7) and 35.5% (95% CI: 32.4-38.6) respectively. After adjustment, the rates were 6.4% (95% CI: 4.0-8.8) and 47.6% (95% CI: 38.2-57) These results may reflect the spread of SARS-CoV-2 from urban to more rural regions as they are lower than the seroprevalence estimates of over 50% that we have previously reported in Bamako. Keywords: COVID-19, Seroprevalence, Age. Rural, Mali West Africa.

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RICKETTSIA AND LEPTOSPIRA COINFECTION IN A TWELVE-YEARS-OLD FEMALE FROM YUCATAN, MEXICO: CLINICAL CASE REPORT

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It is estimated that approximately 75% of emerging and reemerging infection diseases worldwide are zoonotic. Rocky Mountain spotted fever is a disease caused by *Rickettsia ricketssii*, a gram-negative bacteria transmitted by an arthropod, while Leptospirosis is a zoonotic disease caused *Leptospira spp.*, a gram-negative spirochete. *Rickettsia* and Leptospirosis are not a first line diagnoses, even tough Mexico show ecologic and socioeconomic characteristics that favor their transmission. The objective of the present case report is to document an unusual case of *Rickettsia* and *Leptospira* coinfection with emphasis on clinical manifestations and epidemiological context that may orient future multidisciplinary measures and diagnosis. Here we present the case of a

12-year-old female, whose mother had a history of recent a Rickettsial infection. The patient debuted with fever, developed unspecific signs and symptoms of infection, but quickly deteriorated with gastrointestinal, hepatic, renal, and neurological dysfunction. After discarding rabies, the patient was tested for *Leptospira* approaching antibodies using Microscopic Agglutination Test (MAT), which reported positivive with 1:400 to *L. Interrogans* serovar Australis. Also serologic and molecular diagnosis of *Rickettsia* were performed. Immunofluorescence reagent for *R. rickettsii* IgM 1:256 and IgG 1:512 resulted positive and CSF was positive to *R. rickettsii*. Despite anitbiotic treatment was indicated, the patient died due to septic shock after 33 days at the intensive care unit. This case exemplifies the possible clinical course in a probable coinfection between *Rickettsia* and *Leptospira* in a pediatric patient. When signs and symptoms, cannot fully be explain by a single pathogen and epidemiological and clinical context suggest more than one possible causal agent, coinfection should be included in differential diagnosis when poor sanitation and cohabitation and infested animals converge. In conclusion simultaneous instead of sequential diagnosis is recommended in order to prevent future fatal outcomes in patients.

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EPIDEMIOLOGICAL CHARACTERIZATION OF HUMAN SETTLEMENTS EXPOSED TO TRIATOMINAE SPECIES AND TRYPANOSOMA CRUZI IN THE COLOMBIAN CARIBBEAN

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Chagas disease or American trypanosomiasis is caused by the protozoan parasite *Trypanosoma cruzi* which represents a public health problem because it is part of the group of Neglected Tropical Diseases (NTD). Chagas disease affects annually approximately 6 to 8 million people worldwide, with an estimated 50.000 deaths; likewise, between 60 to 100 million people live in risk areas around the world including Colombian Caribbean region. As part of the response for the control of CD the research groups in Tropical Medicine of the Colombian Caribbean proposed to analyze epidemiologically and serologically the human settlements exposed to Triatominae species, the presence of *Trypanosoma cruzi* and Chagas disease in municipalities of the Atlantic Department in Colombia. This was a descriptive epidemiological study of prevalence with prospective data collection. Patients with suspected diagnosis or risk factor of Chagas disease were included due their environmental risk of exposure to Triatominae located in the department of Atlantico such as the municipality of Corral de San Luis. A serum sample was taken and / or whole blood for the application of serological tests aimed to characterize patients as carriers or not of IgG antibodies against *T. cruzi*. As part of the results the serological profile of the recruited patients in relation to Chagas disease was described for the first time for a municipality in the Atlantic department where the parasite and the transmitting vector of the disease has been described in the past. This information is of relevant importance as a public health surveillance measure since, up to the date of this work, no cases of Chagas disease have been notified in government reports in the region of study, but there have been positive cases in neighboring municipalities therefore with this work a response is given to a population with many risk factors for the presence of this vector borne disease.

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DISCOVERY FOR LOA LOA CROSS-REACTIVE BIOMARKERS RESPONSIBLE FOR FALSE-POSITIVE LYMPHATIC FILARIASIS RAPID DIAGNOSTIC TESTS

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Reactivity of *L. loa* antigens (LAgs) to lymphatic filariasis (LF) rapid tests (LF-RDT) (Immunochromatographic test, ICT and Filariasis Test Strip, FTS) is a hindrance to LF elimination in West and Central African countries. Previous attempts to characterize those Ags were inconclusive due to the limited number of cross-reactive sera analyzed. To determine which LAgs are consistently present in cross-reactive sera, we collected sera from 73 FTS-positive individuals and 13 FTS-negative controls, both groups harboring high *L. loa* microfilariae counts, and with no evidence of LF infection. Immunoprecipitation was used to isolate cross-reactive antigens. Western blot, using the monoclonal antibody (AD12) was employed to detect the different proteins present in those samples, and liquid chromatography-electrospray ionization-tandem mass spectrometry was used for the identification and characterization of those proteins. The AD12 western blot showed that cross-reactive sera contain multiple LAgs, the most common one being a ~80 kDa protein. Interestingly, the ~80kDa protein was also detectable by western blot in 92.3% FTS-negative samples. Mass spectrometry of a set of 20 FTS-positive samples already analyzed revealed the presence of 23 proteins, including two proteins present in >80% of samples. The comparison of this antigen profile with that of FTS-negative controls is ongoing and will help classifying the antigens as sole markers of cross-reactivity or as more broadly applicable antigen biomarkers for loiasis. To the best of our knowledge, this is the first large-scale proteomic identification of *L. loa* antigens in cross-reactive sera.

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EXPRESSION AND CHARACTERIZATION OF OV-103 AND OV-RAL-2, TWO LEADING VACCINE CANDIDATES FOR ONCHOCERCIASIS

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Ov-103 and Ov-RAL-2 are two vaccine candidates for onchocerciasis that induce significant protection against *Onchocerca volvulus* L3 infection in the mouse model. To produce large quantity of recombinant proteins with high purity and stability for testing in a clinical trial for efficacy in calves against natural infection with Onchocerca ochengi that in endemic in sub Saharan regions of Africa, the large scale production of recombinant proteins of these two antigens was carried out at 10L fermentation. Recombinant Ov-103 was highly expressed in yeast *PichiaPink* strain#1 at the 10 L scale under induction of methanol, and purified by one-step ion exchange chromatography. The recombinant Ov-RAL-2 was expressed in *E. coli* BL21 under induction of 1 mM IPTG at the 10 L scale with estimated raw yield of up to 1.0 g /L, then purified by IMAC as first capture step followed by a Q column polishing step. By these simplified two-steps purification, the purity of rOv-RAL-2 was over 97% with low contamination of endotoxin. Both recombinant proteins could be recognized by the sera from infected patients living in endemic areas. Far-UV CD spectrum analysis reveals that both Ov-RAL-2 (E. coli) and Ov-103 (yeast) have similar α-helical structure with a typical positive peak at 193 nm and a negative peak at 202-208 nm. Stability studies showed that Ov-RAL-2 was stable at 4°C and 37°C for at least 14 days, and remained

stable for 35 months so far at -80°C; no significant changes were observed in its visual appearance, concentration and purity. For Ov-103, it has a trend to form aggregation over time even when stored at -80°C. The efficacy in the vaccinated calves co-administered with these two vaccine antigens will be also presented.

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INCREASING IMMUNE ACTIVATION IN PATIENTS WITH ADVANCED STAGE FILARIAL LYMPHEDEMA

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Filarial lymphedema is caused by the adult worm of *Wuchereria bancrofti,* which can live for up to seven years in the human body. Worldwide, around 68 million people are infected with this helminth and approximately 25 to 30 million suffer from this chronic pathology. Treatment of filariasis commonly consists of antihelminthic drug combinations (Albendazole plus Ivermectin and/or Diethylcarbamazine) which diminishes microfilariae – the larval stage – but has only minimal effects on the adult worm of *W. bancrofti.* Other treatment options are under investigation. As part of an ongoing clinical trial testing the efficacy of Doxycycline to improve filarial lymphedema, individuals with different stages of lymphedema were recruited in Tanzania and Ghana. Four hundred twenty participants per country were characterized at baseline using the lymphedema staging (stage 0-7) according to Dreyer *et al*. (2002), circumference measurements with a tape measure, and an infrared scanner (LymphaTech®, Atlanta, Georgia, USA). Apart from clinical characteristics, immunological aspects of the different treatment groups were measured at baseline. Full blood from the participants was characterized for the presence of memory CD4 or CD8 T cells (CD45, CD27), regulatory CD4 T cells (FoxP3, CD25) and immune activation markers (CD38, HLADR). Data from the first 94 patients in Tanzania and 59 patients in Ghana show increased levels of $CD38^{+}$/$HLADR^{+}$ $CD4^{+}$ T cells in participants with advanced stage lymphedema (stage 4 to 7), compared to participants with early lymphedema (stage 2 to 3). The increased immune activation in patients with advanced disease emphasizes the role of the immune system in the process of lymphedema development, which might lead to new treatment opportunities.

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DEVELOPMENT OF A MULTIPLEX BEAD ASSAY FOR DETECTION OF SPECIFIC ANTI-ONCHOCERCA VOLVULUS IMMUNOGLOBULIN IN HUMAN SERA

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Onchocerciasis elimination programs are hampered by the lack of effective tests for making mass drug administration stopping decisions and conducting surveillance. All current tests for onchocerciasis-specific IgG4 use recombinant antigen Ov16. Although anti-Ov16 testing is useful for community screening and mapping of endemic areas, it was not developed under an elimination use case and lacks the sensitivity and specificity for making treatment stopping decisions in Africa. Therefore, identification of additional antigens that could be used alone or in combination with Ov16 to improve diagnostic accuracy and routine serosurveillance is needed. We conducted biomarker discovery to find potential targets for detecting antibodies to *Onchocerca volvulus*. Soluble fractions of homogenized adult worms were separated by size-exclusion liquid chromatography and 1-D gel electrophoresis. Protein immunoblots were used to identify reactive bands after the disruption of carbohydrate moieties. Fractions with reactive bands were subjected to tandem mass spectrometry. We identified 136 peptide sequences and selected 28 proteins using bioinformatic screening. These proteins were expressed, purified, and tested by immunoblot using known *Onchocerca*-positive patient sera. Specific IgG1 and IgG4 reactivity were observed for 5 proteins: Ov16, OvMSA-1, OvFABP, Ov33.3, and OvMSP3 with no reactivity in negative control sera. Ov16, OvMSA-1, OvFABP, and Ov33.3 demonstrated high (500-5000) signal-to-noise ratio (S/N) compared to potentially cross-reactive sera from persons with *Wuchereria bancrofti* infection using bead conjugated antigens either alone or in a multiplex assay. OvMSP3 showed a lower S/N ratio (~40) than other antigens but remains a viable candidate. Several of these antigens have been identified by other laboratories, and their combined use has been proposed for onchocerciasis mapping activities. Independent identification of the same antigens in our studies strengthens their likely utility. Characterization of the target antigens' performance for the onchocerciasis elimination use case is currently ongoing.

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SURVIVAL OF ADULT FEMALE WORMS OF ONCHOCERCA OCHENGI IN GERBILS AND HAMSTERS: IMPLICATIONS FOR THE DEVELOPMENT OF AN IN VIVO MACROFILARICIDE SCREENING MODEL

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Onchocerciasis, the second leading infectious cause of blindness, afflicts approximately 21 million people globally. Its control is limited to the use of the microfilaricidal drugs, ivermectin and moxidectin. Both drugs are unable to kill the adult worms which can survive for up to 15 years in patients, justifying the urgent need for potent and novel microfilaricides that kill adult worms. The development of such drugs has been mired by the lack of an appropriate small laboratory animal model to evaluate potential drug candidates *in vivo*. This study assessed the survival of *O. ochengi* female worms and their embryos over time in two laboratory rodents: gerbils and hamsters and tested using 'proof-of-concept' studies, whether known macrofilaricidal drugs can kill these worms. Gerbils and hamsters were surgically implanted with mechanical or enzyme-liberated adult *O. ochengi* female worms, and sacrificed at various time points (days 7 to 60 post-transplantation) to test for survival. Recovered worms were assessed for viability by biochemical analysis (MTT/formazan assay) or for their fecundity (embryogram). By day 26 post-implantation, 58.6±7.5% were recovered from hamsters, and 20±3.5% from gerbils. Those recovered from gerbils were mostly disintegrated or fragmented, with significantly higher ($p<0.05$) fragmentation observed with enzymatically-liberated worms. From day 3 post-implantation, flubendazole (FBZ) at 20 mg/kg body weight was administered subcutaneously at the nape for 7 days, to validate both rodent models. FBZ had no significant effect on the number and/or viability of worms recovered. However, embryo degradation was observed among some embryonic stages recovered from FBZ-treated gerbils ($p<0.05$). This exploratory study has revealed the gerbil and hamster as permissible rodents to adult female worms of *O. ochengi*. The hamsters appeared to maintain the infection longer, compared to gerbils. In gerbils, the worm isolation method significantly affected worm survival rate compared to the survival rate in hamsters.

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COMPARISON OF STANDARD AND MODIFIED HUMAN LANDING CATCH TECHNIQUES FOR BLACKFLIES IN THE ERA OF ONCHOCERCIASIS ELIMINATION

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Entomological evaluations of large fly catches are required for stopping mass drug administration (MDA) and verification of Onchocerciasis elimination. Human landing catch (HLC) is the standard sampling method for collecting human-biting blackflies for these purposes. Despite ethical concerns regarding the exposure of vector collectors to infective flies, the method is still commonly used, as no suitable alternative has been found to replace it. With many countries entering elimination phase, alternative and safer methods are needed. This study aimed to evaluate a modified HLC where vector collectors are protected by coloured-trousers attractive to blackflies allowing them to land on them but prevent biting. The study was conducted in Massangam district, Cameroon in October - November 2020 (rainy season) and January -February 2021 (dry season). Four points 50 meters apart along the banks of river Mbam known to contribute to onchocerciasis transmission, were selected corresponding to four collection arms - standard HLC (sHLC) and three modified versions,- collectors wearing blue (bHLC), black (nHLC) and blue-black pattern trousers (bnHLC). The collectors were rotated amongst collection points to minimize bias. Collection took place for four consecutive days per month between 7am and 5pm by two individuals interchangeably. All flies caught were dissected. Mann Whitney U test was used to compare the mean proportion of flies collected. A total of 17,246 flies were caught over the 4-month period. There was no statistical difference between proportions of flies caught between nHLC (29.9%; n=5,148) and sHLC (29.7%; n = 5,130) (P=01.3 and P=0.14) during both seasons. SHLC collected significantly more flies than bHLC (21.6%; n=3717; P<0.01) and bnHLC (18.9%; n=3251; P<0.01). A total of 673 (15.8%) were parous, 4 (2.1%) of parous flies were infected and 1 (0.5%) infective. There was no significant difference in the proportion of parous collected in the different arms. The study suggests using black colour-coated material for blackfly collection is a potential alternative to replace a sHLC, reducing the risk of exposure of collectors to fly bites.

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METALLOPROTEASE INHIBITORS AS POTENTIAL MACROFILARICIDAL TREATMENTS FOR LYMPHATIC FILARIASIS

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Lymphatic filariasis is a neglected tropical disease that affects over 60 million people in tropical regions. While there are several medications with activity against microfilariae, development of short course medications with activity against adult stages would enhance global efforts to eliminate LF. In this study, we screened various classes of protease inhibitors and observed substantial macrofilaricidal effect with the matrix metalloprotease (MMP) inhibitor 1,10 phenanthroline. Biochemical analyses confirmed that inhibition of MMP activity occurs when 1, 10 phenanthroline is applied to worm homogenate. On the basis of these results, we then tested several agents that are approved for human use and have MMP inhibitor function for activity against adult *Brugia malayi* worms. Worm motility was quantified using the computer-based screening application,*The Worminator*. Agents that demonstrated effects at 100uM were then tested at a range of concentrations to determine daily EC50's from day 1 to day 7. Two flavonoid supplements, luteolin and quercetin, were found to have EC50s of 36.4uM and 25.9uM at day 7. Both of these agents are safe for human consumption at high concentrations. Additionally, the MMP inhibitors, 1,10-Phenanthroline and its analog 4,7-dimethyl 1,10-phenanthroline were also shown to be highly potent against *B. malayi* adults (EC 50 of 0.9uM and 0.3uM at day 7, respectively). Effects on third-stage larvae (L3) were even more pronounced, as 10uM of luteolin and quercetin were sufficient to kill most L3 within 3 days, as was 1uM of 1,10-phenanthroline and 4,7-dimethyl 1,10-phenanthroline. The effects of these compounds on microfilariae as well as on the endosymbiotic *Wolbachia* bacteria are under investigation. The results of this study suggest that matrix metalloprotease inhibitors may be promising macrofilaricidal agents for lymphatic filariasis. Disclaimer: The opinions and assertions expressed herein are those of the author(s) and do not necessarily reflect the official policy or position of the Uniformed Services University, the Henry M. Jackson Foundation, or the Department of Defense.

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REPLACEMENT OF MISSING VALUES FOR ONE-TIME-MEASUREMENT OUTCOMES IN ONCHOCERCIASIS CLINICAL TRIALS

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To reach the goal of the WHO road map for NTDs 2021-2030 to eliminate onchocerciasis and lymphatic filariasis, alternative treatment strategies are needed with the aim to sterilize or kill the adult worms. To test new drugs or drug combinations, clinical trials are carried out most likely in onchocerciasis patients because there the drug effect on the adult worm can directly be assessed in the onchocerca nodules in which the worms reside. The nodules are surgically extirpated (nodulectomies) at the end of a clinical trial and afterwards histologically evaluated. Intention-to-treat (ITT) analyses are the standard for clinical trials. In this case, all randomized participants have to be analyzed and missing values should be replaced. Data from participants, who missed the nodulectomy are difficult to replace with common methods, because no data regarding the direct drug effect on the adult worms but only baseline data are available. The aim of our project is the replacement of missing data in the best suitable manner. Onchocerciasis nodule data are structured on patient-, nodule- and worm-levels, with possible dependencies between the outcome variables, which makes any quick imputation difficult. Two datasets from different clinical trials were used to implement the approaches on all three levels, facing the challenges concerning the additive and level dependencies between worm, nodule and patient data. All approaches were done with a stepwise multiple imputation with predictive mean matching (PMM-MI) and subsequent averaging to the mean with exception of the last step on worm level where a classical multiple imputation for the embryogenesis outcome was used. The PMM-MI-method showed, compared to a worst-case imputation, a much more suitable approach to replace missing data. The approach first on patient and secondly on worm level was the only possibility to impute all variables on worm level. In a last step validation of the method will be done by using a test dataset with randomly removed patients, which will be compared to the original observed data after imputation.

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MODELLING THE MASS DRUG ADMINISTRATION AND ALTERNATIVE INTERVENTIONS FOR THE ELIMINATION OF LYMPHATIC FILARIASIS IN AMERICAN SAMOA: AN AGENT-BASED SIMULATION STUDY

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Lymphatic filariasis (LF) is a mosquito-borne parasitic helminth, endemic to >70 countries in tropical and sub-tropical regions. In 2000 the World Health Organization began a campaign for the global elimination of LF as a public health problem, using annual MDA as the primary tool for interrupting transmission. Though 17 countries have successfully achieved WHO elimination targets by 2020, others have reported resurgence after completing the prescribed rounds of MDA. American Samoa underwent seven rounds of MDA from 2000-2006 which greatly reduced prevalence of circulating filarial antigen (Ag) from 16.5% in 1999 to 2.3% in 2007, but subsequent surveys found that Ag prevalence had rebounded to 6.2% by 2016, prompting two further rounds of MDA with a triple-drug combination (ivermectin, diethylcarbamazine, albendazole) in 2018 and 2019. We used GEOFIL, a spatially-explicit agent-based model of LF transmission in American Samoa, to model the effects of these additional rounds of MDA and to compare MDA scenarios by varying the number of rounds (2-7), treatment regime (two-drug vs triple-drug), MDA coverage (55%-75%), and assumptions around drug efficacy. We compared annual MDA to alternative intervention scenarios, including ongoing targeted drug administration delivered by teams embedded in the community. Our model predicts that the two additional rounds of triple-drug MDA in 2018 and 2019 are unlikely to be sufficient to interrupt transmission and that at least six annual rounds of triple-drug MDA at 75% coverage would be required to ensure more than 50% probability of elimination by 2035. Our findings have major implications for LF elimination efforts in American Samoa and other Pacific Islands with similar LF challenges, and calls for a reevaluation of end-game strategies in these settings.

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STRONGYLOIDES STERCORALIS, STILL A PROBLEM IN THE SOUTHEAST UNITED STATES?

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Strongyloides stercoralis, a parasitic nematode transmitted through skin penetration after contact with contaminated soil. It is estimated to affect 100 million people worldwide annually. In the United States, Strongyloidiasis is thought to be relatively rare, although there have been endemic foci, notably in the Appalachian region. The region is believed to harbor a higher prevalence of strongyloidiasis for numerous reasons. First, the rugged landscape of the area became a center for resource extraction in the early 1900s leading to subsequent water pollution. The mining industry contributed to long hours in difficult work conditions with defecation common within the mines providing an optimal environment for the free-living form of the parasite. Second, the heat in the summer months in the South contributed to a higher cultural acceptance of not wearing shoes, facilitating the transmission of the parasite. Third, many rural states lagged in the development of proper waste systems, such as disposal of the sewage directly into ditches or streams and reliance on outhouses. For example, the 1990 North Carolina census showed 1.8% of residential units lacked indoor plumbing. A retrospective chart analysis of 97 patients with *Strongyloides stercoralis* diagnpsed by serology or direct parasite identification was performed from records at Wake Forest Baptist Medical Center in Winston-Salem, North Carolina. This 885-bed tertiary care teaching hospital with around 40,000 inpatient admissions annually encompasses a catchment area of 27 counties ranging from southern West Virginia to South Carolina. Data extraction included demographics (i.e. gender, age) as well as, place of birth, and travel and exposure history. Complete analysis of this information is underway and the final results will be available before the date of the conference. The goal of this study is to investigate the current prevalence of *Strongyloides stercoralis* in the southern Appalachia in order to fill an important gap in the literature and explore whether it's still a problem in our present day.

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COMPARISON BETWEEN QUANTITATIVE POLYMERASE CHAIN REACTION AND SODIUM NITRATE FLOTATION MICROSCOPY IN DIAGNOSING SOIL-TRANSMITTED HELMINTH INFECTIONS

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There is evolving interest in alternate microscopy techniques and quantitative polymerase chain reaction (qPCR) to evaluate soil-transmitted helminth (STH) burden. Using data from a cross-sectional survey of 540 schoolchildren across 6 primary schools in 3 municipalities of Timor-Leste, we compared the performance of microscopy using sodium nitrate flotation (SNF) and qPCR in determining STH prevalence and infection intensity. Prevalence by qPCR was higher than SNF for *Ascaris lumbricoides* (17.5% vs 11.2%), hookworm (8.3% vs 1.2%) and *Trichuris trichiura* (4.7% vs 1.6%). Agreement between SNF and qPCR was moderate for *A. lumbricoides* ($\kappa = 0.59$) and *T. trichiura* ($\kappa = 0.44$), and fair for hookworm ($\kappa = 0.21$). Nearly all infections were light intensity by SNF, whereas qPCR identified 36.1% as moderate or heavy infections using cycle threshold to eggs per gram conversion formulas. qPCR is a promising diagnostic technique, though further studies validating infection intensity correlates are required.

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USING POPULATION-BASED SURVEY DATA TO MAKE EVIDENCE-BASED PROGRAMMATIC DECISIONS TOWARD THE ELIMINATION OF SOIL-TRANSMITTED HELMINTHIASIS

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The Kenya Ministry of Health initiated a strategy to break community-wide transmission of soil transmitted helminthiasis (STH) in 2018. Knowledge of the community-wide epidemiology of STH is necessary to plan and direct resources toward this goal. We conducted the first population-based STH survey in Vihiga county in 2019 using multi-stage cluster sampling and Kato Katz to assess prevalence and intensity for STH among pre-school age children (PSAC; 2-4 years), school age children (SAC; 5-14 years), and all adults >14 years. The survey was powered to give representative estimates for each target group and the whole population of the county. The overall prevalence of STH (9.5%) was below the 10.0% WHO threshold for annual preventive chemotherapy treatment (PCT) indicating that PCT once per two years is warranted. Prevalence of moderate-to-heavy intensity infection (MHII) was 2.5%, which is above the WHO 2.0% elimination threshold, indicating continued annual PCT. We found that PSAC had the highest overall and MHII prevalence (14.6% and 5.6%), followed by SAC (8.3% and 2.3%), and adults (4.3% and 1.0%). There was no significant difference in prevalence by sex in any age group ($p=0.06$), and the prevalence and intensity among women of reproductive age (15-49 years) was similar to that of all adults. Participants with finished flooring had a 54% lower prevalence than those with unfinished flooring ($p<0.001$), and

those who reported regular use of shoes had a 74% lower prevalence than those who did not (p<0.001). Half of all participants reported having an improved toilet facility at home, and their prevalence was 40% lower than those with unimproved or no facilities (p=0.001). Prevalence among those who use toilet paper was 47% lower than those who used other materials (p<0.001). Surveys such as these provide valuable insight into target-group specific prevalence of infection and risk factors, and STH surveys of two additional endemic counties are underway in Kenya. Input from experts and opinion leaders is sought to inform what multi-sectoral interventions should be conducted and how future PCT should be targeted to those at risk of morbidity from STH.

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PREVENTIVE CHEMOTHERAPY FOR THE CONTROL OF STRONGYLOIDIASIS IN SCHOOL-AGE CHILDREN: ESTIMATING THE IVERMECTIN NEED

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Strongyloides stercoralis is a soil-transmitted helminth (STH) that affects approximately 600 million people worldwide. Interventions targeting *S. stercoralis* have not been implemented yet. Specific treatment (ivermectin) could be included in already ongoing preventive chemotherapy (PC) campaigns targeting other STHs. The aim of this study was to estimate the quantity of ivermectin needed for an integrated STH/*S. stercoralis* control program. Our study targeted school- age children (SAC), usually the focus of STH deworming campaigns. The normal approximation of the binomial distribution was adopted to calculate the hypothetical prevalence distribution in each endemic country. Considering prevalence thresholds for PC equal to 10%, 15%, and 20%, we estimated the number of SAC in need of treatment. We adjusted the estimates accounting for ivermectin distributed in lymphatic filariasis and onchocerciasis elimination programs and excluded from our calculation areas where *Loa loa* is endemic. The global number of SAC that should be targeted in PC campaigns was estimated at 42.8M (95% CI: 25.1-58.0), 31.1M (95% CI: 24.1-57.8), and 24.1M (95% CI: 12.9-33.8) when the threshold for intervention was set to 10%, 15%, and 20%, respectively. India, China, Indonesia, Bangladesh, and Nigeria accounted for about 50% of the global SAC would have to be covered by PC intervention. Our analysis may support endemic countries to evaluate the ivermectin quantity needed for integrating strongyloidiasis in the existing STH programs. These estimates might also show to generic drug manufacturers the size of the potential market for ivermectin and encourage its production.

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USING DEEP AMPLICON SEQUENCING TO SCREEN FOR THE PRESENCE OF BENZIMIDAZOLE RESISTANCE IN HOOKWORM POPULATIONS IN NORTHWEST ETHIOPIA

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The hookworm, *Necator americanus*, causes severe anemia in school-aged children and pregnant women. Control in endemic regions is achieved by Mass Drug Administration (MDA) programs using the benzimidazole drugs, albendazole and mebendazole, which have variable efficacy. Mebendazole, in particular, is often reported to have low efficacy in clinical studies and the extent to which this is due to anthelmintic resistance is as yet unclear. Benzimidazole resistance is widespread in many species of gastrointestinal nematodes of veterinary importance with mutations at codons 167, 198 and 200 of the isotype-1 β-tubulin gene commonly occurring in resistant populations. Although some of these mutations have been described in *N. americanus*, their prevalence, distribution and contribution to low drug efficacy is poorly studied. We have developed deep amplicon sequencing assays to enable large scale screening of *N. americanus* populations for benzimidazole resistance mutations at codons 167, 198 and 200 in both the isotype-1 and isotype-2 β-tubulin genes. Here we describe their application to investigate whether the low efficacy of mebendazole in an efficacy study in NW Ethiopia could be due to the presence of these mutations. Fecal samples were collected and parasite prevalence was determined from study participants in four districts - Chuahit, Debark, Sanja, and Maksegnit - in the Gondar region of Ethiopia. Following a single dose of mebendazole, the cure rate and egg reduction rate for hookworms were 23.1% and 49.6%, respectively. Deep amplicon sequencing was performed on hookworm positive fecal DNA samples from 26 and 44 participants from Chuahit and Sanja districts, respectively. No mutations were detected at any of the three codons of interest in either β-tubulin gene in any of the samples. These results demonstrate that mebendazole efficacy in this particular trial was not associated with the presence of previously described benzimidazole resistance mutations. This work illustrates the value of deep amplicon sequencing to screen parasite populations in individual stool samples for the presence of known benzimidazole resistance mutations.

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DEVELOPMENT OF CRYSTAL PROTEIN TREATMENTSFOR GASTROINTESTINAL NEMATODES

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Gastrointestinal nematode (GIN) parasitic infections are one of many neglected tropical diseases urgently needing new treatments due to increasing resistance to current anthelmintics. One and a half billion people worldwide are infected with GIN parasites, which can lead to malnutrition, anemia, growth stunting, cognitive deficits, and significant reductions in future earnings and education in children. Crystal proteins (cry) produced by *Bacillus thuringiensis* (Bt), a natural soil bacterium, have been used safely in agriculture for over half a century. We have previously reported that Cry5B cures parasitic nematode infections *in vivo*, including hookworm, *Ascaris suum*, *Parascaris sp.*, and *Haemonchus contortus*. More recently we reported on a new way to produce and deliver cry proteins to nematodes, as a cytosolic crystal protein expressing paraprobiotic (Cry5B IBaCC) that is effectively ingested by hookworms and ascarids. However, delivery of Cry5B in the form of IBaCC to whipworm has proven to be ineffective because whipworms can't ingest such large particles. Finding superior treatments against whipworm is of great importance due to the lack of effective current anthelmintics. To address this problem, we have developed new methods of processing purified Cry5B cytosolic crystals that are highly active against whipworms *in vitro*, while retaining activity against hookworm and ascarids. Additionally, we are exploring the mining of cry variants with various amino acid changes which may lead to the discovery of more potent crystal proteins.

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RETROSPECTIVE REVIEW OF TOXOCARIASIS AT TEXAS CHILDREN'S HOSPITAL

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Toxocara (*T.cati*, *T.canis*) is a zoonotic helminth found in cats and dogs. Accidental ingestion of eggs in contaminated soil causes toxocariasis, which can be asymptomatic, cause covert disease, ocular larva migrans or visceral larva migrans in humans, leading to long-term morbidity. The aim of this study was to characterize *Toxocara* cases in Houston, Texas and identify risk factors in those seeking care at Texas Children's Hospital

(TCH). An observational retrospective review was performed from January 1, 2010-April 9, 2021 in patients 0-18 y/o with corresponding *Toxocara* ICD 9/10 codes or those who had positive *Toxocara* serologies identified via the TCH microbiology laboratory. Demographics, laboratory analysis, symptoms, and risk factors were noted. A total of 48 cases were found with an average age of infection of 6.6 years; 44% of patients were female. Most cases were of Hispanic/Latino ethnicity (30/48; 62.5%) and white race (41/48; 85%). Using geospatial analysis, a toxocariasis hot spot was identified in Northeast Houston. An initial AEC <500 was found in 6/48 cases (12.5%); all were symptomatic, with 4/6 (67%) having vision loss/impairment. A total of 12/48 (25%) had mild eosinophilia (AEC ≥500 but < 1500). Of these, 50% were symptomatic; wheezing/coughing (4/12; 33%) was the most common symptom. Over 35% (17/48) had moderate eosinophilia (AEC ≥1500 but < 5000) of which 8/17 (47%) had symptoms, most commonly abdominal complaints (6/17; 35%). Lastly, 10/48 (21%) had severe eosinophilia (AEC ≥5000), of which 6/10 (60%) had symptoms including 33% with skin lesions and 50% with abdominal complaints. Three patients had unknown AECs. Animal exposure occurred in 37/48 (77%); most had close contact with either dogs (19/37; 51%) or with both cats and dogs (46%). The majority of children had unknown pica behavior. Toxocariasis is the most common zoonotic infection in the U. S. We identified a *Toxocara* hot spot in the Northeast region of Houston. Contact with dogs was also an important risk factor for the development of infection. This information should guide future "one health" efforts to reduce the risk of toxocariasis in children in the Houston area.

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DETERMINING EFFICACY OF ALBENDAZOLE IN DAK LAK PROVINCE, VIETNAM, USING QUANTITATIVE PCR

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Soil-transmitted helminths (STHs) are parasitic worms infecting over 1 billion people worldwide. Regular large-scale deworming is the main strategy to control STH-associated morbidity. Dak Lak province, Vietnam, has implemented large-scale deworming of primary school-age children with mebendazole or albendazole since 2007 as part of the national STH control program. We tested the efficacy of one locally manufactured brand of albendazole ("Zentel"), used as part of a large clinical trial we are conducting (the Community Deworming against STH (CoDe-STH) trial). Quantitative PCR (qPCR) was used to detect and quantify infections; infection intensity in eggs per gram of faeces (EPG) was calculated from cycle threshold (Ct values) using conversion formulas derived from egg-seeding experiments. We also tested the chemical composition of Zentel compared with FDA-approved "Eskazole". Baseline stool samples were collected and assessed for infection status and intensity from 4 hamlets in Dak Lak province in April-May 2020. Individuals providing a baseline stool sample were given a single dose of Zentel. 14 days post-treatment, all individuals who provided a stool sample and took Zentel were asked to provide a second stool sample, to determine drug efficacy. 783 samples were collected. Stool samples were analysed by qPCR to determine infection status and intensity. The most prevalent STH species was the hookworm *Necator americanus* (overall prevalence 39.0%). Prevalence increased with age, with the highest prevalence in adults aged 50+ years at 62.2% overall. The cure rate for *N. americanus* with was 64.9% (range 58.5%-76.5% across different age groups). A 91.7% reduction in intensity of *N. americanus* infection was observed after treatment from an average of 11,203 to 520 EPG. The majority of infections were cured or had an intensity reduction of at least 80%. Use of qPCR allowed calculation of both cure rate and egg reduction rate. The efficacy of Zentel used in the main CoDe-STH trial shows comparable efficacy to that reported for other albendazole brands. Chemical analysis showed the tablet composition was comparable with Eskazole.

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PREVALENCE OF AMERICAN VISCERAL LEISHMANIASIS IN AN INDIGENOUS POPULATION OF THE COLOMBIAN CARIBBEAN COAST

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Leishmaniasis is considered a serious zoonosis, caused by a protozoan of the genus *Leishmania*, which can affect humans who come into contact with the parasite's transmission cycle, thus becoming an anthropozoonosis. American visceral leishmaniasis (AVL) is caused by a species of the *Leishmania* subgenus, *Leishmania chagasi*. Serological tests were performed on 120 individuals belonging to an indigenous population of the municipality of San Andrés de Sotavento on the Colombian Caribbean Coast, to determine the presence of L. chagasi reactors. The sample was taken at random in the towns of Jején, Las Cruces, Bajo Grande, Tuchín, Venecia and Cerro Vidales. A venous blood sample (5 cc) was obtained from each participant, from which the serum that would be used to determine the presence of antibodies against L. chagasi was recovered, using the indirect immunofluorescence technique (IFI). The prevalence of antibodies against L. chagasi found in the present study was 25.8%, equivalent to 31 samples out of 120 examined. The serological reaction of the positive samples showed titers between 1: 8 to 1:16. No reaction was detected for titers greater than or equal to 1:32. The prevalence found by locality corresponded to the following: Jején 13 (10.8%), Las Cruces 3 (2.5%), Bajo Grande 6 (5.0%), Tuchín 1 (0.8%), Venecia 7 (5.8%), and Cerro Vidales 1 (0.8%). The analysis of the results showed a relationship between the seropositivity found and living with canines, since of the 31 seropositive participants, 29 (93.5%) reported living with this animal species. When submitting these data to the statistical analysis of variance, statistical significance was found between the study areas and living with canines ($p < 0.05$). The high prevalence of antibodies against *L. chagasi* (25.8%) found in the present study and its association with having or living with canines highlights an important public health problem in these populations that urgently demands the performance of more studies similar to this one in the canine species to determine its epidemiological importance in the transmission of American visceral leishmaniasis in these communities.

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EXPERIENCES WITH DIAGNOSIS AND TREATMENT OF CHAGAS' DISEASE AT A COLORADO TEACHING HOSPITAL-CLINICAL FEATURES OF PATIENTS WITH POSITIVE SEROLOGIC TESTING

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Chagas' disease (CD), infection by *Trypanosoma cruzi* (TC), is the third most common parasitic infection in the world and can cause cardiac and gastrointestinal complications. While CD is endemic to Latin America, an estimated 300,000 carriers of CD (most born in Latin American) live in the U.S. Diagnosis of chronic CD is established with two different IgG serologies. At University of Colorado Hospital (UCH) positive serologies are confirmed by repeat testing at the CDC. We wanted to describe our institutions experience in diagnosis of CD. The UCH health record was queried for diagnoses of CD and positive TC serologies between 2006 and 2020. All cases with one positive IgG were included. Demographic and clinical data were extracted by manual chart review. 21 cases were included: 20 with a positive IgG serology, 1 with positive histopathology. 4 were confirmed cases of chronic CD, 3 through serology and 1 from histopathology and serology from a heart transplant recipient. 2 chronic CD patients were born in in Mexico, 1 in Guatemala, 1 in El Salvador. 2 chronic CD patients were transplant recipients who were diagnosed after transplant had occurred. 3/4 chronic CD patients were treated with benznidazole. Most, (11/20) positive serologies were sent as part

of pretransplant screening but no confirmed cases of CD resulted from these protocols. 60% of positive serologies never had confirmatory testing with 58% of these subjects lost to follow up. We identified a substantial number of patients with positive serologies for TC at a large academic medical center in Colorado. All confirmed cases of CD were among patients born in Latin America. Most of the testing for CD at UCH is part of pre-transplant screening, much of it in patients not from CD endemic countries. In this population, positive results identified during screening are not always confirmed. There is likely underdiagnosis of CD given lack of standardized screening protocols within our institution and other medical centers nationwide. Given the large number of individuals residing in the US with potential latent infection, improved screening efforts are warranted.

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CUTANEOUS LEISHMANIASIS IN KENYA: A STUDY OF KNOWLEDGE, ATTITUDES, AND PRACTICES IN TWO FOCAL SUB-COUNTIES

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Cutaneous leishmaniasis (CL), a disfiguring and stigmatizing disease occurs in only two known focal areas in Kenya. Over 3 years (2018-2020), this study sought to determine the behavioral, ecological, and socio-economic characteristics of household members in CL endemic villages in the Gilgil and Kipipiri sub-counties using data on knowledge, attitudes, and practices (KAP). A descriptive research design was used for both qualitative and quantitative data. Through purposive sampling of high-risk CL sites, we used a structured questionnaire to interview 132 respondents in 8 villages. 16 key informants (KIs) were interviewed and 9 FGDs (5 male and 4 female) were conducted. Data analysis was done using STATA and Excel spreadsheets. Respondents were 64% female and 36% male. The majority (62%) had primary education. The mean monthly income for an average 4-member family was Ksh.6, 251 (~$62). The majority (60%) lived in mud-wall houses with 25.36 meters average distance to animal sheds. CL awareness was high (86%), 24% reported a family member with suspected CL, 21% had been properly diagnosed and 20% received treatment. 63% thought CL was a very serious disease with fear (56%) the first reaction then shame at 16% if they found out they had CL. While 45% knew CL was transmitted by the sandfly, 72% would not recognize it. A small percentage (20%) reported using treated nets and a negligible 2% occasionally sprayed their homes with insecticide. The recurring theme in the FGDs and KIs was fear; fear of the recurring and multiplying nature of the CL lesions, its easy spread, the disfiguring ulcers, stigmatization, and prolonged, painful weekly intralesional injections resulting in many defaulters. The burden of disease is related to social demographics, degree of exposure, knowledge of the disease, and access to treatment. Community sensitization and preventive measures, a short-term effective treatment that is less painful, reconstructive surgery, and psychosocial support for patients with disfiguring scars are recommended.

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CHRONIC CHAGAS CARDIOMYOPATHY MORBIDITY AND MORTALITY BURDEN IN LATIN AMERICA

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Chagas disease is a largely neglected tropical disease infecting 6-8 million individuals annually in Latin America. Patients with acute or indeterminate infection progress to chronic Chagas cardiomyopathy (CCC) at approximately 5% and 2% yearly. Clinical outcomes vary considerably in CCC and precise estimates of annual mortality rates and its determinants have been challenging to identify. A systematic search was conducted for studies published between January 1946 to October 2018. Inclusion criteria were longitudinal studies of patients diagnosed with CCC that reported data on death. Studies were excluded if they did not state sufficient outcome data. Main outcomes extracted were overall all-cause mortality and specific mortality for cardiovascular, heart failure, sudden death, stroke and noncardiac outcomes. From the initial 10,761 studies identified, 52 studies met the criteria for inclusion. A random-effects meta-analysis using the death rate over the mean follow-up period in years was used to obtain pooled estimated annual mortality rates. The meta-analysis revealed an annual all-cause mortality rate of 7.9% (95% CI: 6.3-10.1; I^2= 97.74%; T^2=0.70) among CCC patients. The pooled estimated annual cardiovascular death rate was 6.3% (95% CI: 4.9-8.0; I^2= 96.32%; T^2=0.52). Annual heart failure mortality rate was 3.5% (95% CI: 2.4-5.1; I2=94.10%; T^2=0.93), annual sudden cardiac death rate was 2.6% (95% CI: 1.9-3.5; I^2=89.72%; T^2=0.53), and annual stroke mortality rate was 0.4% (95% CI: 0.3-0.8; I2=63.84%; T^2=0.49). The annual mortality rate from noncardiac deaths was 1.3% (95% CI: 0.9-1.9; I^2= 75.06%; T^2=0.45). Meta regression showed that low left ventricular ejection fraction (coefficient = -0.04; 95% CI: -0.07, -0.02; P= 0.001) was associated with an increase in mortality risk. Subgroup analysis based on AHA classification revealed pooled estimate rates of 4.8%, 8.7%, 13.9%, and 22.4% (P< 0.001) for B1/B2, B2/C, C, and C/D stages of cardiomyopathy, respectively. Findings from this study highlight the substantial mortality risk in CCC, particularly from cardiac causes in endemic regions.

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END-GAME STRATEGIES AGAINST GAMBIENSE HUMAN AFRICAN TRYPANOSOMIASIS IN THE MANDOUL FOCUS OF CHAD: A MATHEMATICAL AND ECONOMIC MODELING STUDY

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Human African trypanosomiasis, caused by the *gambiense* strain of *Trypanosoma brucei* (gHAT), is a deadly parasitic disease transmitted by tsetse. The Chadian government, along with partners from around the world have stepped up efforts to eliminate the disease, in particular in the formerly high-prevalence focus of Mandoul. In this study, we re-evaluate past assessments of the epidemiological status of gHAT using a sophisticated transmission model and up-to-date fitting methodology. We then re-evaluate the efficiency of intensified strategies aimed at

interrupting transmission that were put in place in 2014, and we make recommendations on the best way forward based on both epidemiological projections and economic efficiency. In our analysis we use a transmission dynamic model fit to epidemiological data from Mandoul to evaluate the cost-effectiveness of combinations of active screening, "enhanced" passive screening (expanding the number of health posts capable of screening for gHAT), and vector control activities employing Tiny Targets. For cost-effectiveness analysis, our primary outcome is disease burden, denominated in disability-adjusted life-years (DALYs) and costs, denominated in 2020 US$. Although new strategies for active and passive screening have paved the way for better evaluation and more rapid and accessible treatment, interventions that include vector control provided a good value-for-money (at less than $750/DALY averted) and substantially increased the probability of reaching the elimination target. Our transmission modelling and economic evaluations suggest that it may be cost-effective to already halt active screening and vector control as long as passive screening remains robust, as it appears that resurgence of infection in the focus is very unlikely. However, it is critical to factor in data requirements for elimination of transmission to be verified and to protect against possible importation of infection from neighbouring endemic foci.

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SURVEILLANCE OF TRYPANOSOMA CRUZI INFECTION IN TRIATOMINE VECTORS, FERAL DOGS AND CATS, AND WILD ANIMALS IN AND AROUND EL PASO COUNTY, TEXAS AND NEW MEXICO

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The causative agent of Chagas disease, *Trypanosoma cruzi*, is transmitted by triatomine vectors. The insect is endemic in the Americas, including the United States, where epidemiological studies are limited, particularly in the Southwestern region. Here, we have determined the prevalence of *T. cruzi* in triatomines, feral cats and dogs, and wild animals, the infecting parasite genotypes and the mammalian host bloodmeal sources of the triatomines at four different geographical sites in the U.S.-Mexico border, including El Paso County, Texas, and nearby cities in New Mexico. Using qualitative polymerase chain reaction to detect *T. cruzi* infections, we found 66.4% (n = 225) of triatomines, 45.3% (n = 95) of feral dogs, 39.2% (n = 24) of feral cats, and 71.4% (n = 7) of wild animals positive for *T. cruzi*. Over 95% of *T. cruzi* genotypes or discrete typing units (DTUs) identified were TcI and some TcIV. Furthermore, *Triatoma rubida* was the triatomine species most frequently (98.2%) collected in all samples analyzed. These findings suggest a high prevalence of *T. cruzi* infections among triatomines, and feral and wild animals in the studied sites. Therefore, our results underscore the urgent need for implementation of a systematic epidemiological surveillance program for *T. cruzi* infections in insect vectors, and feral and wild animals, and Chagas disease in the human population in the southwestern region of the United States.

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PUBLIC AWARENESS ON DISEASE AND TRANSMISSION OF LEISHMANIASIS: A NATIONWIDE SURVEY IN SRI LANKALEISHMANIASIS: A NATION-WIDE SURVEY IN SRI LANKA

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Sri Lanka is now considered endemic for leishmaniasis; yet the community knowledge on the disease and its spread remains poor. This study was conducted to assess the public awareness on the disease and its transmission among individuals through a nation-wide study. The survey was done in selected sites covering all 9 provinces of Sri Lanka and included 301 confirmed cutaneous leishmaniasis patients and 2791 healthy individuals as controls (1:10 case: control ratio) from same areas. A validated questionnaire was used. Only 2.1% of the patients knew the disease by its name and the figures were even lower in the controls (1.4%). Majority of both cases and controls (89.7% and 78.7% respectively) referred to the disease as a fly induced skin disease. More than 90% of the cases correctly identified the disease symptoms (skin lesions) but the percentage of controls (79.6%) who knew correct symptoms were significantly low. Percentage of patients who knew that the disease is curable (91.7%) and who correctly named the vector (68.8%) were significantly high when compared to the control group (74.6% and 46.0% respectively). A very low percentage acknowledged that they could identify a sandfly (1.7% cases, 0.9% controls) and were aware of the breeding places of vector (1.7% cases, 1.1% controls). Over 90% of both cases and controls did not know the bite times of vector, but the percentage of cases who knew the details of bite time (3.3%) were significantly high when compared to the controls (1.2%). Knowledge regarding control of vector in the study population is poor with over 90% of cases and controls being unaware of any control methods. Majority of the patients (52.2%) said the source of information on the disease is from hospitals, healthcare workers, while the major source of information for the controls were friends, neighbors or coworkers. The awareness of the disease/ vector among the general community is low when compared to the patients who seems to gather the relevant knowledge through healthcare systems. Increasing the public awareness of the disease will be beneficial in prevention and elimination strategies.

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THE ROLE OF DIHYDROLIPOYL DEHYDROGENASE IN THE IMMUNOPATHOGENESIS OF LEISHMANIA MAJOR INFECTION: THE ROLE OF DIHYDROLIPOYL DEHYDROGENASE IN THE IMMUNOPATHOGENESIS OF LEISHMANIA MAJOR INFECTION..

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Cutaneous leishmaniasis caused by *Leishmania major* is a major public health problem and causes a range of diseases from self-healing infections to chronic disfiguring disease. Currently, there is no vaccine for human leishmaniasis, which is due to poor understanding of key immunodominant antigens and correlates of protective immunity in infected animals. We previously demonstrated that a naturally processed peptide fragment DLD63-79 derived from *Leishmania* dihydrolipoyl dehydrogenase (DLD) protein-induced strong protective IFN-g-producing $CD4^+$ T cell response following *L. major* infection. The role and contribution of DLD in parasite virulence and pathogenesis of leishmaniasis is currently not known. We hypothesize that DLD is a virulence factor and its deficiency in *L. major* will result in an attenuated disease pathology and altered host immune response. Using the protein analysis software (Uniprot, entry Q4Q4U1) we observed more than 90% amino acid homology among DLD from pathogenic *Leishmania* species suggesting that targeting the *DLD* gene product will be a viable vaccination strategy against all forms of leishmaniasis. We generated *DLD* deficient (KO) *L. major* using the CRISPR-Cas9 gene-editing technology. We confirmed the deletion of the gene in the null mutant by PCR and Western blot. We assessed this deficiency on parasite proliferation in axenic culture and bone marrow-derived macrophages *in vitro*. Growth kinetics in axenic culture and macrophages, which are the primary target cells infected by

Leishmania parasites, show that deficiency of *DLD* gene products results in reduced proliferation in comparison to wild-type (WT) parasites. These findings indicate that DLD is an important metabolic enzyme of *Leishmania* and its deficiency results in impaired parasite proliferation in axenic culture and infected macrophages. Further *in vivo* studies will assess the disease pathology and impact on host immune response in infected mice.

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IN VITRO EFFICACY OF SELECTED ANTIMALARIALS AND OTHER ANTIPARASITIC COMPOUNDS AGAINST CIRCULATING SARS-COV-2 VIRUS IN PANAMA

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There is an urgent need for antivirals to treat COVID-19 a respiratory disease caused by the SARS-CoV2 virus that has caused millions of deaths around the globe. Even though at present several chemotherapeutic agents had been screened for activity against SARS-CoV2, the only one that had been approved by the FDA is remdesivir. Studies conducted in China early in the pandemic, demonstrated that chloroquine (CQ) and hydroxychloroquine, inhibit SARS-CoV-2 replication *in vitro* in African green monkey kidney cells (Vero-E6). Since then, thousands of compounds have been screened using various cell lines including Vero-E6 and human lung cancer (Calu-3) cells, the former that requires proteolytic cleavage of the S protein for viral entry through the action of an acid-dependent endosomal protease and in which CQ is active, or in the latter by a pH-independent extracellular plasma membrane-associated serine protease, in which CQ is inactive. In this study, we aim to compare the *in vitro* efficacy of a series of selected antimalarials and other antiparasitic compounds against SARS-CoV-2 variants circulating in Panama using these two different cell systems. For this purpose, we first screened twenty-three compounds for cytotoxicity in both cell lines using the methyl thiazolyl tetrazolium (MTT) assay to determine their 50% cytotoxic concentration (CC50). Then, to screen the compounds for their antiviral activity, cell monolayers grown in 48 well plates were infected with the A1-Panama and P1-Brazil SARS-CoV-2 variants and treated with ten-fold serial dilutions of the active compounds to determine their median effective concentration (EC50) and percent inhibition using qPCR and a plaque reduction assay as quantification methods. A sigmoidal concentration-response function was fit to the data using nonlinear regression to plot CC50 and EC50 of the compounds. We expect that the results of this ongoing study will provide evidence of *in vitro* activity of a series of selected antimalarials and other antiparasitic compounds against SARS-CoV-2 variants circulating in Panama.

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TRANSCRIPTOMIC ANALYSIS TO IDENTIFY CANDIDATE GENES ASSOCIATED TO PYRAZINAMIDE RESISTANCE IN MYCOBACTERIUM TUBERCULOSIS

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Multidrug-resistant tuberculosis (MDR-TB) threatens the progress towards the Global TB Program goals. New treatment schemes have been proposed against MDR-TB, including re-purposed or new drugs. Pyrazinamide (PZA), is a first-line drug used in resistant TB treatments due to its ability to eliminate semi-latent mycobacteria. However, its mechanism of action is not understood. Most PZA resistance is caused by mutations that hinder the pyrazinamidase activity, which is required to convert PZA into its active form. However, PZA resistant strains with a wild-type PZAse have been reported, indicating the involvement of other mechanisms. Identifying such non-canonical agents may require taking a holistic approach such as transcriptomics analysis. Our study sought to identify genes involved in PZA resistance by evaluating changes in the transcriptome of *M. tuberculosis* (MTB) in different stress states, dictated by pH (6.3 and 7.0) and PZA concentrations (0 and 50 µg/mL). Bacterial suspension were incubated at 37°C for 4 weeks, after which RNA was extracted and purified. Paired-end cDNA libraries were prepared and sequenced in Illumina's NextSeq 550 using 5 million read depth. Reads were aligned to the reference genome and abundances were quantified using Salmon. Differential expression analysis was done with the edgeR package, while GOseq was used to perform a gene enrichment analysis. Differentially expressed (DE) genes across conditions were identified using a $|logFC| > 2$ and p-value < 0.05 threshold. A total of 3979 MTB genes were observed. Drug concentration and pH comparison was done to identify up- and down-regulated genes. We found 13 DE genes of interest, which are involved in regulation of immune system processes, organic substance transport, macromolecule localization, organic compound biosynthesis, and other similar molecular pathways. We also found genes coding for PE/PPE proteins, which we believe could be of interest due to their pivotal role in bacteria survival under stress conditions, such as those generated by PZA intracellular activity.

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RESPIRATORY PATHOGEN DETECTION AMONG FEBRILE INPATIENTS IN NORTHERN TANZANIA, 2016-2019

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Febrile illness is a common reason for hospital admission in sub-Saharan Africa, yet the etiology of illness often remains unknown. Incorporation of respiratory pathogen testing, including viral pathogens, could contribute to filling knowledge gaps on etiologies and informing rational antimicrobial utilization among febrile patients with respiratory symptoms. From September 2016 throughMay 2019, we enrolled a prospective cohort of febrile pediatric and adult inpatients within 24 hours of admission to hospital. We sampled the nasopharynx and oropharynx (NP/OP) at enrollment using Dacron flocculated swabs and placed these swabs in universal transport media. Total nucleic acids were extracted (QIAGEN, QIAmp Virus) and subjected to multiplex polymerase chain reaction using the Luminex NxTAG Respiratory Pathogen Panel (RPP) for detection of 21 pathogens. The cohort included 930 enrolled participants— 432 (46.5%) were female, median (interquartile range) age was 22.3 (2.1-44.0) years, 378 (40.7%) met World Health Organization criteria for severe acute respiratory infection (SARI) and 331 (35.6%) had a non-SARI respiratory illness. A pathogen was detected by RPP in 359 (38.6%) of 930: 225 (59.5%) of 378 SARI, 97 (29.3%) of 331 of non-SARI respiratory illness, and 37 (16.7%) of 221 participants without respiratory symptoms. Among SARI, the most common pathogens were influenza A/B virus (n=71, 18.8%), rhinovirus/enterovirus (RV/EV, n=66, 17.5%), respiratory syncytial virus (n=37, 9.8%). Among non-SARI respiratory illness, the most common pathogens were RV/EV (n=39, 11.8%), adenovirus (n=18, 5.4%), and influenza A/B virus (n=17, 5.1%). Among 38 in-hospital deaths, 6 had a pathogen detected by RPP—RV/EV (n=3), and one each adenovirus, coronavirus OC43, and coronavirus 229E. These results demonstrate that RV/EV detection is associated with SARI and other febrile respiratory illnesses requiring hospital admission in northern Tanzania. The findings have implications for vaccine development and vaccine policy efforts aimed at decreasing morbidity and mortality from respiratory infections in low- and middle-income countries.

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SARS-COV-2 ANTIBODY PROFILES IN CONVALESCENT COVID-19 PATIENTS TESTING POSITIVE BY PCR AT POINT G TEACHING HOSPITAL, BAMAKO, MALI: A SURVEY PERFORMED FROM OCTOBER 2020 TO JANUARY 2021

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To understand antibody responses following SARS-CoV-2 infection in Mali, serum samples were collected between October 2020 to January 2021 from 23 convalescent individuals with previous COVID-19 confirmed by RT-PCR after completion of hospital quarantine. Samples were tested for IgG antibodies to SARS-CoV-2 spike protein and receptor binding domain (RBD) by enzyme-linked immunosorbent assay (ELISA). This study was performed as a positive control cohort as part of a public health surveillance activity in Mali to develop serological tools for use in Mali. Participants were aged from 7 to 74 years and 74% (17/23) were male. The time between RT-PCR diagnosis and collection of samples for ELISA ranged from 27 to 270 days. Disease severity ranged from asymptomatic (17% (4/23)) to critical (13% (3/23)) according to WHO stratification criteria. A single symptom was reported by 26% (6/23) of cases and 2 or more symptoms in 57% (13/23) of cases. This combination of low-acuity illnesses and variable time since infection is likely to be reflective of a community serosurveillance sample.SARS-CoV-2 spike protein and RBD reactivity were strongly correlated (r=0.72). Assay absorbance values were higher to spike protein compared to RBD (optical density 2.43±1.27 vs 1.72±1.23, p=0.004). For both antigens, reactivity was moderately correlated with time since diagnosis (spike r=0.56, RBD r=0.49) and weak to negligibly correlated with disease severity (spike r=0.17, RBD r=0.31 respectively). Using two-antigen assay cutoffs developed in the United States, 78.3% (18/23) of convalescent cases were considered seropositive. Using assay cutoffs developed for optimized performance in Mali, 73.9% (17/23) were considered seropositive. This study provides preliminary results on SARS-CoV-2 antibody responses in Malian convalescent volunteers, which will be critical to develop a reference ELISA for use in population serosurveillance.

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INCIDENCE AND HOUSEHOLD TRANSMISSION OF SARS-COV-2 IN A COMMUNITY COHORT IN PONCE, PUERTO RICO

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Most data on the COVID-19 epidemic in Puerto Rico (PR) and globally come from surveillance and clinical studies and do not capture the full disease spectrum and infection rates of SARS-CoV-2. To inform public health strategies for effective control of COVID-19, cohort studies are needed to investigate SARS-CoV-2 transmission at a community and household level. In June 2020, we implemented a cohort study for COVID-19 within the Communities Organized to Prevent Arboviruses (COPA) project platform among residents of 18 communities in Ponce, PR. Cohort participants provide weekly self-collected nasal swabs for SARS-CoV-2 polymerase chain reaction testing at an external facility and questionnaires to report acute symptoms and personal protective behaviors. Cohort enrollment began in July 2020 and was completed in February 2021 with 1,008 participants, of which 53% are female with median age 35 years (range 1–95). Among 373 households with one or more participants, 77% of all household members were enrolled. During 14,005 person-weeks of follow-up, enumerated through January 2021, we identified incident SARS-CoV-2 infections in 19 participants from 10 households for a community incidence rate of 1.4 per 1,000 person-weeks. Of these 19 participants, 58% were male, median age was 24 years (range 10–56), 10 (63%) sought medical care, and 16 (84%) had symptomatic infections, of which 81% reported cough, 81% loss of taste or smell, and 50% throat pain. All SARS-CoV-2 infections among participants of the same household were identified within 2 weeks of each other, and no re-infections were identified during follow-up. The estimated household attack rate was 33% among potentially susceptible household members. No secondary infections were detected in participating household members of the three asymptomatically infected participants. Preliminary study results showed moderate incidence of SARS-COV-2 in the community with mostly symptomatic infections and that about one in three exposed household members of index cases were infected with SARS-CoV-2. Results will be updated and expanded as follow-up for the cohort continues through February 2022.

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REASONS FOR CONSENTING TO PARTICIPATE IN COMMUNITY RESEARCH AIMED TO PREVENT TUBERCULOSIS

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The World Health Organization's (WHO) End TB strategy is focussed on intensifying research. However, evidence generated by research is influenced by those who agree to participate and can lead to consent bias. The objective of this study was to evaluate the reasons for providing consent or not in community research aimed to prevent TB.All patients starting TB treatment in 32 community health centres in Callao, Peru were approached in the clinic and invited to participate in the PREVENT TB study between July 2016 until November 2018 (N=2821). Callao state has one of the highest rates of TB and drug resistance in Peru (a WHO-defined TB high burden country). The study offered GeneXpert processing of sputum to all participants, and provided half of them with socioeconomic support within a community cluster randomised trial. Using a semi-qualitative questionnaire, the decision and reasons of patients with TB to participate in community research was recorded.There were 42 patients who were ineligible and 3 died before invitation, leaving 2775 eligible patients. In this population, 91% (2536/2775) of patients consented to the study, with significant heterogeneity between health centres (range 80-100%, p<0.001). The most frequently stated reason for participation was the GeneXpert PCR test (43%, 1088/2536), which was only available to this community through this research collaboration. The second most frequently stated reason for participation was the ability to help others affected by TB (17%, 418/2536). Within the 8.6% (239/2775)

eligible patients who did not consent, the most frequently stated reasons were: not having time (22%, 52/239,) or not wanting to give their personal information (21%, 49/239). In conclusion, to have equitable and representative samples in research we must understand the motivation and barriers to participation, mitigating the latter. In this setting, high frequency of participation was attributed to incentivisation and altruism, whereas reasons for declining participation were more diverse.

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IMPACT OF SARS-COV-2 IN PERIPHERAL BLOOD ON IMMUNE RESPONSES AND DISEASE SEVERITY IN HOSPITALIZED PATIENTS WITH COVID-19

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SARS-CoV-2 is a novel coronavirus which causes COVID-19, a disease ranging from asymptomatic to life-threatening. Production of a hyperinflammatory response, Cytokine Release Syndrome (CRS), is central to acute respiratory distress, heart and renal failure, liver damage, shock, and multiorgan failure in COVID-19 patients. Detection of SARS-CoV-2 in peripheral blood (viremia) is associated with increased disease severity. To identify molecular pathways for targeting by immunotherapy, we are conducting a prospective observational study (15 May 2020 to date) in hospitalized COVID-19 patients (n=336): intensive care unit (ICU) and non-ICU. Clinical and laboratory measures are obtained daily. Peripheral blood is collected on days 0, 1, 2, 3, 6, 9, and 14 to determine viral load dynamics and perform transcriptomics by next-generation sequencing. Results presented here examined the relationship between SARS-CoV-2 viremia and transcriptional profiles in a subset of patients (n=31). Correlation trend tests were used to determine the association between SARS-CoV-2 viral load at each timepoint (n=129) and gene expression levels. Enrichment analysis was performed using MetaCore™ with the threshold set at a correlation trend $P<0.05$ and signal intensity input as the correlation trend R (-1 to +1). Viremia was associated with elevated expression of Furin, ACE2, and Angiotensin II, a combination of events known to increase adverse reactions in the lungs, heart, kidneys, and blood vessels. Viremia was also associated with impaired antigen processing, as indicated by downregulation of TCRα/β, CD8, CD3, and CD4, along with MHC class II (e.g., decreased expression of 9 HLAs). Presence of SAR-CoV-2 in blood was further associated with elevated transcript levels of inflammatory mediators that promote CRS in COVID-19 (i.e., IL1RN, IL-10, IP10, MIG, CCL7, and GRO-2). Results presented here provide novel information about the impact of SARS-CoV-2 in peripheral blood on the immune response and illustrate potential causes for the relationship between viremia and enhanced disease severity in patients with COVID-19.

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SEVERE ACUTE RESPIRATORY SYNDROME - COVID-19: CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS IN THE TWO EPIDEMIC WAVES, INSTITUTO DE INFECTOLOGIA EMÍLIO RIBAS-IIER, SÃO PAULO, BRAZIL, 2020 AND 2021

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In December 2019, cluster of pneumonia cases in Wuhan-China was reported to the World Health Organization-WHO. Etiologic agent was identified as a new coronavirus, SARS-CoV-2. On March 11, WHO declared the pandemic of COVID-19 and since then, the disease spread worldwide, with a major impact on morbimortality. Social distance measures to reduce community transmission, although effective, also impact the economy. In the World, 140,332,386 cases of COVID-19 and 3,004,088 deaths were reported, data until April 18, 2021. COVID-19 is a mandatory reportable disease in Brazil within 24 hours after suspicion. We developed a prospective study of notified cases of severe acute respiratory syndrome-SARS, based on the epidemiological investigation form, collected in the routine of epidemiological investigation of the suspected cases of COVID-19 hospitalized in IIER. The information was entered into the database of the Ministry of Health, SIVEP-Gripe. From February 18 to December 30, 2020, 1712 suspected cases of SARS were reported, with 928 (54.2%) laboratory confirmed, 96.5% by RT-PCR in nasopharyngeal secretion. Regarding confirmed cases, 58.6% concentrated in the male gender and 66.3% in the white race. The age group from 15 to 60 years old corresponded to 51.6% of the cases and 60 years old or more to 48.3%. 50.1% of the cases were attended in the ICU and 34.7% with the use of mechanical ventilation. Hospital lethality was 26.3%, with 16.3% in the 15 to 60 age group and 36.9% in the elderly aged 60 or over. Until March 22, 2021, 275 suspected cases were reported, with 178 (64.7%) laboratory confirmed, 98.7% by RT-PCR in nasopharyngeal secretion. Regarding confirmed cases, 60% concentrated in the male gender and 66.1% in the white race. The age group from 15 to 60 years corresponded to 53.8% and 46.2% in over 60 years of age. 55.2% of the cases were attended in the ICU and 30.1% with use of mechanical ventilation. Hospital lethality was 25.1%, with 22.9% in the 15 to 60 age group and 27.8% in the elderly aged 60 or over. Comparing the two epidemic waves there was a statistical difference in the lethality of age groups (15 to 60 and ≥ 60 years), $p = 0.05$ and 0.048, respectively.

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LATE SURGES IN COVID-19 CASES AND VARYING TRANSMISSION POTENTIAL DUE TO PUBLIC HEALTH POLICY CHANGES IN FIVE MIDWEST STATES, MARCH 10, 2020-JANUARY 10, 2021

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North Dakota, South Dakota, Montana, Wyoming, and Idaho experienced low transmission early in the COVID-19 pandemic. This study aims to estimate the area's SARS-CoV-2 transmission potential from March 2020

through January 2021. SARS-CoV-2 time-varying reproduction numbers, R_t, of a 7-day-sliding-window and of non-overlapping-windows between policy change were estimated using the instantaneous reproduction number method developed by Cori et al. and daily number of new cases. The power-law relationship was evaluated with linear regression between the $\log_{10}$-transformed cumulative case number and $\log_{10}$-transformed population size. The median R_t estimates for 7-day-sliding-window across this region were between 1-1.25 during September through November. Between November 13th and 18th North Dakota had a reduction of R_t estimates by 14.71% ($\bar{x}$ = -0.1396, CI [-0.1454, -0.1338]) following a mask mandate , Idaho saw a 1.93% ($\bar{x}$ = 0.0729, CI [0.0501, 0.0956]) reduction and Montana saw a 9.63% ($\bar{x}$ = -0.0868, CI [-0.1015, -0.0720]) reduction of R_t estimates following the tightening of restrictions. Neither Wyoming nor South Dakota implemented any policies or restrictions; however, Wyoming saw an average of 12.12% ($\bar{x}$ = -0.0086, CI [-0.0310, 0.0137]) reduction, and South Dakota saw an average of 5.02% ($\bar{x}$ = 0.0394, CI [0.0135, 0.0653]) increase in R_t estimates. Evidence suggests that high-population counties had higher cumulative case number per-capita in North Dakota at four time points (m=0.2758, 0.2171, 0.0729, 0.0986; p=0.0034, 0.0018, 0.046, 0.0024; respectively). R_t decrease after facemask mandate during the region's case count spike suggests a greater reduction in community transmission of SARS-CoV-2.

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DNA METHYLATION PATTERNS OF CIRCULATING IMMUNE CELLS AS A POTENTIAL TOOL IN THE DIAGNOSIS OF TUBERCULOSIS

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Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* (MTB) and continues to remain a serious health issue worldwide. Timely diagnosis of tuberculosis is a major problem therefore, it is necessary to find new approaches which also contribute to understand the course of MTB infection. In the present pilot study, we have compared the DNA methylation profiles of peripheral blood mononuclear cells (PBMCs) isolated from five healthy individuals, five patients with latent infection (LTBI), and ten with active tuberculosis (ATB) from Lima, Peru. We utilized the DNA methylation profiles in order to identify potential epigenetic signatures that can serve as diagnostic tools among no infection, latent and active tuberculosis. PBMCs were cryopreserved in liquid nitrogen, thawed and DNA extracted using a commercial kit. Genome-wide DNA methylation analysis of the PBMC samples was performed using Illumina Infinium Methylation EPIC 850K array. We identified differentially methylated CpGs (DMCs), with FDR adjusted p-value of 0.05 and a delta beta value > 0.2. A total of 939 DMCs were identified in our 20 samples from LTBI, ATB, and healthy controls. In addition, combining the identified differentially methylated genes (DMGs) from the PBMCs in a Venn analysis, we discovered one common DMG which could serve as a potential biomarker. Pathway enrichment analyses using the KEGG, Reactome, and Panther databases showed Wnt signaling, TGF-beta signaling among the top pathways in ATB and healthy controls enriched genes, we also observed statistically significant pathways related to the nervous system in LTBI and ATB samples. Further analysis of DMCs and DMGs and their notable involvement in specific pathways can open a new horizon in the diagnosis and prevention of tuberculosis disease.

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SARS-COV-2 SEROASSAY OPTIMIZATION AND PERFORMANCE IN A POPULATION WITH HIGH BACKGROUND REACTIVITY IN MALI

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In Mali the first case of SARS-CoV-2 infection was identified in March 2020. Due to a limited access to gold-standard molecular diagnostics, qualified serological assays are urgently needed to conduct serosurveys to understand population exposure to the virus, the antibody response to infection, and help direct Public Health interventions. However, poor assay performance may hinder the utility of these tests, including high rates of false-positivity previously reported in sub-Saharan Africa. From 312 Malian samples collected prior to 2020, we measured antibodies to the commonly tested SARS-CoV-2 antigens and four other betacoronaviruses by ELISA, and assessed functional cross-reactivity in a subset using a SARS-CoV-2 pseudovirus neutralization assay. We then evaluated the performance of a two-antigen ELISA developed in the US, using SARS-CoV-2 spike protein and receptor-binding domain. To optimize test performance, we compared single and two-antigen approaches using existing assay cutoffs and population-specific cutoffs for Malian control samples (positive and negative). Background reactivity to SARS-CoV-2 antigens was common in pre-pandemic samples compared to US controls. SARS-CoV-2 reactivity correlated weakly with other betacoronavirus reactivity, varied between Malian communities, and increased with age. No pre-pandemic samples demonstrated functional activity. Regardless of the cutoffs applied, specificity improved using a two-antigen approach. Test performance was optimal using a two-antigen assay with population-specific cutoffs derived from ROC curve analysis [Sensitivity: 73·9% (51·6-89·8), Specificity: 99·4% (97·7-99·9)]. In the setting of high background reactivity such as sub-Saharan Africa, SARS-CoV-2 serological assays need careful qualification to characterize the epidemiology of disease, prevent unnecessary harm, and allocate resources for targeted control measures. This optimization has allowed testing of almost 8000 samples at two time points for use in Public Health serosurveillance in collaboration with the Malian MoH.

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COMPARISON OF THE URINE POINT-OF-CARE CIRCULATING CATHODIC ANTIGEN ASSAY TO THE STANDARD KATO KATZ STOOL TEST IN A SCHISTOSOMIASIS CONTROL PROGRAM IN WESTERN KENYA

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Schistosomiasis control program guidelines for evaluation and monitoring are based on the Kato Katz (KK) fecal exam method, but sensitivity may suffer in low prevalence settings. The point of care urine circulating cathodic antigen assay (POC-CCA) has been used as an alternative test to map schistosomiasis prevalence but has not been evaluated for use in monitoring and evaluating control programs. This repeated cross-sectional study sought to determine how guidance based on KK can be translated to POC-CCA and its performance in different prevalence settings. Urine

and stool specimens were collected from public primary school students in western Kenya prior to MDA at baseline and annually thereafter for up to 3 years. Prevalence and infection intensity were determined by KK and POC-CCA; changes were compared within strata of schools grouped according to baseline KK prevalence ("low" 0-10%, "medium" >10-20%, "high" >20%) using negative binomial models. Prevalence by POC-CCA was higher than by KK at all time points for all strata. Prevalence by KK decreased significantly for all strata from baseline to year 2 (low: prevalence ratio [PR]=0.58 (95% confidence interval 0.39, 0.87) p<0.01; medium: PR=0.32 (0.18, 0.58) p<0.01; high: PR=0.53 (0.37, 0.75) p <0.01) and year 3 (low: PR=0.59 (0.36, 0.97) p<0.05; medium: PR=0.2 (0.12, 0.33), p<0.01; high: PR=0.33 (0.25, 0.44) p<0.01) as did infection intensity at years 2 and 3 for medium (arithmetic mean ratio [AMR]=0.18 (0.1, 0.34) p<0.01; AMR=0.34 (95% CI 0.15, 0.79) p=0.01, respectively) and high (AMR=0.56 (0.41, 0.77) p<0.01, AMR=0.11 (0.08, 0.15) p<0.01, respectively) prevalence strata schools. A corresponding decrease by POC-CCA was not always replicated for any of the strata and even increased in some years. Concordance between the two tests was poor; 2.8% (n=356) of POC-CCA results were false negative compared to KK and the highest rate of false negatives occurred among high prevalence schools. The POC-CCA did not perform as expected which raises questions concerning the POC-CCA's usefulness for monitoring schistosomiasis control programs in its current format and/or quality control management.

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ASSOCIATION OF FEMALE GENITAL SCHISTOSOMIASIS WITH THE CERVICOVAGINAL MICROBIOTA AND SEXUALLY TRANSMITTED INFECTIONS IN ZAMBIAN WOMEN

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Female genital schistosomiasis (FGS), caused when eggs from the waterborne parasite *S. haematobium* are entrapped in genital tissue, is prevalent in sub-Saharan Africa and has been associated with adverse reproductive outcomes, including ectopic pregnancy, infertility, and prevalent HIV-1. Non-optimal cervicovaginal microbiota, characterized by a shift from lactic acid producing microbiota (e.g. Lactobacillus species) to an increase in anaerobic species can likewise have negative sexual and reproductive health consequences. The cervicovaginal microbiota, including sexually transmitted infections (STI), have not been well-described in PCR-defined female genital schistosomiasis (FGS). Women who were aged 18-31, sexually active, and not pregnant were invited to participate at the final follow-up of the HPTN 071 (PopART) Population Cohort in two communities in Zambia from January - August 2018. We measured key species of the cervicovaginal microbiota (*Lactobacillus crispatus*, *L. iners*, *Gardnerella vaginalis*, *Atopobium vaginae* and *Candida* spp.) and STI (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis* and *Mycoplasma genitalium*) using PCR. We evaluated associations between the presence and concentration of microbiota and STI and FGS (PCR-detected *Schistosoma* DNA in any of three genital specimens). The presence and concentration of key cervicovaginal species did not differ between participants with (n=30) or without (n=158) PCR-defined FGS. A higher proportion of participants with FGS had *T. vaginalis* compared to FGS negative women (p=0.08), with further analysis showing that *T. vaginalis* was more prevalent among women with ≥2 *Schistosoma* PCR positive genital specimens (50.0%, 8/16) than among FGS negative women (21.5% 34/158, p=0.01). In conclusion, we found weak evidence of an association between *T. vaginalis* presence and FGS, with a stronger association in women with a higher burden FGS infection. Additional research is needed on potential between-parasite interactions, especially regarding HIV-1 vulnerability.

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FEMALE GENITAL SCHISTOSOMIASIS AND HIV-1 INCIDENCE IN ZAMBIAN WOMEN: A RETROSPECTIVE COHORT STUDY

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In sub-Saharan Africa, there has been a geographical association between areas of high *S. haematobium* prevalence and HIV-1 infection. Female genital schistosomiasis (FGS) has been associated with prevalent HIV-1, though data both support and refute this association. While *S. haematobium* seropositivity has been associated with HIV-1 acquisition, the association of FGS with incident HIV-1 has not yet been described. In this study, we estimated the incidence of HIV-1 infection in Zambian women with and without FGS. Women who were aged 18-31, sexually active and not pregnant were invited to participate in this study at the final follow-up of the HPTN 071 (PopART) Population Cohort in two communities in Zambia from January – August 2018. HIV-1 negative participants at enrolment (n=492) were included in this analysis with testing to confirm incident HIV-1 performed in HPTN 071 (PopART). Association of incident HIV-1 infection with FGS (*Schistosoma* DNA detected by PCR in any genital specimen) was assessed with exact Poisson regression. Incident HIV-1 infections were observed in 4.1% (20/492) participants. Women with FGS were twice as likely to seroconvert as women without FGS but with no statistical evidence for a difference (aRR 2.16, 95%CI[0.21–12.30], p=0.33). Exploratory analysis suggested an association with HIV-1 acquisition among women with ≥2 positive genital PCR specimens (RR 6.02, [0.58–34.96]), p=0.13). Despite higher HIV-1 seroconversion rates in women with FGS, there was no statistical evidence of association, possibly due to low power. Large prospective studies in areas of higher *S. haematobium* endemicity are needed to evaluate associations with HIV-1 incidence in women.

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IMPLICATIONS OF OVERCOMING EXISTING CHALLENGES IN MAPPING SCHISTOSOMIASIS: THE EXPERIENCE OF RWANDA

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Schistosomiasis is a focal neglected tropical disease (NTD) where endemic communities are those surrounding water bodies and wetlands. It is among NTDs targeted for elimination as a public health problem. Most endemic countries have mapped it and implemented several rounds of preventive chemotherapy to control associated morbidity and aim for elimination following the WHO roadmap on NTDs 2021-2030. The latter recognizes the need for innovative mapping methods and tools to get a granular view of schistosomiasis epidemiology to focus interventions at the right risk areas. Rwanda's NTD program aimed to fill the actual global gap using a novel locally designed sampling methodology. In 2020, the Rwanda national NTD program used an innovative sampling methodology, using a rigorous scientific and collaborative design defining communities presumed at risk as ones adjacent to water bodies and wetlands delineated using GIS tools, further rankedtaking into account

available epidemiological and socio-economic data and local knowledge. The community-based mapping involved all age groups from children aged 1 to adults. Kato-Katz and Point-Of-Care Circulating Cathodic Antigen tests were used. The present mapping data indicate endemicity levels requiring regular mass treatment in 1013 administrative cells (current implementation units below the sub-districts), distributed in 307 sub-districts and all 30 districts of the country, with a total of 4.6 million school age children and adults estimated to be at risk. The experience of schistosomiasis mapping in Rwanda shows the benefits of an improved sampling design to identify all communities at risk of schistosomiasis to know where control and elimination efforts need to be focused. In the previous mapping, which used different sampling designs, endemicity was only found in 127 sub-districts of 22 districts. Since the disease transmission remains focalized and very dynamic in space and time, technical and financial resources must be considered along with other priorities set in the new WHO roadmap to obtain accurate data to help plan elimination.

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BEHAVIORAL RESPONSES TO A SEASONAL ENVIRONMENT REDUCE FREQUENCY BUT INCREASE EXTENT OF WATER CONTACT IN A SCHISTOSOMIASIS-ENDEMIC REGION OF WEST AFRICA

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A variety of seasonal processes govern infectious disease transmission, ranging from fluctuations in pathogen density in the environment to seasonal changes in human behavior. In seasonal climates, snail ecology is an important driver of seasonality in schistosomiasis transmission dynamics. Seasonal fluctuations in temperature, rainfall and water levels also influence the water contact behaviors that expose people to schistosomes. Yet, the extent to which seasonal changes in water contact behavior overlap with seasonal peaks in snail and parasite densities is poorly understood. Along the Senegal River, schistosomiasis has become endemic in a highly seasonal Sahelian environment. We report qualitative data from focus group discussions (n = 12) with adult men, adult women and youth in four villages in this setting, examining how perceptions of the local environment and its seasonality affect how people interact with freshwater resources. Focus group participants (n = 76) associated the risk for schistosomiasis with seasonal changes in temperature and water quality and reported reducing certain water contact behaviors during the rainy season, when they perceived water quality to be low. Participants similarly noted that rainy season increases in water levels affects farmers' ability to cultivate land near shore while others reported that the combination of reduced water quality and high water levels increased the depth to which it was necessary to wade to collect water clear enough for household use. These competing behavioral responses to seasonal changes in the environment make it difficult to discern the net impact of seasonality on human water contact. During the rainy season, when schistosome transmission peaks, people reduce their frequency of occupational and hygienic water contact but also increase the extent of contact necessary to meet household needs. Focus group data revealed broad patterns in the seasonality of human water contact behavior, how it intersects with seasonality in snail and schistosome ecology and where opportunities exist to ameliorate the tension between livelihoods and health in this setting.

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LONGITUDINAL ANALYSIS OF OCCUPATIONAL EXPOSURE TO SCHISTOSOMA JAPONICUM THROUGH IRRIGATED AGRICULTURE IN A SITE OF REEMERGENT AND PERSISTENT TRANSMISSION IN SICHUAN, CHINA

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Schistosomiasis reemerged in Sichuan Province, China in 2004 after decades of multifaceted control efforts. In hilly regions of the province, irrigated agriculture is commonly practiced. The cultivation of rice and corn dominate crops grown during the summer months, though vegetables and peanuts are also grown. Because the cultivation of rice involves flooding fields and labor that is often performed barefoot, we hypothesized that the rice planting season may be a key window of exposure. Using longitudinal infection and household survey data collected in seven villages at three points (2007, 2010 and 2016) since the time since reemergence of *S. japonicum* was detected in 2006, we examine the relationship between irrigated agriculture reported at the household level as a predictor of *Schistosoma japonicum* infection status. In this time, the socio-economic conditions in these villages have improved substantially but agriculture has remained an important economic activity. Controlling for age, sex, socio-economic status, number of hatch tests performed and random intercepts accounting for clustered observations by village, we fit generalized linear mixed models of infection status as a function of binary, categorical and continuous indicators of household rice cultivation for each year of observation. We found that among individuals in households cultivating any amount rice were more likely to be infected in 2007 (OR = 1.94, 95% CI 1.05, 3.57) but not in later years. Similarly, the odds of infection were greater among individuals in households that were cultivating small (0-1 mu; OR = 3.11, 95% CI 1.25, 7.79) and large (2+ mu; OR = 2.80, 95% CI 1.03, 8.59) areas of rice in 2007 compared to individuals in households that were not cultivating any rice. This association was not detected in 2010 or 2016. In 2016, the area of rice was associated with a 52% reduction in the odds of infection (OR = 0.48, 95% CI 0.25, 0.91). Our findings suggest that occupational exposure to *S. japonicum* through agricultural activity has decreased since re-emergence, and may be the result of improved socio-economic conditions and the diversification of livelihoods in the region.

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MALARIA AND HELMINTH COINFECTIONS AMONG CHILDREN LIVING IN LOW AND MIDDLE INCOME COUNTRIES: A SYSTEMATIC REVIEW WITH META-ANALYSIS

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Current knowledge on the burden of, and interactions between malaria and helminth co-infections, as well as the impact of the dual infections on anaemia, remains inconclusive. We conducted a systematic review with meta-analysis to update current knowledge as a first step towards developing and deploying coordinated approaches to the control of malaria-helminth co-infections among children living in LMIC. Using PRISMA guidelines, two reviewers independently searched Medline,

Embase, Global Health and Web of Science from database inception until 16 March 2020 for peer-reviewed articles. No language restriction was applied. We used the summary odds ratio and 95% CI as a measure of association (random-effects model), performed Chi-square heterogeneity test and evaluated the severity of heterogeneity using I^2 statistics. The included studies were examined for publication bias using a funnel plot and Egger's test. Fifty-five of the 3,507 citations screened were eligible, 28 of which had sufficient data for meta-analysis. The 28 studies enrolled 22,114 children in 13 countries across SSA, Southeast Asia and South America. Overall, the pooled estimates showed a prevalence of *Plasmodium*-helminth co-infections of 17.7% (95% CI 12.7-23.2%). Fourteen studies showed a lower odds of *P. falciparum* infection in children co-infected with *Schistosoma* spp (OR: 0.65; 95%CI: 0.37-1.16), while 24 studies showed similar lower odds of *P. falciparum* in children co-infected with STH (OR: 0.42; 95%CI: 0.28-0.64). Sixteen studies showed that the odds of anaemia were higher in children co-infected with *Plasmodium* and STH than in children with *Plasmodium* infection alone (OR= 1.20; 95% CI: 0.59-2.45), and were almost equal in children co-infected with *Plasmodium-Schistosoma* spp or *Plasmodium* infection alone (OR= 0.97, 95% CI: 0.30-3.14). This review suggests that the prevalence of malaria-helminth co-infection is very high and vary widely in children living in LMIC. Geo-spatial tools may be needed to generate better understanding of the burden of the co-infection, which is fundamental to the development of appropriate interventions for integrated control.

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FACTORS ASSOCIATED WITH KNOWLEDGE OF SCHISTOSOMIASIS IN CHILDREN AND ADULTS IN THE KALABANCORO HEALTH DISTRICT IN 2020

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Schistosomiasis continues to be a public health problem in many parts of the world, particularly in Africa. In terms of morbidity, it is the most devastating parasitic disease after malaria. It is still endemic in 74 countries and affects 261 million people worldwide with nearly 800 million people at risk. It is estimated that more than 90% of these cases come from sub-Saharan Africa. In Mali in 2019, the overall prevalence rate was 30%. In Kalabancoro, during 2017 assessments, the National Schistosomiasis and Geo-Helminthiasis Control Program reported a urinary schistosomiasis prevalence of 10.83% and an intestinal schistosomiasis prevalence of 50.83%. This district recorded the highest rate for intestinal schistosomiasis among the 46 districts evaluated. To better understand this high prevalence, this study was initiated. To study the knowledge of children and adults about schistosomiasis in the health district of Kalabancoro. A cross-sectional study was conducted from May to November 2020. A two-level cluster sampling (villages and households) was conducted. Thirty villages were randomly selected from the 117 villages in the district and 947 willing participants were surveyed using a questionnaire. Univariate analysis and binary logistic regression were performed using the Statistical Package for Social Sciences. During the study, 76.1% of participants claimed to know about schistosomiasis; among them, 85.6% did not know the mode of contamination (p=0.001), 66.3% knew the traditional treatment (p=0.004), and 70% reported knowing how to avoid the disease (p<0.01). Variables such as gender (3.47 [2.48-4.85]), age (2.72 [1.94-3.82]), and household near a water reservoir (2.15 [1.49-3.11]) were statistically associated with knowledge of schistosomiasis. The majority of respondents claimed to know about schistosomiasis. However, details about the cause, prevention and treatment of schistosomiasis were not well known. It is important to educate communities about the mode of transmission, prevention and treatment of schistosomiasis and to implement interventions that can reduce the level of transmission.

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THE MEASURING OF TREATMENT COVERAGE FOR SCHISTOSOMIASIS WITH PREVENTIVE CHEMOTHERAPY IN BONG, LOFA AND NIMBA COUNTIES IN 2018 IN LIBERIA

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Schistosomiasis or Bilharzia is a parasitic disease caused by infection with the trematode blood-flukes schistosomes. In sub-Saharan Africa, two major forms of human schistosomiasis occur intestinal, schistosomiasis caused by mainly *Schistosoma mansoni* infection and urinary schistosomiasis due to *Schistosoma haematobium* infection. Schistosomiasis is among the neglected tropical diseases (NTDs), which remain serious public health problems, posing unacceptable threats to human health. The World Health Assembly resolution 54.19 urges all member states to regularly treat at least 75% of all school-aged children are at risk of morbidity from schistosomiasis with Praziquantel (PZQ). To determine if these global goals are being reached, each national program *routinely reports* drug coverage. This metric is calculated using the number of treatments distributed during a round of Preventive Chemotherapy (PC) recorded in treatment registers and/or tally sheets for the numerator, and population figures (often obtained from routine census figures) as the denominator. In order to monitor and support NTD program performance, independent drug *coverage surveys* are recommended by the World Health Organization. These coverage surveys should be carried out across all areas given PC, particularly at crucial time points during the programs i.e. in the first year of the program, in cases where coverage might be suspiciously high or low, to ensure any corrective actions where needed. The result of the survey assists in the identification of recommended actions to improve program delivery. The aim of the coverage survey was to evaluate the effectiveness of PC in reaching the target population in endemic counties. The SCH categories of each county are based on the data collected in the 2012 baseline survey, which was carried out prior to the first MDA in Liberia which shows that schistosomiasis is prevalent in 13 counties with the exception of Rivercess and Grand Kru. During the survey number of interviewed children was low. 651 children/ 1023 adults. The survey contains 14 villages per county (42 in total), 15 Households. PZQ coverage for adults and SAC was 75%.

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DIFFERENTIAL SUSCEPTIBILITY OF BOVINES TO SCHISTOSOMA JAPONICUM IN A CHALLENGE INFECTION MODEL

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Schistosomiasis japonica is an endemic, zoonotic parasitic disease. In the Philippines, water buffalo (carabao, *Bubalus bubalis*) is a primary transmission source. To identify the optimal vaccination-challenge protocol for the schistosomiasis japonica vaccine, we evaluated factors that may affect worm recovery rate by perfusion. Factors included host species (*Bubalus bubalis* versus *Bos indicus*), age, pretreatment with praziquantel, trickle vs. bolus exposure to cercariae, and the timing of perfusion. Eighteen carabao and three cattle were purchased (groups A to G, n=3) from an *Schistosoma japonicum* non-endemic region and maintained on non-endemic pasture during the four-month study (Oct, 2020 to Jan, 2021). Bovine health was monitored daily by veterinary technicians and weekly with a complete physical exam by a veterinarian. Six groups of carabao and one group of cattle were assigned to different infection-perfusion conditions to identify the optimal infection protocol. All animals were verified as uninfected with *S. japonicum* by PCR at the start of the study. Surprisingly, a majority of animals showed high antibody levels to schistosome specific crude antigens, including ES, SEA, and SWAP, and recombinant full-length paramyosin (rSj97), likely due to prior Fasciola infection. Among the study groups, we found marked heterogeneity in worm recovery rates. Specifically, worm recovery rates were highest in groups C (trickled infection, total dose 3,000 cercariae), F (juvenile 1-1.5yr carabao, 1000 cercariae), and G (Cattle, 500 cercariae). Notably, a worm recovery rate of ~10% is sufficient for vaccine efficacy evaluation, and this level was achieved in three out of the seven groups. We concluded that juvenile carabao with the trickled dose of cercariae infection is the optimal vaccination-challenge model for vaccine trials with carabao in the Philippines and have initiated a trial of rSj97 and rSj6-8 using this protocol.

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GLOBAL BURDEN OF SCHISTOSOMIASIS 1990-2020: A MODELLING ANALYSIS FOR THE GLOBAL BURDEN OF DISEASE STUDY 2020, RELEASE 1

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Schistosomiasis is one of the most common parasitic diseases in the world. The World Health Organization set a goal to eliminate schistosomiasis as a public health problem by 2025. We aim to describe the burden of schistosomiasis in the world from 1990 to 2020. We estimated the burden of schistosomiasis with data from 5 species (*S mansoni, S haematobium, S japonicum, S mekongi, S intercalatum*). To estimate deaths, we used a negative binomial regression model of country-year-age-sex-specific deaths, using vital registration and verbal autopsy as sources. To model non-fatal outcomes, we ran a DisMod MR 2.1 model to estimate prevalence among the population at risk, which was then further adjusted to account for total population. We present preliminary results here. Results for GBD 2020, release 1 will be finalized in summer 2021. We estimated 12,500 (95%UI: 10,900-14,000) deaths globally in 2020. The age-standardized death rate in 2020 was 0.15 (95%UI: 0.13 - 0.17) per 100,000, 64% lower than in 1990. In 2020, it also was 25% higher in males than females. Countries in sub-Saharan Africa showed the highest mortality, Central African Republic (6.2 [95%UI: 4.4 - 8.7] deaths per 100,000) and Somalia (5.5 [95%UI: 3.9 - 7.2] deaths per 100,000) had the highest age-standardized mortality rates. Globally, we estimated 155 (95%UI: 118 - 199) million prevalent cases of schistosomiasis in 2020. The age-standardized prevalence rate was 1,989 (95%UI: 1,511 - 2,523) per 100,000 in 2020, 19% lower than in 1990. In terms of DALYs, we estimated a 39% decrease in the age-standardized rate, from 35.2 (95%UI: 24.6 - 54.7) per 100,000 in 1990 to 21.4 (95%UI: 12.7 - 37.8) in 2020. Mauritius, Central African Republic, and Nigeria had the highest age-standardized DALYs rates: 289.1 (95%UI: 126 - 593), 284.2(95%UI: 126 - 594), and 214.6 (95%UI: 119 - 393) DALYs per 100,000, respectively. These findings show progress made in the burden of schistosomiasis over the past 30 years. However, progress in mortality was much greater than in morbidity. The GBD estimates for schistosomiasis can serve as a resource for policymakers to track progress and identify priorities for interventions.

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THE PERSISTENCE OF SCHISTOSOMIASIS IN A LARGE METROPOLITAN AREA OF BRAZIL. RESPONSE OF SCHISTOSOMA MANSONI POPULATION STRUCTURE TO A CHANGING URBAN ENVIRONMENT

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Saramandaia, a poor urban neighborhood in Salvador, Brazil is marked by a series of streams and large, semi-commercial vegetable gardens. Most of these gardens have recently been removed for new construction and housing. We evaluated *Schistosoma mansoni* population structure and persistence before (2018) and after (2019) these interventions coupled with praziquantel treatment. In 2018 and 2019, we conducted sociodemographic and parasitological surveys and collected up to 3 stool samples on different days. Positive individuals by Kato-Katz were treated with praziquantel and reexamined four weeks later. Egg DNA from stools was genotyped for 10 microsatellites. Allele frequencies for infrapopulations and component populations were used to measure differentiation and effective population size (Ne). Between 2018 and 2019 prevalence reduced from 5.7% to 2.8% (p=0.001). Intensity of infection remained constant at 64.0 epg in 2018 and 74.2 epg in 2019. Risk factors for infection were male sex (OR=2.5; p<0.001), occupational contact with surface water (OR=1.7; p=0.046), and use/access to water at 2 specific points in the community (OR=2.2; p=0.005). In 2018, Di (mean pairwise differentiation of infrapopulations) was high (0.228) and higher in 2019 (0.297, Cohen´s D=0.462). Similar results were seen evaluating sex, age, and access/contact with the 3 water contact points in the community. Dc (mean differentiation of the whole population 2018 vs 2019) was moderate between the 2 years (0.077). However, incident cases and newly enrolled individuals were highly differentiated from the starting population of 2018 (0.220 and 0.261, respectively). Ne in 2018 was 3,052 and decreased by 30% in 2019. One round of praziquantel treatment coupled with structural intervention was relatively effective in reducing prevalence, however, little effect was seen on parasite´s population structure. This may be due in part to only being able to treat ~10% of the population and the continuous nature of housing in urban areas. Treatment and sanitation of a much wider population will be necessary to significantly impact the biological potential of *S. mansoni* in this locality.

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RESULTS FROM A HYBRID IMPLEMENTATION AND EFFECTIVENESS PILOT STUDY TO ASSESS THE FEASIBILITY, ACCEPTABILITY AND PROTECTIVE EFFECT OF SEASONAL MALARIA CHEMOPREVENTION IN NAMPULA PROVINCE, MOZAMBIQUE

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Malaria is a significant cause of morbidity and mortality in children under five in Mozambique. The World Health Organization recommends seasonal malaria chemoprevention (SMC): the administration of monthly courses of sulfadoxine-pyrimethamine (SP) and amodiaquine (AQ) (SPAQ) to children aged 3-59 months during the peak transmission season. Due to widespread prevalence of markers associated with SP resistance in East and Southern Africa, the implementation of SMC has so far been well documented across the Sahel zone of West and Central Africa. During November 2020 to February 2021, Ministry of Health National Malaria Control Programme in collaboration with Malaria Consortium, for the first time, implemented SMC in two districts in Nampula province, Mozambique. We conducted a hybrid implementation and effectiveness study where 2 districts received the intervention and one district acted as the control to understand the: 1) adaption of SMC to the Mozambique context; 2) acceptability and feasibility of the intervention based on interviews and focus group discussions; 3) coverage and quality of SPAQ delivery using a cross sectional end-of-round survey; 4) baseline prevalence of SP and AQ resistance associated genotypes (including Pfdhps, Pfdhfr, Pfcrt and Pfmdr1 mutants) and subsequent follow up to determine if selection pressure alters these prevalence measures; and finally 5) reduction in odds of clinically-significant malaria outcomes associated with receipt of SPAQ based on a non-randomised controlled trial with 400 eligible children each in the two intervention districts and a third control district. In brief, initial results showed that SMC was successfully adapted and accepted by stakeholders. Coverage among eligible children in terms of administration of Day 1 SPAQ was 84.0% (95% CI: 82.1-85.8). Of the children recruited to the trial, 64 developed clinical malaria in the intervention period versus 191 in the control arm; we estimated a protective effect of around 80%. The session will describe the complete findings from these studies on the acceptability, feasibility and protective effect of SMC implementation in this context.

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BUILDING DATA INTEGRATION AND VISUALIZATION PLATFORMS TO HELP EMERGENCY OPERATION CENTERS MONITOR MALARIA DURING PUBLIC HEALTH EMERGENCIES - EXPERIENCE FROM THE DEMOCRATIC REPUBLIC OF THE CONGO

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The potential impact of COVID-19 on malaria morbidity and mortality highlighted the need to track malaria trends in near real time and design integrated sustainable interoperable data systems to improve malaria surveillance. Emergency Operations Centers (EOCs) are often geared towards fast response to emerging crises, but the long-term monitoring of a multiplicity of concurrent public health challenges is harder to maintain. Creating platforms for data integration and visualization systems for malaria surveillance, as well as for the coordination, planning, and monitoring of malaria campaigns represents a unique opportunity to reinforce EOC capacity and provide a model for their sustainability. We engaged with the Ministry of Health in the DRC to support its EOC's engagement to respond to COVID-19, all the while strengthening malaria burden reduction efforts and increasing the EOC's efficiency in responding to other emergencies. To support DRC's COVID-19 response, DHIS2-based digital tools to collect and integrate data streams into a single warehouse for COVID-19 data management were adopted. DHIS2 modules were created to enable health workers to track and investigate COVID-19 cases and health service usage; operational and epidemiological dashboards to follow the epidemic and inform the response were created. In collaboration with the National Malaria Control Program, we carried out an assessment of existing malaria data sources and gaps. Malaria dashboards to monitor key indicators, as well as service accessibility and malaria logistic indicators were developed and are now available and used by the National Malaria Control Program. A separate dashboard to track morbidity and mortality data for 21 diseases with epidemic potential, including COVID and malaria, was developed to reinforce the EOC's capacity to monitor multiple diseases. We are now supporting the optimization of outbreak detection algorithms and developed a training curriculum for technical focal points and decision-makers, as well as an online training platform to build capacity and reinforce data use for decision-making.

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EFFECTIVENESS OF A NATIONAL MASS DISTRIBUTION CAMPAIGN OF LONG-LASTING INSECTICIDE-TREATED NETS AND INDOOR RESIDUAL SPRAYING ON CLINICAL MALARIA IN MALAWI, 2018-2020

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Malawi's malaria burden is primarily assessed via cross-sectional national household surveys. However, malaria is spatially and temporally heterogenous and no analyses have been performed at a sub-district level throughout the course of a year. The World Health Organization recommends mass distribution of long-lasting insecticide-treated bed nets (LLINs) every three years, but a national longitudinal evaluation has never been conducted in Malawi to determine LLIN effectiveness lifespans. Using District Health Information Software 2 (DHIS2) health facility data, available from January 2018 to June 2020, we assessed malaria risk before and after a mass distribution campaign, stratifying by age group and comparing risk differences by LLIN type or annual application of indoor residual spraying (IRS). 711 health facilities contributed 20,962 facility reports over 30 months. After national distribution of 10.7 million LLINs and IRS in limited settings, malaria risk decreased from 25.6 to 16.7 cases per 100 people from 2018 to 2019 high transmission seasons, and rebounded to 23.2 in 2020, resulting in significant risk differences of -8.9 in 2019 and -2.4 in 2020 as compared to 2018. Piperonyl butoxide-treated (PBO) LLINs were more effective than pyrethroid-treated LLINs, with adjusted risk differences

of -2.3 (95% CI: -2.7 to -1.9) and -1.5 (95% CI: -2.0 to -1.0) comparing 2019 and 2020 high transmission seasons to 2018. Use of IRS sustained protection with adjusted risk differences of -1.4 (95% CI: -2.0 to -0.9) and -2.8% (95% CI: -3.5 to -2.2) relative to pyrethroid-treated LLINs. Overall, 12 of 28 districts (42.9%) experienced increases in malaria risk in from 2018 to 2020. LLINs in Malawi have a limited effectiveness lifespan and IRS and PBO-treated LLINs perform better than pyrethroid-treated LLINs, perhaps due to net repurposing and insecticide-resistance. DHIS2 provides a compelling framework in which to examine localized malaria trends and evaluate ongoing interventions.

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IMPROVING THE QUALITY AND USE OF MALARIA SURVEILLANCE DATA: RESULTS FROM EVALUATING THE EFFECTIVENESS AND ACCEPTABILITY OF A NEW INTEGRATED MALARIA INFORMATION STORAGE SYSTEM IN SELECTED DISTRICTS IN MOZAMBIQUE

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Mozambique has the fourth highest burden of malaria in the world. Assessments carried out in 2016 and later in 2018 identified critical challenges to the malaria surveillance system in Mozambique, including poor data quality and integration and lack of automated analysis mechanisms. In response to these findings, the development of an integrated malaria information storage system (iMISS) was initiated. iMISS aligns to the high burden high impact (HBHI) data repositories recommendations and stores malaria data across thematic program areas including case surveillance, vector control and commodity management and includes automated data outputs for users at all levels. It also allows health facilities to directly submit their monthly malaria data reports digitally with the aim of improving data quality replacing the need for an additional intermediary step of paper-based reporting. The primary goal of iMISS is to enable malaria staff at all levels of the health system to monitor key indicators and to provide quality evidence to plan and implement responses. The nation-wide roll-out of iMISS and introduction of the direct digital reporting component in seven selected districts started in February/March 2021. In collaboration with the National Malaria Control Programme, a structured evaluation will be carried out from April to September 2021 with the aim of evaluating the effectiveness of the new system and its acceptability among key users. The design of the evaluation is based on the established RE-AIM framework focusing on the five dimensions: reach, effectiveness, adoption, implementation and maintenance of the new system. The primary objective focuses on assessing the data quality of health facility data reported in iMISS and use of iMISS data for decision-making at all levels of the health system. Results of the evaluation will be presented in this session, along with evidence for improvements in data quality and data use as a result of the new system and document lessons learned, key challenges and possible mitigation actions during the first months of implementation.

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SUB-HEALTH CENTERMALARIA SURVEILLANCE: SETUP AND COMMUNITY USE CASES OF A MOBILE SURVEILLANCE APPLICATION

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In its Malaria Elimination Strategic Plan, the Zambian government identified the health facility catchment (HFC) as the unit of elimination and of intervention for implementation. However, existing surveillance tools focus on implementation at the provincial or district level and are rarely tailored for use and response at the HFC. We examined the use case scenarios generated from providing community based malaria mobile application at the sub-HFC level in a rural pre-elimination setting of southern Zambia. An RShiny based mobile application was developed and piloted at Mapanza Rural Health Centre (RHC) located in Choma district in the Southern Province Zambia that is earmarked for malaria elimination. The app provided malaria surveillance data to health center workers and Community Health Workers (CHW) at the HFC. Sub-health center and RHC catchment maps were created using a combination of satellite imagery, ArcGIS software and physical verification by CHWs and RHC staff. The app was piloted in 2020 and additional iterations made after feedback by the end users. Questionnaires were administered to community level end users on experiences around the ease of use, potential use case scenarios and desired outputs from the mobile application. Inventory of the available surveillance platforms and catchment maps was also conducted. The community-based surveillance malaria app provided timely visibility on emergent and incident malaria cases, as well as opportunities for community targeted passive and reactive strategies. Sub-HFC maps were generated and comparisons made with geometrically estimated models. Although, Mapanza RHC catchment had a higher number of malaria cases in the area when compared to the other RHCs in the Macha hospital catchment, the RSHINY app was able to show that malaria incidences at the Mapanza sub-HFC level was not homogeneous and better inform intervention targeting. RHC staff reported not having any other surveillance platforms targeted for response at the HFC. Community based malaria surveillance apps can be leveraged to target sub-HFC of malaria transmission and thus guide community led interventions.

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MALARIA FRONTLINE PROJECT IMPLEMENTATION: LESSONS LEARNT FROM ZAMFARA AND KANO STATES, NIGERIA, 2016-2019

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Poor data quality is a problem for Nigeria's health information system. The United States of America's Centers for Disease Control and Prevention (CDC) collaborated with the Nigeria National Malaria Elimination Program of Nigeria (NMEP) to establish the Malaria Frontline Project (MFP) to improve malaria program surveillance and ensure quality malaria data for decision making in Zamfara and Kano states. Every month Local Government Area (LGA) supervisory team analyzed testing rate, clinical diagnosis and directly observed intermittent preventive treatment (IPTp) of facilities from DHIS2. A facility was visited at least once every quarter, but low performing facilities based on the analysis were visited more frequently. During visit on-the-job training and mentoring of healthcare workers (HCWs) was conducted. Visits ranged between 3-7 per facility per year with mean visits of 5. We analyzed health facility (HF) support visits reports for 2017, 2018 and 2019, respectively. Results for the three years

showed HFs testing rates increased: [(Kano: 92.2%, 97.6% and 98.3%; Zamfara: 90.8%, 95% and 97.7%)]. Clinical diagnosis decreased [(Kano: 2.6%, 1.5% and 1.1%); Zamfara: 1.9%, 0.5% and 0.5%)]. Proportion of HFs practicing the recommended directly observed IPTp increased [(Kano: 83.3%, 97.9% and 97.3%; Zamfara: 83.5%, 88.1% and 88.6%)]. According to the visit reports, HFs that analyzed the selected indicators from their malaria monitoring chart and used the results to make program decision increased: [Kano: 93.0%, 95.0% and 99%; Zamfara: 88.7%, 90.4% and 90.1%] respectively. Use of DHIS2 data analysis with supportive visits helped HCWs at HF and LGA levels to improve malaria program performance. HF support visits driven by DHIS-2 data reviews and HCW mentoring should be encouraged to improve public health program performance especially at HF and LGA levels as well as HF itself analyzing key indicators and use it to make program decision. The lessons learnt can be adopted by other public health programs in the country.

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A PHASE IIIB COMPARATIVE TRIAL OF SEASONAL VACCINATION WITH THE MALARIA VACCINE RTS,S/AS01, SEASONAL MALARIA CHEMOPREVENTION AND OF THE TWO INTERVENTIONS COMBINED

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We conducted an individually randomised, double blind placebo controlled trial in Mali and Burkina Faso, to determine whether seasonal vaccination with RTS,S/AS01$_E$ could provide a similar level of protection to Seasonal malaria chemoprevention (SMC) with sulphadoxine-pyrimethamine plus amodiaquine (SP+AQ), and whether a combination of the two interventions would provide greater protection than SMC alone. Children aged 5-17 months were randomized in April 2017 to receive SMC alone, RTS,S/AS01$_E$ alone or both interventions (n=5920); 1965, 1988 and 1967 children in these groups respectively were followed over a three-year period. Children in the SMC alone group received three doses of rabies vaccine in year one and a dose of hepatitis A vaccine in years 2 and 3, together with four cycles of SMC each year. Children in the RTSS alone group received three priming doses of RTS,S/AS01$_E$ vaccine in year one and a booster dose in years 2 and 3 together with four cycles of SMC placebo each year. Children in the combined group received RTS,S/AS01$_E$ vaccine and SMC each year. The incidence of the primary endpoint, uncomplicated clinical malaria (fever with a parasite density of ≥5000 per µl) in the SMC alone, RTS,S alone and the combined groups was 305, 278 and 113 per 1000 person-years at risk respectively. The hazard ratio (HR) comparing RTS,S alone to SMC was 0.92, (95% confidence interval (CI): 0.84, 1.01), excluding the pre-specified non-inferiority margin of 1.20. Episodes of , hospital admissions with severe malaria and deaths from malaria were reduced by 70.5% (95% CI: 41.9, 85.0) and 72.9% (95% CI: 2.91, 92.4) in the combined group compared to the SMC alone group and reduced by 70.6% (95% CI: 42.3, 85.0) and 75.3% (95% CI: 12.5, 93.0) in the combined group compared to the RTS,S alone group. Five children given RTS,S/AS01$_E$ developed febrile convulsion the day after vaccination but recovered without sequalae. Seasonally-targeted RTS,S/AS01$_E$ vaccine was safe and non-inferior to SMC in preventing uncomplicated malaria. The combination of these interventions was superior to either alone in preventing uncomplicated malaria, severe malaria and death from malaria.

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EFFECTS OF SCHISTOSOMA MANSONI AND PRAZIQUANTEL TREATMENT ON THE GASTROINTESTINAL MUCOSA: A COHORT STUDY IN RURAL TANZANIA

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We conducted a prospective cohort study among women and men living in a Tanzanian fishing village to compare the clinical findings on sigmoidoscopy and laboratory values (hematologic, renal, liver function tests, and erythrocyte sedimentation rate (ESR)) in adults with and without *Schistosoma mansoni* infection at baseline and 6 months after praziquantel treatment. Two groups were enrolled in a 2:1 ratio: an *S. mansoni*-infected group that was positive for both serum schistosome circulating anodic antigen (CAA) and stool ova, and a schistosome-uninfected group. Each participant underwent a baseline sigmoidoscopy and provided peripheral blood for laboratory testing. Schistosome testing was repeated at two-month intervals and treatment with standard-dose praziquantel provided when indicated. Sigmoidoscopies and laboratory testing were repeated at 6 months. Grading of the endoscopic findings was done using the Mayo Scoring System for Assessment of Ulcerative Colitis Activity. In total, 48 individuals were enrolled: 16 infected women, 16 infected men, 10 uninfected women, and 6 uninfected men. *S. mansoni*-infected women had a higher Mayo Score on sigmoidoscopy than uninfected women at baseline (2 versus 0.5, p=0.0008). Men with *S. mansoni* infection at baseline had no sigmoidoscopic differences but did have higher eosinophils, aspartate aminotransferase, and ESR and lower body mass index and platelets than uninfected men (all p<0.05). At 6 months, 28 individuals completed repeat sigmoidoscopy and blood tests. Of these, 16 had either cleared their baseline infection (n=7), or achieved a greater than 5-fold decrease in serum CAA (n=9). Among these 16 individuals, women experienced significant improvements in Mayo Score, eosinophils, creatinine, ALT, and ESR. In men, platelets increased after successful treatment. *S. mansoni* infection was associated with mild to moderate sigmoidoscopic abnormalities in women, and hematologic abnormalities in men, which improved after praziquantel treatment. Larger studies are warranted to investigate gastrointestinal mucosal effects of *S. mansoni* infection and potential differences by sex.

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HOST TISSUE PROTEOMICS REVEAL INSIGHTS INTO THE MOLECULAR BASIS OF SCHISTOSOMA HAEMATOBIUM-INDUCED BLADDER PATHOLOGY

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Schistosomiasis remains a major public health concern worldwide. In the case of urogenital schistosomiasis (caused by *Schistosoma haematobium*), adult worms produce eggs in the bladder tissue, which are excreted in the urine and is the major cause of pathology. Disease manifestations include inflammatory fibrosis of the bladder, bladder carcinogenesis, and obstructive renal failure, with death rates of >150,000/year. While there are enormous opportunities to influence health through specifically designed interventions, limited mechanistic studies imply that the molecular mechanisms underlying pathology have not been

well defined. Based on a mouse bladder wall injection model for urogenital schistosomiasis, proteome profiling of schistosome-infected and uninfected mice bladder tissue was carried out to elucidate the pathways involved in pathology from urogenital schistosomiasis. Purified *S. haematobium* eggs (3,000 eggs/50 µl PBS) or uninfected hamster liver/intestinal extracts (controls) were microinjected into the bladder wall of mice. Mice were sacrificed 7 days post-injection, and bladders were lysed and processed for proteome profiling using mass spectrometry. Multivariate analyses in combination with protein-protein interactions and functional analysis showed that oxidative stress, cell adhesion/aggregation, coagulation, smooth muscle proliferation, and tissue regeneration proteins were more highly represented in egg-injected mice. Proteins involved in immunity/defence responses, including leukocyte migration/aggregation, inflammation, and response to interleukins were more prevalent in egg-injected mice. These findings highlight the host responses induced by active *S. haematobium* infection, characterised by several inflammatory and characteristic bladder tissue changes that lead to pathology, host and parasite survival. This study provides an in-depth analysis of potential host protein indicators and provide new insights into the pathophysiology of urogenital schistosomiasis, and will be relevant for development of improved interventions for disease control.

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HOST METABOLIC ALTERATIONS FROM EARLY SCHISTOSOMA HAEMATOBIUM INFECTION IN PRESCHOOL-AGED CHILDREN

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The worldwide disease burden from schistosomiasis remains significant. Studies in animal models suggest that the pathological consequences from infection are due in part, to parasite-induced changes in host metabolic pathways. There are limited human studies on the impact of infection on host metabolism, none relating to *S. haematobium*, and none describing the changes that occur early in infection. Here, we analysed the changes in host metabolic profiles, in response to the first *S. haematobium* infection and treatment in Zimbabwean children. A cohort of 83 schistosome-negative children (2–5 years old) as determined by parasitological examination, guardian interviews and examination of medical records, was recruited at baseline. Children were followed up after 3 months for parasitological diagnosis of their first *S. haematobium* infection (urine egg count). Children positive for infection were treated with praziquantel, and treatment efficacy checked 3 months after treatment. Blood samples were taken at each time point, and capillary electrophoresis mass spectrometry in conjunction with multivariate analysis were used to compare the change in serum metabolite profiles in schistosome-infected vs. uninfected children. Following baseline at the 3-month follow up, 11 children had become infected with *S. haematobium* (incidence=13.3%). Our results showed that early infection with *S. haematobium* was associated with significant increases (>2-fold) in discriminatory metabolites, linked primarily with energy (G6P, 3-PG, AMP, ADP) and purine (AMP, ADP) metabolism. These observed changes were commensurate with infection intensity, and were resolved within 3 months, following curative treatment. Taken together, these changes are consistent with parasite-related clinical manifestations of malnutrition, poor growth and poor physical and cognitive performance observed in schistosome-infected children. This study provides further understanding into the early host metabolic responses to the infection, the need for early treatment, and development of appropriate interventions aimed at reducing disease consequences.

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IN UTERO METABOLIC RESPONSES TO MATERNAL SCHISTOSOMA JAPONICUM OCCUR IN A SEX-DEPENDENT MANNER

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Metabolic hormones are critical mediators of fetal growth and are known to be impacted by inflammation experienced during pregnancy. We have previously reported maternal *Schistosoma japonicum* to be associated with increased production of pro-inflammatory cytokines by the placenta. In addition, the placental responses to insults *in utero* have been shown to occur in a sex divergent manner, with male fetuses more sensitive than females. IGF binding proteins, particularly IGFBP1, is thought to inhibit IGF availability by binding IGFs in circulation and altering their interaction with receptors on target cells. Accordingly, IGFBP1 is negatively associated with fetal growth. In this study, we evaluated the metabolic hormones adiponectin, leptin, insulin-like growth factor (IGF)-1 and -2, and the mediators IGF binding protein (IGFBP)-1, -3 and -5 in a cohort of pregnant women in Leyte, the Philippines. *S. japonicum* infection was determined via Kato-Katz. All infected women refused treatment with praziquantel. Logistic regression models included maternal education and gestational age for all analyses. Cord blood hormones were not different based on maternal infection. Interestingly, cord blood IGFBP1 levels were lower in females and higher in males (*P=0.02*) born to infected mothers, compared to uninfected counterparts of the same sex. There was no significant difference in the hormones measured in maternal serum based on infection status, although IGFBP3 was significantly lower (*P=0.03*) in circulation of infected mothers carrying female fetuses. Males born to women who experienced *S. japonicum* during pregnancy tended to be born lighter than males born to uninfected mothers (*P=.06*). Our study substantiates this premise, while also displaying a potential sex discordant mechanism by which female fetuses display lower IGFBP1, thereby increasing bioavailability of IGFs and protecting growth *in utero*. Given the relative low prevalence and majority of infections of low intensity in our study (11% and 93%, respectively) these data remain preliminary, however represent an intriguing potential mechanism by which growth may be protected.

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THE ROLE OF MICROBIAL TRANSLOCATION IN THE PATHOGENESIS OF SCHISTOSOMA MANSONI ASSOCIATED MORBIDITY

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Children with intestinal schistosomiasis are at risk for undernutrition, linear growth stunting, and impaired cognitive development. The mechanisms by which the infection results in these morbidities are understudied. There is evidence that environmental enteric dysfunction (EED) contributes by decreasing gut absorptive capacity and integrity that can result in translocation of luminal microbes and components into the bloodstream with subsequent systemic immune activation. We examined the longitudinal impact of praziquantel (PZQ) treatment on EED biomarkers and a key driver of childhood linear growth, insulin growth factor I (IGF-1). 290 infected children, age 6-15 years, were enrolled from sites in Minas Gerais State/Brazil. Participants were treated with PZQ at baseline. EED biomarkers lipopolysaccharide (LPS), intestinal fatty acid binding protein (I-FABP) were measured, as well as IGF-1 at baseline, 6 and 12-month. Multivariate regression analysis was applied to assess associations between EED biomarkers and both baseline *Schistosoma mansoni* intensity and

changes in biomarkers post treatment after controlling for potential confounding variables (age, sex, weight-for-age z-score, socioeconomic status, and hookworm infection status). At baseline, *S. mansoni* infection intensities were 27.2% low, 46.9% moderate, and 25.9% high. LPS concentrations were significantly reduced at the 12-month when compared to baseline (p = 0.0002). No differences were observed for I-FABP or IGF-1 after treatment. At 6-month, I-FABP concentration was significantly higher in high vs low intensity infection at baseline (p = 0.0017). IGF-1 concentrations were significantly lower in the moderate and high infection categories when compared to low intensity in all three study visits. We report that PZQ treatment for *S. mansoni* infection resulted in delayed changes in EED biomarkers, suggesting a requisite period needed for gut healing. These findings provide further evidence for the relationship between schistosomiasis and EED and suggests a mechanistic role for EED in schistosomiasis-related morbidities, particularly linear growth.

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INTRANASAL ADMINISTRATION OF AN ADENOVIRUS-VECTOR EXPRESSING CATHEPSIN B PROTECTS FROM SCHISTOSOMIASIS INFECTION IN A PRE-CLINICAL MODEL

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Schistosomiasis, caused by the parasite *Schistosoma spp*, is one of the most prevalent parasitic diseases worldwide. With over 700 million people at risk of infection, this parasite causes debilitating illnesses lasting more than 30 years. This project tested the protective ability of *Schistosoma mansoni* cathepsin B when delivered by a recombinant Adenovirus (AdV-SmCB) as a novel vaccine vector. Previous work consisted of AdV-SmCB delivered intramuscularly followed by two boosts of recombinant antigen. Upon immunization with our vaccine, we observed robust cell-mediated and humoral immune responses, giving significant protection. As *Schistosoma* larva travel through the lung, we sought to harness the capability of our Adenovirus vector to stimulate a mucosal response. Single-dose intranasal administration of our recombinant Adenovirus (AdV-SmCB-IN) was able to elicit robust humoral responses, producing antigen-specific serum IgG, and local IgA production in the lung. More importantly our AdV-SmCB-IN reduced parasite burden by 79% in adult worms and more modestly, 55% and 56% in hepatic and intestinal eggs. Not only does our recombinant Adenovirus vaccine result in higher parasite burden reduction levels than the World Health Organization standard of 40%, but also offers versatility as a needle-free vaccine. An effective vaccine for schistosomiasis would benefit populations in endemic regions aiding the interruption of disease transmission as well as international travelers visiting tropical regions.

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DIMINISHING CROSS-REACTIVITY IN SCHISTOSOMIASIS SEROLOGY BY APPLYING A SINGLE GLYCAN TARGET APPROACH

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Many current serological schistosomiasis assays are based on crude soluble egg, cercarial or worm antigen preparations. This poses an inherent liability for assay specificity as a large proportion of antibodies binding to such crude antigen preparations are recognising a subset of glycan elements present in the parasite's glycoprotein and glycolipid repertoire, that is also present in other helminths, other invertebrates, food stuff of plant origin or the mammalian host. However, extensive glycomics work has shown that schistosomes in addition contain several unique and potentially species specific glycan elements. Therefore, glycan driven cross-reactivity can be overcome by identification of single or a combination of few defined glycan antigens for development of future sensitive and specific serological tools. In order to identify such antigens, we applied an iterative target selection process based on custom-made glycan microarrays combined with well-characterised sample sets. We have assessed the specificity and sensitivity of candidate antigens by analysing schistosome non-endemic area samples from a controlled human schistosome infection model, from Dutch, Belgian and Spanish primary infection traveller samples as well as samples from an Indonesian soil-transmitted helminth endemic area. Furthermore, samples from schistosomiasis endemic areas in Kenya were investigated. Through this process a candidate target with promising accuracy for primary schistosomiasis infection has been found. Development of a highly sensitive and specific antibody detection assay would be a beneficial addition to the existing diagnostic tool repertoire for schistosomiasis. An accurate antibody detection tool would have particular impact and use in traveller diagnostics as well as in very low endemic, near- and post-elimination settings for transmission monitoring purposes.

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A PHASE 1 OPEN-LABEL TRIAL WITH DENGUE-1-VIRUS LIVE VIRUS HUMAN CHALLENGE ASSESSMENT OF HEALTHY U.S. ADULTS PREVIOUSLY PRIMED WITH TETRAVALENT DENGUE VIRUS PURIFIED INACTIVATED VACCINE AND BOOSTED WITH TETRAVALENT DENGUE VIRUS LIVE ATTENUATED VACCINE FORMULATION

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Dengue Human Infection Models (DHIM) are critically needed to down-select tetravalent dengue virus (TDEN) vaccine candidates before advanced clinical trials. An attenuated DENV-1 DHIM was previously dose-optimized and was safe, well-tolerated and induced symptomatic, mild dengue illness. As part of a heterologous prime-boost strategy, volunteers received TDEN Purified Inactivated Vaccine (PIV) prime followed by a TDEN Live Attenuated Vaccine (LAV) boost at 90 or 180 days in 2018. Vaccinated volunteers (n = 6) aged 18-50 years, with detectable pre-DHIM TDEN antibody titers and flavivirus-naïve control volunteers (n = 4) were challenged with DENV-1 strain 45AZ5 in Jan 2021. Volunteers were followed daily from Days 4-16 with quantitative PCR used for detection, and DENV-1 solicited adverse events assessed. A novel Illness Index, incorporating dengue signs and symptoms as well as laboratory abnormalities was employed to score illness. Nine of 10 volunteers developed detectable viremia (5/6 vaccinees and all 4 controls). The mean viremia onset in vaccinees was day 5 (range 5-6) versus day 8 (range 7-10) for controls, P = 0.007. The viremia duration was 8.2 days (range 7-10 days) in vaccinees vs. 9.75 (range 9-11) in controls, P = 0.088. There was no difference in viremia area under the curve (vaccinees 3.35 x 10^7 vs. controls 2.66 x10^7, P = 0.895). Despite earlier onset and shorter duration, the vaccinee Illness Index scored at 17.5 vs controls' 15.1, P= 0.55. The most common symptoms were headache , myalgia, and rash. Mild to moderate leukopenia and transaminitis were commonly observed. Grade

3 adverse events were detected in vaccinees only: fever >102.1°F (n = 3) and headache (n = 1), with one transient Grade 4 AST/ALT (393/124). Immunogenicity assays, including microneutralization and *in vitro* antibody dependent enhancement tests, are underway. We conclude that TDEN-PIV primed, TDEN-LAV boosted volunteers were unprotected against DENV-1 with a trend towards more severe symptoms than flavivirus-naïve controls. These findings highlight the importance of utilizing DHIM before advanced Phase II/III clinical trials of dengue vaccine candidates.

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CUMULATIVE SAFETY ASSESSMENT OF THE TAKEDA TETRAVALENT DENGUE VACCINE CANDIDATE IN PARTICIPANTS 4 TO 60 YEARS OLD

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Takeda's tetravalent live-attenuated dengue vaccine candidate, TAK-003 (TDV), has been assessed in 13 phase 2 and 3 clinical trials with >25,000 participants 4-60 years old. Safety assessment of TDV includes >2 years of data accumulated from >20,000 4–16 year-olds (TDV:placebo [PBO], 2:1) living in dengue-endemic areas from the ongoing phase 3 trial (ClinicalTrials.gov NCT02747927). An integrated safety data analysis was conducted for all 5 placebo-controlled trials (14,627 TDV and 7,167 PBO recipients) in which ≥98% of participants received 2 doses of vaccine 3 months apart. Demographic and baseline characteristics were well-balanced between TDV and PBO groups; 30.6% TDV vs 28.8% PBO recipients were baseline dengue seronegative. Data from a subset of ~5000 participants showed solicited reactogenicity was generally less frequent after the second vaccine dose; local adverse events (AEs) within 7 days (43.4% TDV vs 25.7% PBO) were mostly mild and transient injection site pain, and resolved within 1-3 days, while systemic AEs within 14 days (43.8% TDV vs. 37.1% PBO) were mostly mild or moderate in severity, most frequently headache and myalgia, and generally resolved within 3-4 days. Evaluation of unsolicited AEs, AEs leading to vaccine and/or trial discontinuation, medically attended AEs, and serious AEs (SAEs) revealed no important safety risks. Unsolicited AEs (21.3% TDV vs 22.8% PBO) within 28 days after each vaccination comprised mainly medical conditions normally observed in the evaluated age groups; ≤3% were assessed as related to vaccination. SAEs, mostly infections or infestations, occurred in <0.4% of participants within 30 days of vaccine or PBO. Fewer SAEs occurred for TDV than for PBO (2.84 vs 3.26 per 100 person-years) mainly owing to fewer dengue-related SAEs in the TDV group. SAEs in 5 study participants were study vaccine related (1 TDV vs 4 PBO). There were 9 deaths in PBO-controlled trials (6 TDV vs 3 PBO): all were considered unrelated. The integrated analysis, including up to 4 years' safety data, has identified no important risks; TDV was well-tolerated in 4- to 60-year-olds irrespective of prior dengue exposure.

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UNPRECEDENTED PRECLINICAL EFFICACY OF JNJ-1802, A NOVEL PAN-SEROTYPE DENGUE ANTIVIRAL SMALL MOLECULE, AGAINST DENGUE VIRUS IN NON-HUMAN PRIMATE AND MURINE MODELS

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The development of safe and effective dengue antivirals would bring significant benefits to public health. A novel dengue-specific antiviral small molecule, JNJ-1802, inhibited dengue virus (DENV) infection of Vero cells with laboratory-adapted and clinical strains of all four serotypes at pico- to nanomolar range. JNJ-1802 prevents the interaction between DENV proteins NS3 and NS4B. When immunocompromised AG129 mice were non-lethally challenged with DENV-2 (10^2 PFU), prophylactic once daily (q.d.) administration of JNJ-1802 at 1 mg/kg resulted in undetectable viral RNA. Twenty-five days after lethal infection with DENV-2 (10^6 PFU), survival in AG129 mice dosed with 0.3 to 10 mg/kg JNJ-1802 twice daily (b.i.d.) was significantly increased (≥80%) *versus* vehicle-treated mice (0%). Additionally, JNJ-1802 offered significant protection in mice after lethal infection with DENV-1, -3 and -4, demonstrating pan-serotype coverage in this *in vivo* mouse model. Next, protective efficacy of JNJ-1802 against DENV infection was assessed in rhesus macaque (*Macaca mulatta*) models. In macaques dosed prophylactically with JNJ-1802 and challenged with DENV-2 (10^2 $TCID_{50}$), a potent and dose-dependent efficacy was observed. No viral RNA and no IgG seroconversion were detected (until day 28 post-inoculation) after q.d. oral dosing (from 1 day pre- to 10 days post-challenge) with either 0.93 or 3 mg/kg, while vehicle-treated animals had a mean peak viral load of 5.64 $\log_{10}$ copies/ml and developed IgG seroconversion. Similarly, when challenged with DENV-1 ($0.6x10^5$ PFU), no viral RNA and no IgG seroconversion were observed (until day 29 post-inoculation) in macaques dosed orally with JNJ-1802 at 6 mg/kg q.d. (from 3 days pre- to 10 days post-challenge) *versus* vehicle (mean peak viral load of 5.17 $\log_{10}$ copies/ml and IgG seroconversion). These preclinical

data support a pan-serotype protective role for JNJ-1802 against DENV infection. This work is the first to demonstrate *in vivo* efficacy of a dengue antiviral small molecule in non-human primates. The first-in-human study of JNJ-1802 has been completed and it is moving further into clinical development.

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EFFECTIVENESS OF A TETRAVALENT DENGUE VACCINE, BRAZIL, 2016 - 2019

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The Brazilian state of Paraná conducted a mass vaccination campaign against dengue with the tetravalent attenuated vaccine CYD-TDV. The campaign targeted 30 endemic municipalities. The objective of this study was to assess the effectiveness of CYD-TDV in preventing symptomatic virologically confirmed dengue cases according to specific age groups in five of the 30 municipalities. A case-control study was carried out in the five most populous municipalities targeted by the vaccination. Vaccine uptake with the full schedule (3 doses) in these municipalities was 25%. Symptomatic dengue cases in the age groups targeted by the campaign were notified by the municipal health departments. The age groups targeted were 15-18 and 19-27 in all municipalities and 9-14 and 28-44 in one municipality. All cases were confirmed by real time reverse transcription quantitative polymerase chain reaction (RT-qPCR). For each case, two controls were selected: a neighborhood control and a workplace or school/college control, matched by age group. Vaccine status was ascertained by the participants' vaccine cards and by the State's electronic vaccine registry. A conditional logistic regression model was used to determine the odds ratio for vaccination and the vaccine effectiveness. Study participants included 618 RT-qPCR-confirmed dengue cases and 1,236 matched controls (with a non-reactive dengue IgM serologic test). Vaccine effectiveness against dengue due to any serotype was 11.1% (95% CI: -19.0%; 33.6%). Effectiveness against DENV-1 was 33.3% (95% CI: -5.0%; 57.6%) and against DENV-2 was -56.7% (95% CI: -142.2%; -1.5%). No DENV-3 cases were detected in these municipalities. The vaccine was significantly effective in the prevention of DENV-4 cases (VE = 93.3%; 95% CI: 47.7%; 99.2%). CYD-TDV was effective in the prevention of symptomatic cases due to DENV-4, but not due to any serotype. The low dengue sero-prevalence in the target population could possibly be related to these results.

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STRUCTURE-GUIDED STABILIZED DENV2 ENVELOPE SUBUNIT VACCINE ELICITS DENV2 NEUTRALIZING ANTIBODY IN VIVO.

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The four dengue virus (DENV) serotypes are mosquito-borne flaviviruses that infect several hundred million people living in tropical regions of the world. Vaccines have great potential for protecting people from flavivirus infections. Several live attenuated dengue virus vaccines are currently being evaluated in human clinical trials. Investigators have also evaluated the use of recombinant envelope (E) proteins from DENVs as subunit vaccines with limited success. Potent neutralizing human antibodies against DENVs target quaternary structure epitopes that are presented by the E dimers on the virion. However, wild-type DENV2 E protein, when expressed as secreted antigen (WT rE), is predominantly monomeric and does not display these quaternary structure epitopes. Here we report on studies to test if recombinant DENV2 E dimers are better vaccine antigens than monomers. Using molecular modeling, Rosetta, we have identified mutations that greatly increase the stability of DENV2 E dimers (RD) under physiological conditions of vaccination. Unlike WT rE, RD was recognized by quaternary epitope-targeting human neutralizing antibodies. Mice were immunized with WT rE and RD to compare the binding and functional properties of antibodies induced by each antigen. RD vaccinated mice sera had more dimer-specific antibodies and neutralized DENV2 significantly better than WT-vaccinated mice. Our data suggest that the oligomeric state of DENV rE is crucial in eliciting DENV neutralizing antibodies, and that structure-guided antigen design is a viable option to develop a successful dengue subunit vaccine.

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IMMUNE PROFILING OF HOST RESPONSE TO TRIVALENT AND TETRAVALENT DENGUE CANDIDATE VACCINE AND DENGUE CHALLENGE

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Safe and effective vaccines are needed for dengue prevention. Understanding the immune response to dengue is essential for developing a vaccine that is protective against all four dengue serotypes without disease enhancement. Here, we used samples from two randomized, placebo-controlled clinical trials; one in which participants received the live attenuated tetravalent dengue vaccine TV005 (N=18) or placebo (N=18) [CIR-299], and another in which participants received a live attenuated trivalent vaccine (rDEN1Δ30, rDEN3Δ30/31, rDEN4Δ30) (N=15) or placebo (N=6) [CIR-300]. All participants in both trials were challenged with DEN2Δ30 (Tonga/74) on day 180 after enrollment. Here we evaluate the immune response post-vaccination and post-challenge for both dengue vaccines. Longitudinal Luminex-based immune profiles for 41 cytokines were obtained from samples collected at 0, 8 and 21 days post-vaccination and post-challenge. Significant increases in IP-10 expression were observed following receipt of either the trivalent or tetravalent vaccine. Following receipt of the challenge virus, a large increase in IP-10 expression was observed in the placebo (FCH = 4.5) and trivalent groups (FCH = 2.3) but not in the tetravalent group (FCH = 1.1). MCP-1, IL-1RA, and MIP-1β exhibit a similar pattern as IP-10. These results illustrate the protective effects of trivalent and tetravalent vaccines against dengue virus, and suggest a better protective effect with the tetravalent than the trivalent vaccine. Since epidemiology studies have found that the non-Black race is a risk factor for more severe dengue, we explored the differences between African Americans (N=26) and Caucasian subjects (N=26). The vaccination and challenge IP-10 responses observed in the placebo and trivalent group were specific to Caucasians, with African Americans showing an attenuated response. These results indicated racial differences in the immune response to vaccination and infection post-vaccination and may explain why Caucasians may have a more vigorous dengue virus immune response than African Americans.

1295

MONOMERIC IGA1 ANTAGONIZES IGG1-MEDIATED ENHANCEMENT OF DENV-3 INFECTION IN VITRO

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Dengue virus (DENV) is a prevalent human pathogen, infecting approximately 400 million individuals per year and causing symptomatic

disease in approximately 100 million. A distinct clinical feature of dengue is the increased risk for severe disease in some individuals with a preexisting DENV-specific immunity. One proposed mechanism for this phenomenon is antibody-dependent enhancement (ADE), in which poorly-neutralizing IgG isotype antibodies from a prior infection opsonize DENV to infect F_c gamma receptor-bearing cells, increasing the infection burden and consequent immunopathology. While IgM and IgG are the most readily observed DENV-reactive antibody isotypes induced after DENV infection, our group and others have described induction of a DENV-specific serum IgA antibody response during dengue. This IgA fraction is induced between IgM and IgG seroconversion, peaks near convalescence, and wanes more rapidly than the other isotypes. Notably, though detectable in both primary and secondary dengue cases, the IgA fraction appears to achieve a higher titer during primary infection. Despite being the second most abundant isotype in circulation, the role of IgA in dengue is not well characterized. We hypothesized that monomeric IgA would be able to neutralize DENV virions without the possibility of ADE, given its lack of an F_c-gamma domain. To test this, we synthesized IgG1 and IgA1 versions of two different DENV-reactive monoclonal antibodies. We demonstrate that isotype-switching does not impact the antigen binding and neutralization properties of the two mAbs. However, we show that DENV-reactive IgG, but not IgA, mediates ADE in an F_c gamma receptor-bearing cell line, and that introducing a fraction of IgA into an infection-enhancing IgG solution can potently antagonize ADE. These results suggest an unappreciated role for DENV-reactive IgA during the humoral response to DENV infection and raise the potential that IgA is a regulator of disease severity and inflammation.

1296

DEFINING POLYSACCHARIDE-SPECIFIC ANTIBODY TARGETS AGAINST VIBRIO CHOLERAE O139 IN HUMANS FOLLOWING O139-CHOLERA AND FOLLOWING VACCINATION WITH A COMMERCIAL BIVALENT ORAL CHOLERA VACCINE, AND EVALUATION OF CONJUGATE VACCINES TARGETING O139

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Cholera caused by *Vibrio cholerae* O139 could re-emerge, and proactive development of an effective O139 vaccine would be prudent. To define immunoreactive and potentially immunogenic carbohydrate targets of *Vibrio cholerae* O139, we assessed immunoreactivity of various OSP-related polysaccharides with plasma from humans hospitalized with cholera caused by O139, comparing responses to those induced in recipients of a commercial oral whole cell killed bivalent (O1, O139) cholera vaccine (WC-O1/O139). We also assessed conjugate vaccines containing selected subsets of these saccharides for their ability to induce protective immunity using a mouse model of cholera. We found that patients with wild type O139 cholera develop IgM, IgA, and IgG immune responses against O139 OSP and many of its fragments, but we were only able to detect a moderate IgM response to purified O139 OSP-core, and none to its fragments in immunologically-naïve recipients of WC-O1/O139. We found that immunoreactivity of O139-specific polysaccharides with wild type infection-elicited antibodies markedly increased when saccharides contain colitose and phosphate residues, that a synthetic terminal tetrasaccharide fragment of OSP is more immunoreactive and protectively immunogenic than complete OSP, that native OSP-core is a better protective immunogen than the synthetic OSP lacking core, and that functional vibriocidal activity of antibodies predicts *in vivo* protection in our model, but depends on capsule thickness. Our results suggest that O139 OSP-specific responses are not prominent following vaccination with a currently available oral cholera vaccine in immunologically naïve humans, and that vaccines targeting *V. cholerae* O139 should be based on native OSP-core or terminal tetrasaccharide.

1297

EFFECTIVENESS OF TYPHOID CONJUGATE VACCINE AGAINST CULTURE-CONFIRMED TYPHOID IN A PERI-URBAN SETTING IN KARACHI: A CASE-CONTROL STUDY

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Enteric fever, caused by *Salmonella* Typhi and *S*. Paratyphi, is a cause of high morbidity and mortality among children in South Asia. Rising antimicrobial resistance presents an additional challenge. Typhoid Conjugate Vaccine (TCV) is recommended by the World Health Organization for use among people 6 months to 45 years old living in endemic settings. This study aimed to assess the effectiveness of TCV against culture-confirmed *S.* Typhi in Lyari Town, Karachi, Pakistan. This peri-urban town was one of the worst affected by the outbreak of extensively drug-resistant (XDR) typhoid that started in November 2016. A matched case-control study was conducted following a mass immunization campaign with TCV at Kharadar General Hospital, Lyari General Hospital and Jan Bai Aga Khan Secondary care Hospital from August 2019 through December 2019 in Lyari Town Karachi, Pakistan. Children aged 6 months to 15 years presenting with culture-confirmed *S.* Typhi were enrolled as cases. Controls were afebrile children at least seven months of age and born within ± six months of the date of birth of a case 6-36 months old or born within ± three years of the date of birth of a case 3-15 years old, visiting the same hospitals as case or living in the neighborhood of the case. For each case, at least one age-matched hospital control and two age-matched community controls were enrolled. Of 82 typhoid fever patients enrolled, 8 (9.8%) had received the vaccine for typhoid. Of the 164 community controls and 82 hospital controls enrolled, 38 (23.2%) community controls and 27 (32.9%) hospital controls were vaccinated for typhoid. The age and sex-adjusted vaccine effectiveness was found to be 72% (95% confidence interval: 34% - 88%). Using multivariate conditional logistic regression, the consumption of meals prepared outside the home more than once per month (adjusted odds ratio: 3.72, 95% CI: 1.55- 8.94; p-value: 0.003) was significantly associated with culture-confirmed typhoid. A single dose of TCV is effective against culture-confirmed typhoid among children aged 6 months to 15 years old in an XDR typhoid outbreak setting of a peri-urban community in Karachi, Pakistan.

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ANTIMICROBIAL RESISTANT TYPHOID AS A CAUSE OF ACUTE FEBRILE ILLNESS IN BANGLADESH

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Typhoid is a major cause of acute febrile illness (AFI) in Bangladesh, with a reported incidence of 200 episodes per 100,000 person-years. We report the clinical manifestations and antimicrobial resistance patterns of patients with *Salmonella* Typhi in selected Bangladeshi hospitals. During May 2019-March 2020, study physicians screened patients aged >2 years presenting to outpatient departments of 4 public hospitals in different regions. We randomly enrolled patients who had measured fever

(≥100.4°F) during assessment with onset within the past 14 days but did not take any antibiotics in the past 24 hours. We collected and transported their blood to the icddr,b laboratory within 24 hours for bacterial culture. Grown blood cultures were subjected to bacterial identification and antibiotic susceptibility testing in a fully automated VITEK 2 system. Among 690 AFI patients enrolled, 67 (9.7%) were culture-positive for *Salmonella* Typhi. Typhoid was more common among males (52, 78%) and urban residents (59, 88%). The proportion of typhoid cases sharply increased after 10 years of age; 67% of all typhoid cases were ≥15 years old. Typhoid was found to be more likely in patients with high temperature >103°F than lower (adjusted odds ratio, AOR 3.1, 95% CI 1.3-7.7), among students than non-students (AOR 2.6, 95% CI 1.4-4.8), among males than females (AOR 2.1, 95% CI 1.2-3.9), in medicine than pediatrics (AOR 2.4, 95% CI 1.3-4.6), in Rajshahi (AOR 4.0, 95% CI 1.4-12) and Dhaka (AOR 3.5, 95% CI 1.2-10.6) than Sylhet. *Salmonella* Typhi isolate antimicrobial sensitivities were as follows: ceftriaxone (61/61, 100%), amoxicillin-clavulanic acid (56/64, 88%), azithromycin (25/30, 83%), cotrimoxazole (43/63, 68%), ampicillin (37/66, 56%), and ciprofloxacin (7/25, 11%). After enrolment, patients were treated with azithromycin (38%), ceftriaxone (33%), ciprofloxacin (16%), cefixime (13%), and cefuroxime (8%) stated by the patients who could mention the antibiotic names (n=24). No hospitalizations or deaths were reported. Preventive measures such as typhoid vaccination and appropriate antimicrobial use could reduce the typhoid burden in Bangladesh.

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ANTIBIOTICS IMPROVE GROWTH IN TANZANIAN CHILDREN WITH SMALL INTESTINE BACTERIAL OVERGROWTH

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Small intestine bacterial overgrowth (SIBO) is common in children living in low-income countries. SIBO has been associated with environmental enteric dysfunction and poor linear growth. We conducted a 2x2 factorial designed randomized interventional trial of antimicrobials (azithromycin + nitazoxanide), nicotinamide, antimicrobials + nicotinamide, or a placebo to determine these intervention's effect on linear growth. Children were enrolled at birth and followed through 18 months of age. Nicotinamide was administered daily. Azithromycin was given as a single dose at months 6, 9, 12, and 15 and nitazoxanide was given as a 3-day course at months 12 and 15. A subset of children enrolled in this trial were tested for SIBO with the hypothesis that children with SIBO would respond to antimicrobials with improved growth. SIBO was measured by glucose-hydrogen breath test at 6, 12, and 18 months. A >12ppm increase in breath hydrogen over baseline was considered SIBO positive. We stratified children by those who received the antimicrobial intervention and those that did not and created separate linear regression models for each group to predict the change in Length-for-Age Z score (delta LAZ) between birth and 18 months of age. 400 children were enrolled in the SIBO testing subset. 202 received antimicrobials while 198 did not. 316 children were SIBO tested at 6 months of age with 4% positivity. 227 were tested at 12 months of age with 19% positive. 353 children were tested at 18 months of age with 16% positivity. 359 children had at least 2 SIBO tests. The average LAZ at enrollment was -0.8 SD and by 18 months of age the average LAZ had dropped to -1.9 SD. For the children who received antimicrobials, the number of positive SIBO tests was not a significant predictor of growth (parameter estimate 0.15 SD [95% CI -0.15 to 0.47 SD, p = 0.31]). However, for the children that did not receive the antimicrobial intervention, for each positive SIBO test the delta LAZ from 0 to 18 months decreased by 0.43 SD (95% CI -0.74 to -0.13, p = 0.005). These results suggest that antimicrobials can abrogate the negative effects of SIBO on longitudinal growth.

1300

EXPANDING THE VACCINE VALUE PROPOSITION BY QUANTIFYING THE BURDEN OF ANTIBIOTIC USE THAT CAN BE ATTRIBUTED TO SHIGELLA AND IS POTENTIALLY AVERTABLE BY VACCINES

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Vaccines for *Shigella* may reduce selection for antimicrobial resistance (AMR) by preventing diarrhea that prompts antibiotic treatment. To quantify these benefits, we estimated the burden of antibiotic use attributable to *Shigella* in the MAL-ED birth cohort study. We analyzed 9392 diarrhea episodes, 38085 stools, and 15697 antibiotic courses in 1715 children. We estimated attributable fractions and incidence rates of overall and diarrhea-specific antibiotic use attributable to *Shigella*. We estimated associations with antibiotic treatment, adjusting for age, sex, site, and socioeconomic status. Finally, we estimated the incidence and proportion of antibiotic exposure to other enteropathogens that was attributed to treatment of *Shigella* diarrhea. *Shigella* was a leading cause of antibiotic use, responsible for 14.8 antibiotic courses per 100-child years, including 11.7% of antibiotic courses for diarrhea and 3.2% of all antibiotic courses. *Shigella* was responsible for a larger proportion of all fluoroquinolone (12.2%) and macrolide (5.5%) use, drug classes that are appropriate for diarrhea treatment and of concern for AMR. *Shigella*-attributable diarrhea was 47% more likely to be treated compared to other etiologies (RR:1.47; CI:1.34, 1.61), and 26% of this association was mediated by either higher diarrhea severity or dysentery. However, less than one-fifth (18.7%) of all antibiotic treatments attributable to *Shigella* were for dysentery episodes. Additionally, treatment of *Shigella* diarrhea resulted in frequent antibiotic exposures to other pathogens that were carried asymptomatically at the time of treatment, including 5.6 antibiotic exposures per 100-child years to *Campylobacter*, and 11.1 per 100-child years to diarrheagenic *Escherichia coli*. *Shigella* was disproportionately responsible for antibiotic use due to its high burden and severity, which strengthens the value proposition for *Shigella* vaccines. A *Shigella* vaccine could further reduce the risk of AMR by preventing a substantial proportion of antibiotic exposures to other pathogens commonly carried among children in low-resource settings.

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A CLINICAL PREDICTION RULE TO GUIDE DIAGNOSTIC TESTING IN CHILDREN UNDER-FIVE PRESENTING WITH DIARRHEA IN LOW AND MIDDLE INCOME COUNTRIES

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Diarrheal diseases are a leading cause of death for children under-5 worldwide, and identification of etiology helps guide pathogen-specific therapy. However, the availability of diagnostic testing is often limited in low and middle income countries (LMICs), where the majority of diarrhea morbidity and mortality occurs. We developed a clinical prediction tool to guide clinicians in identifying when to use a hypothetical point-of-care diagnostic for making diarrhea treatment decisions. We used clinical and demographic data from the Global Enteric Multicenter Study (GEMS) study to build predictive models to identify pediatric diarrhea patients that could potentially benefit from antibacterial therapy (*Shigella*/EIEC or *Vibrio cholerae* attributable fraction ≥0.5) in children ≤59 months presenting

with moderate-to-severe diarrhea (MSD) in Africa and Asia. We screened variables using random forests, and assessed predictive performance with random forest regression and logistic regression using 5-fold cross-validation. Of the 5304 cases with etiology, 1524 (29%) had an etiology that would benefit from antibiotic therapy. Our ability to predict which diarrhea patients would potentially benefit from antibiotic therapy was high, with an AUC=0.79 (95% CI: 0.78, 0.80) for a model including only the top two predictive variables, age and presence of bloody diarrhea. We show that by using our CPR to triage who receives diagnostic testing, 39% more children would receive necessary antibiotics and 33% fewer children would receive unnecessary antibiotics compared to using bloody diarrhea (dysentery) as the only criteria for antibiotic use, with only 41% of children targeted for the point-of-care test. We demonstrate how a clinical prediction rule can be used to guide use of a point-of-care diagnostic test for diarrhea management in LMICs. Using our CPR, available diagnostic capacity can be optimized to improve appropriate antibiotic use.

1302

DEVELOPMENT AND VALIDATION OF A CLINICAL PREDICTION MODEL FOR ACUTE VIRAL DIARRHEA IN BANGLADESH

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Diarrhea remains a major cause of morbidity and mortality in low- and middle-income countries (LMICs) where antibiotics are commonly used for viral causes of illness. Our aim was to develop a clinical prediction model to estimate risk of viral-only diarrhea in patients of all ages in Bangladesh to improve clinical decision-making. This retrospective analysis used data from a collaborative nationwide diarrhea surveillance network (2,516 patients) of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) and Bangladesh's Institute of Epidemiology Disease Control and Research (IEDCR) and icddr,b routine clinical surveillance data (3,000 patients) from 2014-2018. The datasets included historical, clinical, and microbiological data from patients with acute diarrhea. Microbiological testing was performed using TaqMan Array Cards (TAC) PCR for 23 enteric pathogens. A multivariable logistic regression model was derived using all clinically relevant candidate predictors available from the nationwide dataset for the primary outcome of viral only pathogen(s) using TAC PCR cycle threshold (CT) of <30. The model was then externally validated using the icddr,b surveillance dataset. Viral diarrhea was most common in children under 5 with 67% having viral-only pathogens. Predictors included in the model were: age, sex, diarrhea duration, presence of abdominal pain, stool frequency, vomiting, dehydration status. Multivariable logistic regression showed that younger age, longer diarrhea duration, lack of abdominal pain, non-bloody stool were significantly associated with viral only etiology. The clinical prediction model's AUC was 0.82 with internally validated AUC of 0.80. In external validation, discrimination of the model was still robust with an AUC of 0.75 with calibration slope 0.75. This model is now being planned to be developed into a mobile health tool for clinical implementation to identify patients with viral diarrhea who do not warrant antibiotics and can be managed with rehydration alone. This may help to support rational use of antibiotics and reduce concerns of antibiotic resistance in LMICs.

1303

POPULATION SYNCHRONY AS AN ARBOVIRUS RISK METRIC IN CONNECTICUT, USA

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Synchrony is the degree of co-variance of disparate populations in space and time, and measures of synchrony are associated with species' persistence and stability. Whether synchrony can inform public health risk by providing indices of similarity among arbovirus surveillance sites has not been previously explored. We used multiple functions in the R packages *vegan* and *synchrony* to define synchrony among 19 mosquito and 7 arbovirus species at 87 long-term surveillance sites in Connecticut, US from 2001 - 2020; we then used machine learning approaches to determine the influence of distance, habitat, and climate on metrics of synchrony among the 19 species. We concluded our analyses by examining spatial patterns of synchrony among the disease vector species *Culiseta melanura* and *Culex pipiens* to develop risk maps for specific regions of CT. Mosquito communities were highly synchronous among sites, and 77% of sites displayed significant patterns of community synchrony (avg. S = 0.35, $p<0.05$). 84% of species displayed significant patterns of synchrony among sites (avg S. = 0.26, $p < 0.05$), and three flood-water nuisance (*Aedes trivitattus, Aedes vexans,* and *Psorophora ferox*) and two disease vector (*Cs. melanura* and *Cx. pipiens*) species had the highest indices of synchrony. Among the arboviruses, synchrony was considered significant for Cache Valley, eastern equine encephalitis, and Potosi virus isolates. Similarity in collections decayed with distance across all species and viruses, yet habitat and climate similarity scores were more informative of synchrony. In our species-specific analyses, *Cs. melanura* collections among sites were positively and significantly correlated up to 20km while *Cx. pipiens* collections were positively and significantly correlated up to 25km then negatively correlated near the extremes of the data set (> 100 km). These metrics provide useful and easy-to-interpret information for local public health departments regarding arboviral risk based on information obtained outside of a specific department's jurisdiction.

1304

NATURAL AEDES-BORNE VIRUS INFECTION IN AEDES AEGYPTI WITH IMPLICATIONS FOR ESTIMATES OF TRANSMISSION RISK

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Emerging *Aedes*-borne viruses (ABVs) such as chikungunya (CHIKV), Dengue (DENV) and Zika (ZIKV) contribute significantly to the global burden of infectious diseases, usually affecting low income areas. In this study we describe the natural infection rate of CHIKV, DENV, and ZIKV in individually tested field-caught mosquitoes in Merida, Yucatan, Mexico. We used indoor *Aedes aegypti* sequential sampling with Prokopack aspirators to collect all mosquitoes inside houses with suspected active ABV transmission to quantify the arbovirus infection rate of captured females *Aedes aegypti* mosquitoes and males (a proxy of transovarial transmission), we tested all collected specimens by RT-PCR followinged by SANGER sequencing analysis for final confirmation. We tested 2,161 females (62.8% of the collection) and 1,278 males (37.2% of the collection). From these, a total of 250 (7.3%) mosquitoes were positive for an ABV coming from 52 of the 200 (29.0%) houses tested. The majority of ABV-positive mosquitoes were positive for CHIKV (203, 81.2%),

followed by 31 (12.4%) mosquitoes positive for DENV and 16 (6.4%) mosquitoes positive for ZIKV. The distribution of infected *Aedes aegypti* for both sexes was overdispersed, the maximum number of positive mosquitoes detected per house was 32 specimens, 25 females and 11 males. We also found a positive association between infected females and male mosquitoes. Remarkably we found a large number of ABV-positive male mosquitoes from a natural urban setting (CHIKV= 74; DENV= 12, ZIKV= 1). These findings suggested vertical transmission and its possible role to viral maintenance in endemic settings contributing to periodic re-emergence among humans.

1305

SKIN IMMUNE SIGNATURES AFTER AEDES AEGYPTI MOSQUITO BITE IN CAMBODIAN VOLUNTEERS IS CONSISTENT WITH AN ANTI-INFLAMMATORY PROFILE

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Vector-host-pathogen interactions in the skin microenvironment influence host innate and adaptive immune responses. In order to determine the immune signatures against *Aedes aegypti* mosquito saliva in the skin, we enrolled 10 healthy Cambodian participants to undergo a controlled *Aedes aegypti* uninfected mosquito feed. Three millimeter punch biopsies were taken from unbitten skin and from bitten (control) skin at 30 minutes, 4 hours and 48 hours after the mosquitoes fed on participants' forearms. After tissue dissociation, skin immune cells from the biopsies were stained and analyzed by flow cytometry on a FACS Aria. Whereas at 30 minutes post-feeding minimal changes in innate and adaptive immune populations were observed, at four hours post-feeding, we found an increased frequency of Langerhans cells ($CD207^+$, $p<0.001$) and activated dermal dendritic cells compared to normal skin (NSK) ($CD1c^+CD69^+$, $p<0.05$). In addition, the frequency of M2 type macrophages ($CD163^+CD14^+$, $p<0.001$) and their activation status ($CD16^+$, $p<0.01$ and $CD69^+$, $p<0.05$) increased whereas the frequency of degranulating cells decreased ($CD56^+CD107a^+$, $p<0.001$). At 48 hours post-feeding, frequencies of activated $CD8^+$ T cells expressing PD1 exhaustion marker were increased ($p<0.01$) compared to NSK. Within the CD4 compartment, compared to NSK the frequencies of Th1 cells decreased ($p<0.01$) while frequencies of Th2 cells increased ($p<0.05$). After *in vitro* re-stimulation with salivary gland extract, mostly $CD8^+$ T cells upregulated co-stimulatory molecules and produced TNF-α ($p<0.01$) 48h post mosquito bite. Via a novel clinical design utilizing uninfected mosquito-bitten skin from endemic participants, these data demonstrate that the early innate skin immune response to mosquito saliva is anti-inflammatory. The T-cell response, primarily observed at 48h, is Th2-skewed and driven by $CD8^+$ T cells confirming data generated from animal models. Identification of key cell populations that mediate the response to mosquito saliva in human skin is a fundamental step towards understanding arboviral infections transmitted by *Aedes aegypti* mosquitos.

1306

UNDERSTANDING RESPONSES TO A CHANGING CLIMATE USING COMMON GARDEN AND SELECTION EXPERIMENTS ON AEDES MOSQUITOES

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The dynamics and distribution of mosquitoes and the diseases they transmit are strongly influenced by environmental temperature. Given the predicted increase in temperature with climate change, there is concern over the possible shift in populations of vectors such as *Aedes aegypti,* and associated viruses such as dengue, Zika and Chikungunya. Many mechanistic models that explore the affects of temperature on transmission assume 'average' thermal performance curves that are representative for a species. However, thermal adaptation to a local environment could lead to population-level differences in thermal performance. In this study, we used common garden experiments and experimental evolution to assess evidence for local thermal adaptation of *Ae. aegypti*. We examined thermal tolerance using a physiological knockdown assay previously shown to be able differentiate between local populations of *Drosophila.* This assay was used to explore whether there were population level differences in five field populations of *Ae. aegypti* from Mexico, together with a standard laboratory strain. The assay revealed differences in knockdown between populations, with an indication that populations from locations with higher/more variable temperatures had higher thermal tolerance. To confirm whether local temperature could indeed select for differences in knockdown we established 6 replicate lines from one of the field populations and maintained half of them at a standard insectary temperature of 27°C and the other half at an elevated temperature of 31°C. After 10 generations we found the high-temperature selected lines to exhibit an increase in thermal tolerance in our knockdown assay. Our results indicate that differences in environmental temperature can lead to differences in thermal performance between vector populations, suggesting that average, species-level thermal performance curve might be insufficient for predicting the effects of climate and climate change on vector-borne disease transmission at a local level.

1307

REEMERGENCE OF YELLOW FEVER VIRUS IN SOUTHEASTERN BRAZIL, 2017-2018: WHAT SPARKED THE SPREAD?

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The 2017-2018 yellow fever virus (YFV) epidemic in southeastern Brazil marked a reemergence of YFV in urban states that had been YFV free for nearly a century. Unlike earlier urban YFV transmission, this epidemic was also driven by forest mosquitos. The objective of this study was to evaluate environmental drivers of this epidemic. Using surveillance data from the Brazilian Ministry of Health of human and non-human primate (NHP) cases of yellow fever, we traced the spatiotemporal progression of the epidemic. We then assessed the epidemic timing in relation to drought using a monthly Standardized Precipitation Evapotranspiration Index. Lastly, we evaluated demographic risk factors for rural or outdoor exposure amongst YFV cases. Both human and NHP cases were first identified in a hot, dry, rural area in northern Minas Gerais before spreading southeast into the more cool, wet urban states of Espírito Santo, São Paulo, and Rio de Janeiro. Outbreaks also coincided with drought in all four southeastern states of Brazil. Confirmed YFV cases had an increased odds of being male (OR 2.58; 95% CI 2.28-2.92), being of working age (OR: 2.03; 95% CI: 1.76-2.35), and having recent travel from an urban to a rural area (OR: 5.02; 95% CI: 3.76-6.69). The 2017-2018 YFV epidemic in Brazil originated in hot, dry rural areas of Minas Gerais before expanding south

into urban centers. An unusually severe drought in this region may have created environmental pressures that sparked the reemergence of YFV in Brazil's southeastern cities.

1308

MOSQUITOES ARE EXCITO-REPELLED UPON CONTACT WITH TOPICAL REPELLENTS: A 3D VIDEO TRACKING ANALYSIS OF MOSQUITOES EXPOSED TO COMMON TOPICAL REPELLENTS IN THE STANDARD ARM-IN-CAGE TEST

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Topical repellents may provide personal protection against mosquito bites and their efficacy is usually assessed in the arm-in-cage test. In this assay, study participants expose their repellent-treated forearm into a cage containing host-seeking mosquitoes. This procedure is then repeated at regular intervals (e.g. every hour) until the study participants are bitten, which provides an estimate for the formulation's complete protection time. However, the endpoint says little about how mosquitoes would interact with the repellent under a real world scenario since in the arm-in-cage test the mosquitoes are confined to a very small volume, while in nature they would approach humans over much longer distances, integrating an array of different distance-dependent stimuli. Therefore, understanding of how mosquitoes interact with topical repellents is essential to assess the validity of the arm-in-cage test. Here, we measured the behaviour of host seeking mosquitoes in the arm-in-cage test using a 3D infrared video camera system. We tracked mosquito flight paths of *Aedes aegypti* and *Anopheles stephensi* at a rate of 50 positional data per second and in real time as the mosquitoes interacted with a repellent-treated forearm in the arm-in-cage test. The applied repellents included 20% ethanolic solutions of N,N-diethyl-meta-toluamide, p-menthane-3,8-diol, icaridin and ethyl butylacetylaminopropionate. All four repellents reduced the number of bites while simultaneously increasing the number of contacts the mosquitoes made with the arm. The mosquito behaviour was primarily characterised by gustatory excito-repellency upon contact with the arm rather than being repelled by volatile chemicals. Our observations cast doubts on the validity of the arm-in-cage test as the only basis for making label claim recommendations for the complete protection times of topical repellents.

1309

FIRST REPORT OF HIGH SURVIVAL AND FECUNDITY RATES OF MALARIA VECTOR MOSQUITOES IN IRRIGATED AREAS OF ETHIOPIA

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Irrigated agriculture is key to increase crop productivity and ensure food security in Africa. However, unintended negative public health impacts such as malaria have been a challenge around such developmental projects. This study aimed at assessing the effects of irrigation practices on malaria vector mosquito development and survivorship in Ethiopia. The study was conduct in two agroecosystems, irrigated and non-irrigated areas, in western Ethiopia. Monthly larval surveys were conducted between 2017 and 2020. Life-table experiment was done to examine the effect of environmental modification on survivorship and development of both immature and adults *Anopheles arabiensis.* Habitat diversity, larval abundance, pupation rate, development time of immatures and adult longevity and fecundity were compared between the two agroecosystems. The number of anopheline positive habitats was two-fold higher in irrigated than non-irrigated areas. Anopheline larval abundance in the irrigated area was 16.6 % higher than the non-irrigated area. Irrigated agroecosystem was significantly associated with larval anopheline occurrence. The estimated mean survival time of female *An. arabiensis* in the irrigated and non-irrigated areas was 37.9 and 31.3 days, respectively. The study found that fecundity of *An. arabienis*, was 96.2% higher in the irrigated agroecosystem than in the non-irrigated area. The findings of this study underscore that irrigation in semi-arid areas of Ethiopia increase the survival and fecundity of the major malaria vector, *An. arabiensis*. Integrated Vector Management that incorporates environmental management is critical to control mosquito breeding around irrigation schemes.

1310

MICROFILARIAE-INDUCED EOSINOPHIL EXTRACELLULAR DNA TRAPS ARE INDUCED VIA THE DECTIN-1 RECEPTOR, NADPH OXIDASE AND THE INFLAMMASOME

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Eosinophils and their cytotoxic granules play an important part in the protective immunity against filariae. The release of those granules can be mediated through different mechanisms including the extracellular DNA trap cell death (ETosis), a form of cell death where intracellular DNA is explosively released, entrapping pathogens and supporting their killing. The results of the present study demonstrate that microfilariae (MF) of the filarial nematode *Litomosoides sigmodontis* trigger DNA release by bone marrow-derived eosinophils. *In vitro*, these eosinophil DNA traps (EETosis), consisting of nuclear and mitochondrial DNA, inhibit MF motility in a DNA- and cell contact-dependent manner. Dectin-1 was identified as the pattern-recognition receptor involved in triggering the MF-induced EETosis. Moreover, EETosis in response to MF is dependent on NADPH oxidase since eosinophils derived from $Cybb^{-/-}$ mice, which lack NADPH oxidase, are not able to release extracellular DNA traps. Furthermore, caspase-1 is activated during MF-induced EETosis and inhibition of caspase-1 in wild-type eosinophils and caspase-1-deficient eosinophils do not undergo ETosis. This suggests that the inflammasome pathway is involved during filarial-induced EETosis. *In vivo* studies reveal an increase in local DNA concentration upon *L. sigmodontis* infection in mice. Intrapleural MF injection raises local DNA concentrations, which is partly mediated by eosinophils, indicating a potential role of EETosis as an *in vivo* effector mechanism as well. Furthermore, MF covered by DNA traps and injected into naïve mice are removed faster from the peripheral circulation than MF that are not covered by extracellular traps suggesting a potential role of ETosis in MF clearance. In summary, these results identify eosinophil ETosis as an important mechanism in protective immunity against MF and reveal the underlying signaling mechanism during eosinophil DNA release.

1311

ADOPTIVE TRANSFER OF IMMUNE CELLS INTO RAGIL-2RG-DEFICIENT MICE: A NOVEL APPROACH TO INVESTIGATE IMMUNITY AGAINST HUMAN AND RODENT FILARIAE

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Besides severe clinical symptoms, the majority of filarial infections are asymptomatic due to the filariae-driven modulation of host immunity. A major obstacle for research on human filariae has been the inability to obtain adult worms and the insufficient possibility to analyse infection kinetics and immune signalling. Thus, the *Litomosoides sigmodontis* filarial mouse model is well-established and serves to study host-parasite interactions. Research revealed several important factors, molecules and

immune cells that are crucial for immune responses against the rodent filarial nematode. Nevertheless, the complex immunological mechanisms associated with filarial control and disease progression remain unclear and translation to human infections is difficult, especially since human filarial infections in rodent models are limited. To overcome these obstacles, we performed adoptive transfer experiments into RAG2IL-2Rγ-deficient C57BL/6 mice. These mice have no T, B and natural killer cells, develop full patency upon 72 days post-infection (p.i.), despite the fact that C57BL/6 mice normally clear the infection upon 40 days p.i., and have been shown to be susceptible for infection with the human filaria *Loa loa*. Using this mouse model in combination with the adoptive transfer cell transfer, we revealed that $CD4^+$ T cells from *L. sigmodontis*-infected C57BL/6 donor mice significantly reduce worm burden, length and fertility, dampen tissue inflammation at the site of infection and moreover block microfilariae (offspring of the filaria) release. These findings were accompanied with an increased Th17 $CD4^+$ T cell response, distinct cytokine and chemokine secretion patterns and altered innate cell composition. In summary, this study shows for the first time that $CD4^+$ T cells and their immune responses are central for the immunity against *L. sigmodontis* in C57BL/6 mice. Moreover, adoptive transfer of immune subsets in RAG2IL-2Rγ-deficient C57BL/6 mice will provide an optimal platform to decipher cell populations, cytokines, chemokines and receptors that control worm survival and patency of rodent and human filarial infections.

1312

RNASEQ-BASED ANALYSES OF THE EFFECT OF BRUGIA MALAYI MICROFILARIAL-DERIVED EXTRACELLULAR VESICLES ON HUMAN MONOCYTES AND DENDRITIC CELLS

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Live microfilariae (mf) and mf-derived extracellular vesicles (EVs) have been shown to modulate human antigen presenting cell (APC) function, most notably by suppressing the induction of IL-12 (and other pro-inflammatory cytokines) following activation with LPS and interferon-γ. Moreover, live mf and mf-derived EVs have been shown to suppress mTOR phosphorylation to the same degree as did rapamycin. To explore further how EVs alter human APC function, we studied the effect of mf and EVs on human elutriated monocytes and monocyte-derived DCs following exposure to Mf, mf-derived excretrory/secretory (E/S) products, E/S depleted of EVs through ultracentrifugation and purified EVs. After demonstrating that almost all of the measurable responses (cytokines, mTOR phosphorylation) induced by live mf could be recapitulated by EVs and EV-containing E/S, we next performed RNAseq analysis of human DC and human monocytes following exposure to live mf, EVs, E/S, or EV-depleted E/S. In preliminary analyses of the data for the DC, using a false discovery rate (FDR)<0.05, EV-exposed DCs had induced the expression of 212 differentially expressed genes (DEGs) when compared to unexposed DC and 157 when compared to ES-depleted EVs. These genes were enriched in GO biological processes associated with neutrophil degranulation and 15 DEGs associated with KEGG Lysosome pathways. IPA analysis point to immune dysregulation. We are further analyzing the DEGs of human monocytes. Common pathways modulated by EVs in both DC and monocytes are being explored specifically in directed experiments to understand the intracellular processes altered by EVs and the effect these have on effector T cells. Taken together, our data suggest a modulatory role of EVs on APC function that likely leads to defects in T cell effector function.

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IL-11 REGULATES INNATE MUCOSAL IMMUNITY IN ACUTE HELMINTH INFECTION

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Interleukin (IL)-11, a pleiotropic IL-6 family-member cytokine, appears to play a role in both innate and adaptive immune responses and tissue inflammation. Little is known, however, about its function in helminth infection. Having shown that IL-11 gene expression is upregulated in the lungs of *Ascaris*-infected mice at 8 days post-infection (dpi), we sought to understand the role played by IL-11 at lung barrier surfaces following infection with *Ascaris*. Compared to uninfected mice, IL-11 levels were significantly elevated in the lung tissue of *Ascaris*-infected mice (2,825 pg/mL vs 894 pg/mL, P<0.0001 at 8 dpi). Using both flow cytometry and confocal microscopy, we found that bronchial epithelial cells and subepithelial fibroblasts were the primary IL-11-producing populations following *Ascaris* infection. To assess the function of IL-11 in *Ascaris* infection, we administered anti-IL-11 antibody intranasally during *Ascaris* infection. This IL-11 neutralization impaired the influx of macrophages and neutrophils to the lungs; moreover, there was induction of CXCL5, a chemokine known to inhibit neutrophil trafficking. Intranasal administration of recombinant IL-11, conversely, induced G-CSF and CXCL1, increased neutrophil influx, and reduced airway inflammation and worm burden (40% decrease, P<0.01) in the *Ascaris*-infected mice when compared with PBS-treated, infected mice, suggesting an immune-mediated regulatory effect of IL-11 in helminth infection; similar effects have been reported in bacterial pneumonia and inflammatory bowel disorders. To model the interaction between *Ascaris* and lung epithelial cells, we used an *in vitro* system whereby a human bronchial epithelial cell line grown in a monolayer on an extracellular gel matrix was shown to produce markedly increased (55% above baseline) amounts of IL-11 following exposure to *Ascaris* larvae. These data suggest that IL-11 acts as an alarmin released by bronchial epithelial cells in response to Ascaris larvae (or larval products) that may in turn regulate the neutrophil-dominated inflammation seen during acute helminth infections.

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COMPARING IMMUNE RESPONSES TO PRIMARY INFECTION WITH DENGUE, CHIKUNGUNYA AND ZIKA VIRUSES

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Dengue virus (DENV), Zika virus (ZIKV) and chikungunya virus (CHIKV) are mosquito-borne arboviruses with similar epidemiology and transmission cycles, collectively causing >50 million cases each year, with a resurgence in recent decades. Although clinical symptoms are similar and can be mild, all 3 viruses can lead to severe and life-threatening complications that differ among the viruses. To characterize immune responses to these 3 viruses, we measured and compared immune cell profiles by CyTOF in children naturally infected with DENV1, DENV3, ZIKV or CHIKV

in two longitudinal studies in Managua, Nicaragua. Abundance of 28 cell populations were measured in children following primary infection with CHIKV (n=42), DENV1/DENV3 (n=28) or ZIKV (n=87), during acute infection (~1-3 days post-onset of symptoms) and convalescence (~14-21 days). Linear mixed-effects models were used to compare changes in the $\log_{10}$ frequency of immune cell populations in acute infection vs. convalescent infection, as well as to compare profiles between virus types. In total, 26 cell types showed a significant frequency difference (FDR < 0.05) in acute samples vs. convalescence for at least one virus type. Acute infection with CHIKV was characterized by a higher proportion of CD14+ monocytes, while acute ZIKV infection showed higher proportions of CD4+ naïve T cells and CD8+ central memory T cells. Immune cell profiles following acute DENV1/DENV3 infection shared similarities with both ZIKV and CHIKV infection. Convalescence after infection with any of the 3 viruses was characterized by a higher proportion memory B cells, CD27- B cells, and CD1c+ Type-2 dendritic cells. In this study, we have identified changes in immune cell subtypes that are characteristic of the infection responses to three major arboviruses. More research is needed to understand how the infection mechanisms of each virus influence immune responses, and how specific immune cell types determine disease progression and the potential for severe complications. More broadly, this work demonstrates the utility of mass cytometry for studying immune responses to natural arbovirus infection.

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PEPTIDE-BASED SEROLOGIC PLATFORMS CAPABLE TO DIFFERENTIALLY IDENTIFY DENGUE AND ZIKA INFECTIONS IN AN ENDEMIC REGION

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Dengue is one of the most important infectious diseases nowadays, and early diagnosis is a determining factor for disease outcomes. Co-circulation of viruses with serological cross-reactivity with Dengue virus (DV), such as Zika virus (ZV), further complicates diagnosis. One approach towards creating diagnostic tests able to differentiate between such viruses is to determine peptides that would lack immune response cross-reactivity. However, the immobilization of small proteins or peptides on surfaces has been a barrier to the development of tests. This work aims to identify specific peptides in the non-structural protein 1 (NS1) of DV and ZV and evaluate their use in serological diagnostic platforms. For this, we screened DV and ZV peptide libraries with NS1 monoclonal DV1-4 and ZV antibodies (NS1 mAbs). Three peptides were identified with specific binding: one that is ZV-specific and two that are DV-specific. We tested our peptide ELISA assay with 170 human samples from patients who had known a prior diagnosis of DV and/or ZV infection validated by plaque neutralization assay (PRNT). The pepELISA DV-specific showed a sensitivity of 97% and a specificity of 96,3%. The pepELISA ZV-specific showed a sensitivity of 77,9% and a specificity of 97,7%. After that, 1365 samples from an endemic area were tested, where 60,4% was seroprevalent for DV and 15,6% for ZV – with 13% of this population positive for both infections. With the success of pepELISA, we evaluated a lateral flow-based assay using gold nanoparticles (GNP) in two immobilization variations. For the first, biotinylated peptides were conjugated with streptavidin and spotted onto a nitrocellulose membrane, and GNP conjugated with anti-human IgG was tested with the human samples. For the second test, peptides synthesized covalently linked to lipoic acid were conjugated to the surface of GNP. Both strategies were able to differentiate ZV and DV mAbs and patient samples. These techniques presented here are effective, fast, and inexpensive tests and would allow in the near future the rapid assessment of the exposure - essential in a vaccine campaign of both viruses.

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CHIMERIC DENGUE VIRUS REAGENTS REVEAL IMPORTANT STRUCTURAL TARGETS OF DENV3 HOMOTYPIC IMMUNITY

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The four Dengue virus serotypes (DENV1-4) infect several hundred million people per year. Primary DENV infections stimulate serotype-specific (TS) neutralizing antibodies (NAbs) that are correlated with protection against homologous but not heterologous serotypes. A secondary infection with a new serotype stimulates both TS NAbs as well as serotype-cross reactive (CR) NAbs that are associated with long lasting immunity to all serotypes. We currently have a poor understanding of the epitopes targeted by NAbs in people exposed to DENV3 infections or vaccines. The DENV envelope (E) protein with three distinct domains (EDI, EDII and EDIII) is the main target of NAbs. To study individual variation in humoral immune responses after DENV3 infection or vaccination, we created a panel of recombinant chimeric DENVs in which the DENV3 EDI, EDII or EDIII domains of the E protein were individually transplanted into a DENV1 backbone. Monoclonals (mAbs) binding to known epitopes on the E-protein were run against DENV1/3 EDI, EDII & EDIII chimeras to assess that each virus retained domain-specific DENV antibody epitopes. Using these chimeric viruses, we also mapped three new DENV3 neutralizing mAbs to EDII, bringing the total number of known EDII-directed DENV3 TS neutralizing mAbs to 5. Two of the 5 EDII-directed mAbs neutralized DENV3 genotype 3 (GIII) but not genotype 4 (GIV). The critical residues in these epitopes were mapped using DENV3/3 genotype chimeras with select residues or "clusters" from DENV3 GIII transplanted into the DENV3 GIV backbone. Additionally, important structural targets of natural & vaccine-induced polyclonal TS 1° immunity to DENV3 were identified with the DENV1/3 (EDI-III) chimeric panel. Together, the DENV1/3 EDI-III chimeric virus panel has revealed major antigenic sites on E protein targeted by NAbs in people exposed to primary DENV3 infections. In the future, full-domain transplant chimeric reagents can be used as a tool to assess antibody quality after natural infection or vaccination & to learn about structural targets of immunity for other DENV serotypes.

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ANTIGENIC EVOLUTION OF DENGUE VIRUSES OVER MULTIPLE DECADES

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Antigenic relationships among the four dengue virus serotypes (DENV1-4) are important determinants of dengue disease risk. Whether the DENV serotypes change antigenically in response to one another remains uncertain. We tracked the antigenic dynamics of DENV1-4 selected from 1,944 sequenced viruses isolated in Bangkok, Thailand between 1994-

2014 (n=348) in comparison to regional and global DENV antigenic diversity (n=64 strains). DENV1-4 circulating in Thailand oscillated in antigenic distance relative to one another but overall became more antigenically dissimilar over the observational period. For DENV1, DENV2, and DENV4, greater antigenic relatedness to other serotypes correlated with antigenic movement away from the same serotype and with larger epidemics. For DENV3, antigenic similarity to other serotypes co-occurred with low DENV3 transmission and a genotype replacement, at which point newer DENV3 strains antigenically moved away from older DENV3 strains and other serotypes simultaneously. We observe that the four DENV serotypes circulating in one geographic area evolve antigenically over time in relation to one another and epidemic magnitude, providing insights into theorized tradeoffs for DENV evolution.

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ANTIBODY FUCOSYLATION PREDICTS DISEASE SEVERITY IN SECONDARY DENGUE INFECTION

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Immune status to dengue virus (DENV) currently represents the greatest risk factor for hospitalization after a bite from a DENV-infected mosquito. However, additional susceptibility factors exist, as <5% of patients with pre-existing anti-DENV IgGs develop severe disease. While anti-viral antibodies generally confer protective functions, antibodies against DENV are associated with enhanced disease susceptibility. Antibodies can mediate DENV infection of leukocytes via Fcγ receptors, likely contributing to dengue disease pathogenesis. Severe dengue patients are characterized by increased abundance of afucosylated IgG1 glycoforms, which exhibit higher affinity for the activating FcγRIIIa. Whether afucosylated anti-DENV IgG is the result of secondary DENV infection, or their increased abundance truly represents a prognostic factor for susceptibility to severe dengue disease remains unknown. We examined Fab and Fc structures from a Cambodian pediatric cohort pre- and post-infection (n=18) and from individuals who were inapparent infected (n= 23) or hospitalized (n=48) and classified according to WHO1997 criteria. Neither antibody titers nor neutralizing activity correlated with disease severity in DENV-infected populations. Afucosylation is associated with dengue disease susceptibility as secondary inapparent infected individuals had lower levels of DENV-specific and total afucosylated IgG1 compared to hospitalized cases. Moreover, IgG1 afucosylation is associated with dengue disease severity and correlates with biological features of severe disease. Afucosylation is increased during secondary infection compared to primary infection. Analyzing plasma samples from individuals before and after infection revealed that afucosylation increases after infection and during convalescence. Moreover, IgG1 afucosylation was specifically modulated by DENV infection, as we did not observe any changes after West Nile or Zika virus infection. Thus, the IgG1 fucosylation status represents a robust prognostic tool for dengue disease and highlights the key role of the Fc glycan structure in dengue pathogenesis.

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DISRUPTION OF SPATIOTEMPORAL DEPENDENCE IN DENGUE TRANSMISSION BY WMEL WOLBACHIA IN YOGYAKARTA, INDONESIA

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Dengue cases are known to exhibit focal clustering in space and time at the level of the household and neighborhood, driven by local mosquito population dynamics, human population immunity, and fine scale human and mosquito movement. We examined how the spatiotemporal clustering of dengue cases was disrupted by introduction of the arbovirus-blocking bacterium *Wolbachia* (*w*Mel-strain) into the *Aedes aegypti* population in a randomized controlled efficacy trial in Yogyakarta, Indonesia. We analysed 318 serotyped dengue cases and 5,921 test-negative controls with geolocated residence enrolled over 27 months following randomized *w*Mel deployments. The odds that a pair of individuals within a given space-time window are homotypic dengue cases, versus heterotypic cases or test-negative controls, serves as a measure of local transmission. A lack of homotypic clustering is therefore supportive of limited or absent local transmission. We find evidence of significant spatial dependence up to 300m among the 265 dengue cases detected in the untreated arm of the trial. Spatial dependence is strongest within 50m, with a 4.68-fold increase (compared to 95% permutation null range: 0.13, 1.22) in the odds that a pair of individuals enrolled within 30 days and 50m of each other are homotypic dengue cases – and therefore potentially transmission-related - compared to pairs occurring at any distance. In contrast, we find no evidence of spatial dependence among the 53 dengue cases detected in the *w*Mel-treated arm. Strikingly, in 6 of the 12 *w*Mel-treated areas not a single pair of homotypic dengue cases occurred in any 30-day window. This provides compelling evidence that introgression of *w*Mel *Wolbachia* into *Aedes aegypti* mosquito populations interrupts local dengue virus transmission, leading to reduced case incidence.

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LAND USE, INFRASTRUCTURE, AND THE HETEROGENEOUS BURDEN OF DENGUE VIRUS INFECTION WITHIN A HIGHLY-ENDEMIC COMMUNITY

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Dengue is described as an urban disease, yet the burden in rural areas can be high. Across the urban-rural gradient, hot spots of transmission are observed. We hypothesized that risk of DENV is spatially associated with land use, and modified by infrastructural factors such as water and waste management. Data derive from an ongoing study for DENV infection among families in an agricultural Thai province. Blood and data regarding demographics and household features are collected on enrollment. Using satellite imagery and supervised classification, land cover was defined as developed, cropland, and water. The proportions of each within a 500-meter buffer around each house's coordinates were computed. Clusters of development (towns/villages) were identified using k-means clustering. Seropositivity was defined as hemagglutination inhibition titer >=20 for any DENV serotype. Predictors of seropositivity were evaluated using mixed models, controlling for age and clustering by household. Between 2015-2019, 2872 individuals in 470 families were enrolled, with a median age of 21 years (range: 0-93 years). 87.0% were seropositive on enrollment. 22.5% of the study area was classified as developed and 76.9% as cropland. 58.8% of households were within 2.5km of a town/village ('non-rural'). Many previously-identified risk

factors for DENV (lack of screens, presence of water containers, housing materials promoting mosquito entry) were more common in rural homes. Despite this, residence in or near a town/village was associated with DENV seropositivity (OR=1.65), as were residing in a house on poles (OR=3.6) and a 10-container increase in water containers on premises (OR=1.20) ($p<0.05$ for all). There was a strong interaction between rurality and the requirement to transport trash to the dump in predicting seropositivity (OR=5.2 in non-rural versus OR=1.7 in rural areas, $p<0.05$). We describe high transmission across this largely-agricultural study area, with highest risk in and around areas of development. We report modifiable infrastructural factors such as trash management and water supply / storage that underlie these differences in risk.

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WAIT AND WATCH: A POTENTIAL TRACHOMA SURVEILLANCE STRATEGY FROM AMHARA, ETHIOPIA

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Recrudescence of trachoma after the elimination as a public health problem threshold has been reached is a serious concern for global trachoma control. Currently, many district-level surveillance surveys (conducted ≥2 years since elimination threshold has been reached) are resulting in a trachomatous inflammation-follicular (TF) among children ages 1 to 9 years above the elimination threshold (≥5%). This problem is particularly acute in Ethiopia, where 50% of surveillance surveys have had results above threshold. Once a district returns to TF ≥5%, a program typically restarts costly mass drug administration (MDA) campaigns. In Amhara region, Ethiopia, most surveillance surveys which result in a TF prevalence ≥5% have a prevalence close to 5%, making it difficult to determine whether the result is due to true recrudescence or to statistical variability. This study's aim was to monitor recrudescence within Amhara by waiting to restart MDA within 2 districts with a TF prevalence ≥5% at surveillance survey; the districts would be resurveyed 1 year later using traditional and alternative trachoma indicators, such as measures of infection and serology (a "wait and watch" approach). Surveys were multi-stage cluster surveys whereby certified graders assessed TF. For the post-surveillance surveys, children ages 1 to 9 years also provided a dried blood spot, and children 1 to 5 years provided an ocular swab. In 2017 both Metema and Woreta town districts reached the 5% threshold for the first time. During the 2019 surveillance surveys both Metema (5.2%) and Woreta town (5.1%) had a TF prevalence just greater than the threshold. No MDA occurred in these districts during the 2020 MDA in Amhara, and they were surveyed again in 2021. The TF prevalence in Metema and Woreta town was 3.6% (95%Confidence Interval (CI):1.4-6.4) and 2.5% (95%CI:0.8-4.5), respectively. Infection and serology data will help characterize and confirm this low trachoma burden. Based on these results, the Program should not restart MDA in these 2 districts and should consider the wait and watch approach for other districts above the elimination threshold at surveillance survey.

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STATE AND LOCAL GOVERNMENT AREA ESTIMATES FOR TRACHOMA SEROPREVALENCE IN 1 TO 9-YEAR-OLDS IN NIGERIA USING MULTIPLEX BEAD ASSAY

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Trachoma is the leading infectious cause of blindness, resulting from repeated infections of the conjunctivae by *Chlamydia trachomatis (Ct)*. The clinical sign trachomatous inflammation—follicular (TF) is used to assess transmission of ocular *Ct* in 1–9-year-olds during population-based, two-stage cluster surveys generally conducted at district level (population sizes of 100,000–250,000). Testing for antibodies may be useful for surveillance for *Ct* transmission after elimination of trachoma as a public health problem by leveraging samples collected in serosurveys for other infectious diseases. In Nigeria, where trachoma is endemic in certain local government areas (LGA, similar to districts), dried blood spots (DBS) were collected during the 2018 Nigeria HIV/AIDS Indicator and Impact Survey (NAIIS), a national household HIV survey conducted in all 36 states and the Federal Capital Territory. DBS from 20,695 children age 1-9 years were tested using a multiplex bead assay (MBA) for antibodies against 35 antigens for various infectious and vaccine-preventable diseases, including *Ct* Pgp3. State-level seroprevalence estimates ranged from 0.6% (95% CI: 0.1–1.9) in Abia State to 31.3% (95% CI: 25.9–37.0) in Yobe State. Three states with the highest seroprevalence estimates (Yobe, Jigawa, and Borno) are in the Northeast; their LGA-level seroprevalence estimates ranged from 5% to 100%. Of the 741 LGAs surveyed, 289 (39%) had seropositive children; 14 of the 15 LGAs with the highest seroprevalence estimates were in Yobe, Jigawa, and Borno. Further analyses will assess for clustering in LGAs using spatial methods and geolocation data from the survey. Because trachoma is a highly focal disease, nationally representative surveys such as NAIIS may undersample and miss foci of transmission. Data from this survey, where the number of children sampled per LGA (median 24, range 1-127) is approximately a log-fold lower than in trachoma-focused surveys (approximately 1000 children per LGA), will help us understand the utility and limits using specimens from other disease-specific surveys as an alternative method for trachoma surveillance.

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PREDICTING FUTURE COMMUNITY-LEVEL OCULAR CHLAMYDIA TRACHOMATIS INFECTION PREVALENCE USING SEROLOGICAL, CLINICAL, MOLECULAR AND GEOSPATIAL DATA

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Trachoma is targeted for elimination as a public health problem by 2030. Models to predict future community-level infections could help direct control programs. We hypothesized that (1) communities with high levels of infection would be geographically clustered in stable foci of transmission ("hotspots") and (2) models using trachoma indicators (clinical grading, ocular *C. trachomatis* (*Ct*) infections, and IgG responses to *Ct* antigens) and remotely sensed geospatial data would accurately predict future community-level *Ct* infections. We tested our hypotheses using measurements from a randomized controlled trial designed to

assess improved water, sanitation and hygiene in the absence of mass drug administration among 40 communities in Amhara, Ethiopia. Median *Ct* infection prevalence among children 0-5 years old (0-5y) increased from 7% at enrollment to 29% by month 36. We found no evidence for high degrees of global clustering in trachoma indicators over the study area. At baseline, correlation between community-level seroprevalence and *Ct* infection was stronger among children 0-5y (ρ = 0.76, 95% CI = 0.56-0.88) than children 6-9y (ρ = 0.48, 95% CI = 0.23-0.68), and stronger than the correlation between clinical trachoma and *Ct* infection (0-5y ρ = 0.56, 95% CI = 0.28-0.75; 6-9y ρ = 0.40, 95% CI = 0.10-0.67). Seroprevalence was the strongest concurrent predictor of community-level infections at month 36 among children 0-5y (cross-validated R^2 = 0.76, 95% CI = 0.59-0.85). In longitudinal analyses, seroprevalence among children 0-5y from 12 months prior was a weak predictor of infection prevalence at month 36 (cvR^2 = 0.33), and performance further declined for seroprevalence from 24 and 36 months prior (cvR^2 = -0.02 and -0.03, respectively). Geospatial layers, a spatial Gaussian process, and stacked ensemble machine learning did not meaningfully improve predictions. Serological markers among children 0-5y may be well-suited for community-level monitoring given their objectivity and strong correlation with concurrent *Ct* infection prevalence, but accurate, future prediction in the context of unstable transmission remains an open problem.

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NEONATAL INFECTION ASSOCIATED WITH CHILD DEATHS FROM SUB-SAHARAN AFRICA AND SOUTH ASIA: INITIAL FINDINGS FROM CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE

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In 2015, among 5.9 million child (<5 years) deaths globally, 45% were in the neonatal period with sepsis the third most common cause in neonates. The Child Health and Mortality Prevention Surveillance (CHAMPS) network aims to determine causes of stillbirths and under-5 mortality in sub-Saharan Africa and South Asia. Post-mortem tissue specimens were collected using minimally invasive tissue sampling (MITS). The project used multiple laboratory diagnostic platforms, including microbial culture, TaqMan Array Card-based real time PCR, histopathology of MITS, coupled with demographic and clinical data and verbal autopsy to determine the cause of each death. Cause of death was adjudicated by a multi-disciplinary panel (DeCoDe panel) using all available data. From January 2017 to December 2019, 675 neonatal deaths were adjudicated by DeCoDe panels in 7 CHAMPS sites. Due to infection 19% of neonates died <24 hours following birth, 44% in the early neonatal (1-6 days) and 37% in the late neonatal (7-28 days) period. In 320 (47% of 675) cases, invasive infectious disease was identified in the causal chain leading to death. Country specific apportion of infection in the causal chain was 26% in Bangladesh (20/76), 75% in Ethiopia (12/16), 31% in Kenya (31/100), 39% Mali (25/64), 25% in Mozambique (18/72), 61% in Sierra Leone (22/36) and 62% in South Africa (192/311). Over 65% (311/462) of causal pathogens were identified as Gram-negative bacteria, including 118 (38%) *Klebsiella* spp, 113 (36%) *Acinetobacter* spp and 39 (13%) *E. coli*. Gram-positive organisms were determined to be in the causal pathway in 23% (105/462) of cases, including 30 (29%) *Streptococcus agalactiae*. Other non-bacterial organisms included *Candida* spp in 5% (25/462), and viruses in 4% (20/462). Neonatal deaths were attributed to polymicrobial infections in 110 (37/ 320) cases. Our analysis showed that Gram negative bacterial infection still plays a significant role in neonatal death. Deciphering the route of these infections in early days of life and development of appropriate preventive and therapeutic measures will reduce neonatal death in low- and middle-income countries.

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INITIAL VALIDATION OF A NOVEL RECOMBINASE POLYMERASE AMPLIFICATION-LATERAL FLOW ASSAY FOR DIAGNOSIS OF HUMAN BRUCELLOSIS

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Brucellosis is a common zoonotic disease that is endemic in most parts of the world and disproportionately affects rural and resource-limited settings. *Brucella spp.* are highly contagious and can be transmitted to humans through contact with fluids from infected animals, inhalation of aerosolized particles, or consumption of unpasteurized milk and cheese. The diagnosis of human brucellosis is usually made by serologic tests, which are often qualitative, do not differentiate between acute and chronic infection, and can cross react with other bacteria, causing false positives. These issues lead to both over and under-diagnosis of brucellosis and, in turn, inappropriate or inadequate antibiotic treatment. To address the need for new diagnostic tools that can be used at peripheral health facilities in resource-constrained settings, we developed a real-time Recombinase Polymerase Amplification (RPA)-based diagnostic assay for brucellosis that is performed isothermally at 37°C in under 30 minutes and adapted it to a lateral-flow (LF) detection platform. We selected *bcsp31* as the gene target, which encodes for a surface membrane protein that is conserved across *Brucella spp.* The RPA-LF assay detected genomic DNA (BEI Resources, Manassas, VA) from the following *Brucella spp*: *Brucella suis* biovar 1, *Brucella suis* biovar 2, *Brucella abortus* biotype 4, *Brucella abortus* biotype 9, *Brucella abortus* RB51, and *Brucella melitensis* biotype 1. We also performed serial dilutions of *Brucella abortus* biovar 4, and the assay demonstrated high sensitivity with an estimated lower limit of detection of 1.37 genomes by probit analysis. The assay did not detect bacteria known to at times cross-react with existing serologic assays (*Escherichia coli, Erhlichia chaffeensis, Yersinia enterocolitia,* and *Franciscella tularensis*). In conclusion, preliminary experiments indicate that an RPA-LF assay targeting the *bcsp31* gene is sensitive and specific for detection of genomic DNA from *Brucella spp.* in a laboratory setting. Further evaluation with clinical specimens in real-world contexts is planned.

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HIGH RESOLUTION METABOLOMICS HIGHLIGHTS DIFFERENCES IN LIPID AND NUTRITIONAL METABOLISM ACROSS THE LEPROSY SPECTRUM PROVIDING AVENUES FOR ADVANCES IN LEPROSY HOST-PATHOGEN RESEARCH

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High resolution metabolomics (HRM) has led to better understanding of host-pathogen interactions of various infectious diseases but has rarely been used in leprosy research. Thousands of small molecules from endogenous metabolism, diet, the environment, medications, the microbiome and from infectious pathogens themselves can be detected through HRM and advanced data extraction. Our objectives were to identify unique metabolic signatures related to leprosy and to increase our understanding of associated nutrient metabolism. Between June 2018 and December 2019, adults newly diagnosed with leprosy and healthy controls were recruited from leprosy referral clinics in Minas Gerais, Brazil. Plasma samples were drawn, frozen, and shipped to the Clinical Biomarker Laboratory at Emory University. Metabolites were detected using an established HRM platform and characterized by accurate mass m/z and retention time. The Mummichog informatics package was used to compare metabolic pathway activity between groups. Additionally, select individual metabolites were quantified and compared between groups. All analyses controlled for age and sex. Sixty-seven individuals with leprosy were enrolled of which 26 (62% of cases) were multibacillary (MB), 16 were paucibacillary (PB), and 25 were controls (including 16 household contacts). Exploratory analysis of metabolic pathways showed several statistically significant differences between groups: arachidonic acid metabolism between cases vs controls ($p = 0.01$); vitamin E ($p=0.007$) and retinol metabolism ($p=0.04$) between cases and contacts; and vitamin D3 metabolism between MB and PB ($p=0.004$). In addition, plasma tryptophan concentrations were lower in MB vs PB, consistent with an increase in catabolism by indoleamine-2,3-dioxygenase. These metabolic signatures provide insight into leprosy host-pathogen interactions that will improve our understanding of the pathophysiology of infection that could lead to improved diagnostics and therapeutics. HRM is, thus, an innovative tool for a neglected pathogen and these preliminary data provide a foundation for a wide range of future studies.

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METAPROTEOMIC ANALYSIS OF HUMAN BRONCHOALVEOLAR LAVAGE FLUID REVEALS MICROBIOMES ASSOCIATED WITH SUSCEPTIBILITY AND PROTECTION AGAINST TB INFECTION

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The human lung metaproteomic profile has been extensively studied in individuals having pulmonary TB, and the main biospecimen that is widely used is the human sputum. The sputum microbiota composition does not reflect the microbiota composition in the lower respiratory tract, which the human BALF can reveal. To the best of our understanding, no study has investigated the metaproteomic profile of individuals with a broad spectrum of TB infection following the lung challenge with the live BCG and PPD antigens. The individuals were divided into two cohorts: protective phenotypes (LTB, n=75 & SIM, n=33) and susceptible phenotypes (RTB, n=33, & PTB, n=69). BALF samples were analyzed using Q-Exactive tandem mass spectrometry. The bioinformatics workflow involved the construction of the samples specific database using the MetaLab version 2.0 pipeline. MSfregger version 3.1.1 was used for the target-decoy sequence database search. The identified peptides were analyzed using Unipept for taxonomic classifications. The human BALF's microbiota composition following lung challenge with the live BCG and PPD was mainly composed of the Phylum, *Firmicutes, Bacteroidetes, proteobacteria, Actinobacteria, Fusobacteria, Spirochaetes*, and *Synergisteses*. RTB cohort has a less diverse phylum composition, and the LTB cohort had a diverse phylum composition. There was a significant reduction in the short-chain fatty acid (SCFA) producers in the RTB cohort compared with the PTB, LTB, and SIM at the genera level. The bacteria composition of the SIM and LTB were characterized by the high abundance of the SCFA producers. This research shows that the protection of TB depends on the abundance of SCFA producers in the human lungs. These findings will help the vaccine developers as it emerges that the commensal bacteria's function should be considered when developing the vaccines. It also increases our understanding of the Correlation of protection and susceptibility to TB infection in humans.

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PROSTAGLANDIN E2 SIGNALING REGULATES OENOCYTOID IMMUNE FUNCTION THAT IS ESSENTIAL FOR ESTABLISHING ANOPHELES GAMBIAE INNATE IMMUNITY

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Bioactive fatty acids such as prostaglandin E2 (PGE2) and its cognate prostaglandin receptors play pivotal roles in mediating immune and inflammatory processes across species. While prostaglandin signaling has previously been implicated in mosquito anti-*Plasmodium* and anti-viral immunity, the receptor(s) responsible for mosquito prostaglandin signaling have not been characterized. Here, we identify a PGE2 receptor (*Ag*PGE2R) in *Anopheles gambiae* and demonstrate that its expression is most abundant in specialized mosquito immune cell populations known as oenocytoids. Through the administration of PGE2 and *AgPGE2R*-silencing, we demonstrate that prostaglandin signaling regulates a subset of prophenoloxidases (PPOs) and antimicrobial peptides (AMPs) that significantly limit bacterial replication and *Plasmodium* oocyst survival. Additional experiments demonstrate that PGE2 signaling is essential to the late-phase immune response through the regulation of *PPO1* and *PPO3* expression to promote oocyst killing. We also provide evidence that PGE2 signaling induces oenocytoid cell rupture at high PGE2 concentrations and demonstrate that oenocytoid cell lysis prior to *Plasmodium* infection negates the antagonistic effects of PGE2 signaling on parasite survival. Together, these results provide new mechanistic insights into the influence of prostaglandin signaling on oenocytoid function that promotes pathogen killing in the mosquito host.

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INVESTIGATING THE ENERGETIC COSTS OF INSECTICIDE-RESISTANCE IN CALIFORNIA AEDES AEGYPTI

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We collected 12 pools of 10 adult female *Aedes aegypti* from two colonies to investigate differences in the physiology of a laboratory strain (ROCK) and a colony derived from the Central Valley of California (CLOVIS) with high-throughput metabolomics. This technique offers a snapshot of the insects metabolome, and can reveal different levels of metabolic activity and demands on metabolic pathways. Samples were processed by the UC Davis Metabolomics center which provided data on the abundance of primary metabolites, lipids, and biogenic amines. We analyzed the data in Metaboanalyst, and observed these two groups had markedly different metabolomes. The CLOVIS metabolome had evidence of significantly elevated glucose metabolism, elevated pentose phosphate pathway activity, and lower amounts of storage lipids. This led us to speculate some of these differences may be attributable, in part, to the energetic costs of maintaining metabolic mechanisms of pyrethroid insecticide resistance. The ROCK group is fully susceptible to commonly used pyrethroid insecticides, while CLOVIS has both target site and metabolic

mechanisms of resistance to pyrethroid insecticides. These findings imply that the maintenance of metabolic mechanisms of resistance are costly, and may come at the expense of energy storage, which may help explain observations of reduced lifespan, fecundity, and adult body size in pyrethroid resistant insects. We predict these features may leave these resistant insects more susceptible to inhibitors that interact with pathways experiencing increased demand, and plan to evaluate inhibitors which interact with the pentose phosphate pathway and insect lipid metabolism as novel products for mosquito control.

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CHARACTERIZATION OF ANOPHELES CHAPERONIN COMPLEX PREFOLDIN: A POTENTIAL BROAD-SPECTRUM PLASMODIUM BLOCKING TARGET IN ANOPHELINE MOSQUITOES

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Plasmodium relies on numerous mosquito-derived host factors, when engaging its intimate interactions with the vector's midgut, hemolymph, and salivary glands. We have identified prefoldin subunit 6 (PFDN6) as a potent agonist host factor for *Plasmodium* infection. The evolutionarily conserved eukaryotic prefoldin (PFD) is a chaperone protein that acts as a heterohexameric complex and aids in the assembly of microtubules and microfilaments. Through CRISPR/Cas9-mediated gene editing we have confirmed the essential biological function of PFDN6; especially in the absence of PFDN6, mosquitoes have shown pre-adult (embryonic and larval stages) lethality due to the lack of properly assembled actin. RNAi-mediated gene silencing of *Pfdn6* (as well as any of the other five subunit genes (*Pfdn1-5*) silenced individually or in combinations) suggests PFD works as a heteromultimeric protein complex, in association with its known partner CCT/TRiC, when fulfilling its agonist function for *Plasmodium*; furthermore the same function of PFDN6 was validated through CRISPR/Cas9-based conditional knockout of *Pfdn6* in the midgut. An interactome study based on co-immunoprecipitation has identified several interacting partners with PFDN6, including Tep15, LRIM 26, Enolase, and several cytoskeletal proteins. Enolase has shown similar function as PFD through RNAi- and CRISPR/Cas9- based studies. Interestingly, different species of anopheline mosquitoes (*A. gambiae, A. stepheni,* and *A. dirus*) co-fed with the polyclonal PFDN6 antibodies and *P. falciparum* or *P. vivax* gametocytes infected blood resulted in significant lower parasites loads in the mosquito midguts and salivary glands, suggesting its broad-spectrum *Plasmodium* agonist function in a variety of mosquito vectors. A preliminary transmission-blocking vaccine (TBV) assay based on a mouse model system showed the same phenotypes, further suggesting PFD as a powerful candidate for the development of TBV targeting various malaria parasites in the major vectors. Finally, through several approaches we have elucidated a potential mechanism of the PFD's role as a host factor.

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THE ROLE OF MOSQUITO SALIVA IN THE ACTIVATION OF FIBRINOLYSIS

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Malaria parasites undergo two severe bottlenecks during the sexual reproduction in the mosquito midgut and during sporozoite infection of the human. To overcome these bottlenecks *Plasmodium* parasites can co-opt host proteins to enhance infectivity. Our laboratory has shown that malaria parasites hijack the fibrinolytic system to facilitate infection of both, the mosquito vector and the vertebrate host. Plasminogen, the key molecule of the fibrinolytic system, is activated into the serine protease plasmin by tissue-type (t-PA) and urokinase-type (u-PA) plasminogen activator. It has been shown that mosquito saliva injected in the dermis of a human or ingested by the mosquito during blood feeding, can alter hemostatic responses. To determine how mosquito saliva plays a role in the activation of the fibrinolytic system, we established a fluorescence-based biochemical assay to detect Plasminogen and t-PA activation by saliva or salivary gland (SG) extracts. We detect activation of t-PA in the presence of mosquito SG extract or saliva when compared to the control (SG extract or saliva without t-PA). Plasminogen was not activated when incubated with saliva. Activation of t-PA was lost when the saliva was pre-incubated at 65 °C or 100 °C, and Western blot analysis shows that saliva induces cleavage of inactive single-chain t-PA into the active two-chain form, pointing that the t-PA activator is a protease. To identify the saliva t-PA activator, we fractionated SG extracts by size-exclusion chromatography and identified a fraction which activated t-PA. Mass-spectrometry analysis of the active fraction identified eight proteins as potential t-PA activators. We are currently characterizing the identified SG proteins for activation of t-PA. Studying the interaction between the mosquito saliva, the mammalian fibrinolytic system and the malaria parasite could lay the foundation for the development of new malaria intervention strategies.

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MOSQUITO LIPOGENIC AND LIPOLYTIC PATHWAYS ARE CRITICAL METABOLIC CHECKPOINTS FOR ANOPHELES REPRODUCTION AND PLASMODIUM TRANSMISSION

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Female *Anopheles* mosquitoes require several blood meals during their life span to initiate multiple cycles of egg development, and these obligatory steps are exploited by *Plasmodium* parasites for their own transmission. Blood feeding is therefore a critical step for both mosquito reproduction and parasite transmission that could be exploited to impact malaria dynamics in endemic areas. Here, we elucidated the specific role of blood meal-derived lipids (and/or of lipids synthesized *de novo* after a blood meal) in *Anopheles gambiae* reproduction and *Plasmodium falciparum* development in mosquito stages. Initial targeted lipidomic analyses revealed a coordinated accumulation and depletion of major lipid classes across key mosquito tissues during blood meal digestion, reflective of an engagement of lipogenic and lipolytic pathways. RNA interference (RNAi) against triglyceride (TAG) lipase 2 (TL2) and associated proteins, involved in lipolytic breakdown of TAGs to yield free fatty acids and diacylglycerol (DAG), significantly impairs egg development and abolishes fertility. *TL2*-depleted females have impaired lipid mobilization from midgut and fat body, possibly responsible for the increase in *P. falciparum* growth rates observed in these conditions. Additionally, RNAi inhibition of fatty acid synthase (FAS), the rate limiting enzyme in *de novo* fatty acid synthesis, completely abrogates egg development and *P. falciparum* infection. Altogether, this study identifies both mosquito lipogenic and lipolytic pathways as critical metabolic checkpoints for mosquito reproduction and parasite development, providing new targets for the control of malaria transmission.

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CTL4 CONTROLS TEP1-INDEPENDENT MELANIZATION OF HUMAN MALARIA PARASITES

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Melanization is one of the most effective innate defense mechanisms in mosquito vectors. Numerous studies have shown that the *Anopheles* TEP1-controlled complement-like system is essential for melanization of

the rodent model malaria parasite *Plasmodium berghei,* which evades this defense by recruiting C-type lectins. But the role of TEP1 has not been sufficiently addressed in the context of malaria infection with the clinically relevant human malaria parasite, *Plasmodium falciparum*. Using CRISPR/Cas9 genome editing, we show that the melanization of *P. falciparum* is independent of the TEP1-controlled complement-like system, and a small proportion of *P. falciparum* ookinetes are capable of evading this defense mechanism in the midgut tissue of CTL4^{null} mosquitoes, in contrast to the complete melanization of rodent *P. berghei*. Furthermore, we discovered that the major anti-*Plasmodium* pathway Imd does not influence *Plasmodium* melanization. Our study proves CTL4 as one of the most potent malaria transmission-blocking targets.

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A LABRUM-INTERACTING PROTEIN CONTROLS PROBING IN AEDES MOSQUITOES

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We demonstrate that the Labrum-Interacting Protein-2 (LIPS-2) from the saliva of *Aedes albopictus* females binds a cuticular protein located at the tip of the labrum, regulating the probing phase during the piercing of a vertebrate skin. LIPS-2 is a protein with orthologs found only in species belonging to the subfamily Culicinae and we show that it is characterized by a novel folding topology. Contrary to most mosquito saliva proteins characterized so far, the experimentally-determined structural model of the protein suggests that it lacks any evident enzymatic activity or pockets for small molecule binding. Knocking down the expression of this gene via RNA interference prolongs probing time, while the purified recombinant protein stimulates female mosquitoes to display probing movements. The fluorescently-labeled protein binds to the tip of the labrum at the stylet fascicle forming the food channel. Pre-treatment of the labrum with trypsin causes a remarkable loss of fluorescent signal, suggesting the presence of a protein interactor. Searching for possible interacting proteins, we used a yeast-two-hybrid approach. We built the screening library using mRNA extracted from labra formed mostly within 8 hours from pupation, as highlighted by X-ray tomography analysis of labrum development. From the screening, we identified a cuticular protein as a possible interactor of LIPS-2. Immunofluorescence assays using specific antibodies localized this cuticular protein at the tip of female mosquito proboscis. We further confirmed the interaction between LIPS-2 and its interactor using biochemical and molecular assays. Taken together, our results widen our understanding of the molecular interactions occurring at the site of a mosquito bite, paving the way for the development of strategies to block or to inhibit it. This has major implication considering that the mosquito-borne pathogens are transferred to the vertebrate host together with the insect saliva.

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EVALUATION OF SAFETY AND EFFICACY OF NEW WORLD LEISHMANIA MEXICANA CENTRIN GENE DELETED (LMEXCEN-/-) LIVE ATTENUATED PARASITES AS A PROPHYLACTIC VACCINE AGAINST VISCERAL LEISHMANIASIS

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Leishmaniasis is a vector-borne parasitic disease afflicting millions of peoples worldwide. To date there is no licensed vaccine available against human leishmaniasis. It has been shown that infection with a low dose of dermotropic wild type *Leishmania major* confers protection against cutaneous leishmaniasis (CL) and cross-protects against visceral leishmaniasis (VL). However, such a method of immunization is not practical because of a greater risk of complications in a naïve population. Therefore, genetically modified live attenuated parasites that are non-pathogenic might induce the same protective immunity as leishmanization. Previously, we have demonstrated that immunization with Old World *Leishmania major* lacking the centrin gene can protect against CL and VL. In this study we have explored whether deleting the centrin gene using CRISPR-Cas methodology in *Leishmania mexicana (LmexCen-/-)*, a new world *Leishmania* sp., is safe, immunogenic as well as efficacious against heterologous challenge with *L. donovani* in the hamster model. Intradermal immunization of hamsters with *LmexCen-/-* did not produce detectable lesions, while wild type *L. mexicana* parasites induced severe pathology. Upon immunization, *LmexCen-/-* induced proinflammatory IFN-γ as measured by RTPCR in splenocytes. Seven weeks post-immunization hamsters were infected with *L. donovani* by either needle injection or by infected sand flies. In both cases, ten months post-challenge, non-immunized hamsters developed severe hepatosplenomegaly of VL, while immunized hamsters survived and showed no signs of VL pathology. Additionally, immunized hamsters had a significantly lower parasite burden in the liver and spleen compared to non-immunized animals. Our studies demonstrate that the *LmexCen-/-* mutant parasite is safe and has a potential to be an effective vaccine against VL.

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IN SILICO IDENTIFICATION OF PEPTIDES THAT INTERACT WITH HLA-DRB1 ALLELES ASSOCIATED WITH CHAGAS DISEASE CARDIOMYOPATHY

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Our group has shown that more than 60% of Chagas heart disease patients present at least one HLA-DRB1 allele (*0103, *0402, *1301 and *1302) expressing the DERAA epitope, which may be associated with susceptibility to Chagas disease cardiomyopathy. The objective of this work was to identify peptides from the parasite and from the human host that may be recognized by the *0103, *0402, *1301 and *1302 alleles of HLA-DRB1. Immune Epitope Database tool was used to identify potential antigens that bind to these HLA-DRB1 alleles, while NetMHCIIpan 2.3 was used to determine potential epitopes and to predict peptide-HLA binding affinity. The HPEPDOCK server and Pymol software were used for peptide-HLA-II docking. Our initial analysis showed that no peptide from the parasite was recognized by these HLA-DRB1 alleles. However, we found that antigens from the host immune system such as immunoglobulins and antigens from the HLA system were recognized by theses alleles. In addition, peptides from vimentin, enolase, cathepsin S, collagen and myelin basic protein were potentially recognizable by these alleles. Interestingly, all immunoglobulin-derived putative peptides showed homology with parasite proteins related to escape mechanisms, and proteins crucial for the maintenance and replication of *T. cruzi*. In general, these antigens had a higher binding affinity to the HLA-DRB1 *13 gene. In addition, using a database of cardiac tissue transcripts from patients with Chagas cardiopathy, we observed that mRNA for vimentin, cathepsin S, HLA-DRA and COL2A1 were up-regulated in heart from Chagas cardiopathy patients, but not in idiopathic heart disease, which suggests that these changes are intrinsic to Chagas disease. Determine the nature of the parasite antigen, especially those with high homology to self-antigens with the potential to induce cross-reactivity, and determinate the nature of the HLA-DRB1 molecule that selects a dominant motif, can assist in monitoring patients at potential risk of developing Chagas heart disease, as well as identify potential targets for alternative immunotherapy for human Chagas disease.

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REVEALING THE LOCATION AND CONFIRMING THE PRESENCE OF TC24 IN DIFFERENT PARASITIC LIFE STAGES OF TRYPANOSOMA CRUZI

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Different studies have shown that Tc24-C4, a recombinant version of the flagellar calcium-binding protein of *T. cruzi*, has excellent potential as a therapeutic vaccine candidate to prevent Chagas Disease, showing reduced parasitemia and cardiac inflammation in animal models. Here, we report new findings made with the novel monoclonal antibody (mAb) Tc24-C4/884 raised against Tc24-C4. The antibody, which recognizes recombinant Tc24-C4, wild type Tc24 (Tc24-WT) and native Tc24 from *T. cruzi,* appeared to be very well suited to localize native Tc24 in *Trypanosoma cruzi* by different imaging techniques. Surprisingly, when using fluorescent confocal microscopy to detect binding of Tc24-C4/884 to native Tc24 in T. *cruzi* trypomastigotes, we observed that Tc24 is mostly intracellular, which contradicts the hypothesis made by previous studies that stated that Tc24 is a surface-exposed antigen. Consequently, antibody-mediated immune responses against Tc24 cannot opsonize the parasites or induce antibody-mediated lysis of T. cruzi. Further research using imaging flow cytometry showed that the expression of Tc24 decreases significantly when *T. cruzi* trypomastigotes enter host cells and transform into amastigotes. As part of the flagellum, Tc24 will likely be one of the proteins discarded by the parasite upon host cell internalization, then processed and presented on the MHC-I of infected cells. The cellular response against Tc24 may therefore be a more important part of the mechanism of Tc24-C4 as a vaccine candidate.

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COMPARISON OF FUNCTIONAL CHARACTERISTICS OF CD4+ AND CD8+ T-CELLS IN CHAGAS DISEASES CARDIOMYOPATHY AND IDIOPATHIC HEART DISEASES AND THEIR ASSOCIATION WITH PATHOLOGY

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Chronic Chagas cardiomyopathy (CCC) is the most severe form of Chagas disease, caused by infection with *Trypanosoma cruzi*. While T cells are critical in mediating cardiac pathology in CCC, whether the mechanisms underlying CCC pathology are exclusive of Chagas disease or play a role in other heart diseases is not defined. We sought to study the involvement of potentially cytotoxic $CD4^+$ and $CD8^+$ T cells in CCC or in idiopathic cardiopathies (ID). We evaluated the expression of cytokines and their receptors, cytotoxic molecules, and those associated with cellular recruitment to the heart in circulating CD4+ and CD8+ cells from CCC and ID patients using multiparameter flow cytometry, combined with conventional and unsupervised machine-learning strategies. We also used an *in-silico* approach to evaluate the expression of genes that code for key molecules related to CD4 and CD8 function in CCC and ID. Our data demonstrated an increased frequency of expression of IFN-γ, TNFR1, IL-17, IL-10 by $CD8^+$ T cells in CCC as compared to ID. Analysis of TNFR1/IL10R ratio suggested a predominantly inflammatory profile in CD8+ T cells from CCC. These cells expressed higher frequency of CCR5 and c-MET in CCC, which have been associated with cardiotropism. *In silico* analysis of gene transcripts in the cardiac tissue demonstrated up-regulation of $CD8^+$, IFN-γ, perforin, granzyme A, CCR5, and its ligands CCL3, CCL4 and CCL5 in CCC. There was an increased frequency of TNFR1 in circulating $CD4^+$ T cells from ID, than CCC. These cells expressed CCR4 and CXCR3, and the frequency of $CD4^+$IFN-gamma$^+$ cells was positively correlated with $CD4^+CXCR3^+CCR4^+$ cells in ID. Our data shows that circulating CD8+ T cells from CCC display inflammatory and cytotoxic potential and that the molecules that define those functions are also upregulated in the heart of CCC, suggesting that $CD8^+$ T cells mediate the continuous inflammatory response in CCC. In contrast, $CD4^+$ T cells seem to be the principal agents involved with pathology in ID. These data show the involvement of distinct T cell populations and their functions in CCC and ID, which may contribute to identify specific immunotherapeutic targets.

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TOLL LIKE RECEPTOR-9 (TLR-9) SIGNALING PLAYS AN IMPORTANT ROLE IN LIVE ATTENUATED LEISHMANIA VACCINE INDUCED PROTECTIVE IMMUNITY

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Visceral leishmaniasis (VL), is a vector-borne zoonotic disease with no available vaccine. Recently, we have demonstrated the protective role of live attenuated centrin gene-deleted *Leishmania donovani* (*LdCen*$^{-/-}$) parasite vaccine by induction of strong innate immunity in animal models. Innate responses during VL contribute in parasite control and help in the development of efficient adaptive responses. Amongst those, Toll-like receptors (TLRs), present mainly on the surface of the innate cells, play a crucial role in the initial steps of *Leishmania* infection. Although several studies have shown the importance of TLRs, the role of TLR's in the development of a specific response to a live attenuated *Leishmania* vaccine is not known. Hence, we analyzed the role of TLR's during *LdCen*$^{-/-}$ infection and compared with wild type *L. donovani* (*LdWT*) infection both *in vitro* and *in vivo.* We found that *LdCen*$^{-/-}$ infection significantly upregulates TLR-9 expression on dendritic cells (DCs), along with concomitant downregulation of TLR-2 expression, compared to *LdWT* infection both *in vitro* and *in vivo.* Additionally, we found that *LdCen*$^{-/-}$ infection activates TLR-9 mediated downstream signaling in DCs, facilitating active nuclear translocation of nuclear factor κB. These events culminated in up-regulation of the DC's proinflammatory response, activation and DC mediated $CD4^+$T cell proliferation which was abrogated by treatment with TLR9-specific small interfering RNA. Importantly, TLR-9 silencing in *LdCen*$^{-/-}$ infected DCs significantly increased the expression of TLR-2 and the frequency of IL-10 associated disease promoting nTreg and Tr1 cells. This study demonstrates that TLR9 mediated antagonistic regulation of TLR-2 pathway, and concomitant induction of DC activation, plays a crucial role in shaping the *LdCen*$^{-/-}$ vaccine induced host protective immune response.

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ENDOGENOUS NEUTROPHIL ACTIVATION IN SUBCLINICAL LEISHMANIA BRAZILIENSIS INFECTION

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Individuals with subclinical (SC) *L. braziliensis* infection in Corte de Pedra, Brazil, are characterized by positive leishmania skin test and/or high production of IFN-γ specific to leishmania antigen, in the absence of clinical symptoms. We previously demonstrated that innate immune response contributes to protective immunity in SC. We also showed that neutrophils cooperate to the immunopathogenesis of CL and this phenotype is altered after treatment and cure. We hypothesized that neutrophils from SC individuals display a distinct profile and contribute to immune protection against CL. Neutrophils were isolated from peripheral blood of SC and CL individuals, infected with *L. braziliensis*, stained and evaluated by light microscopy. Additionally, whole blood samples from both groups were stained with antibodies to CD15, CD62L, CD66b, TLR2,

TLR4 and HLA, and assessed by flow cytometry. The oxidative capacity of infected neutrophils was also measured by flow cytometry with CM-H2DCFDA usage. Our results show that the percentage of neutrophils infected, and the total numbers of parasites phagocytosed were lower in neutrophils from SC compared to CL patients. Despite generating greater amounts of ROS, the percentage of infection and parasite burden in CL neutrophils increased over time. SC neutrophils displayed lower amounts of CD62L and trended toward higher expression of CD66b, TLR2 and TLR4 in comparison to CL neutrophils, consistent with a more activated state. In contrast, CL neutrophils showed higher expression of HLA. In summary, neutrophils from SC individuals had a more activated phenotype than CL patients and were less permissive to infection with *L. braziliensis*. Unexpectedly, there was a dissociation between ROS production and parasite killing by CL neutrophils. The expression of HLA on CL neutrophils raises the possibility that cells of the DC-neutrophil hybrid phenotype might play a role in the immunopathogenesis of CL. Whether the observed neutrophil diversity correlates with or drives the outcome of *L. braziliensis* infection is of prime importance in understanding the host protective versus exacerbating response in CL.

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CROSSTALK BETWEEN MYELOID CELLS AND CD4 AND CD8 T CELLS IN THE CONTEXT OF IMMUNE EXHAUSTION

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Leishmaniasis is a chronic disease of reticuloendothelial organs that is usually controlled by TH1-type CD4 T cells. Anti-microbicidal responses are ineffective during disease progression, and it is reported that T cells in humans and dogs with visceral leishmaniasis (VL) express markers of cell exhaustion. We hypothesized that myeloid cells provide counter-receptors for inhibitory receptors on T cells at the local sites of *Leishmania infantum* infection, inhibiting T cell effector functions. In a BL6 mice model of *L. infantum* infection, DCs (CD11b+CD11c+Ly6G^{low}Ly6GC+MHCII+) from infected mice demonstrated an exhausted profile with increased surface expression of inhibitory receptors (IRs) as PDL1, Gal 9, increased secretion of IL-10 and lower surface expression CCR7. Those cells also revealed a dysfunctional state with lower antigen processing and phagocytosis ability, comparing with cells from health animals. Sorted and *L.infantum* mCHerry transfectants infected DCs, presented similar dysfunctional phenotype of DCs from infected mice. Antigen experienced (CD49+ CD11a+) CD4 and CD8 T cells presented a Tex like profile with increased surface expression of IRs, such as PD1, TIM3and a shift for secretion of IL-10. Surprisingly naïve (CD49- CD11a-) and low activated (CD49+ CD11a^{low}) CD4 and CD8 T cells also revealed markers of Tex phenotype in infected animals. Disruption of pro-exhaustion interaction between DCs and Tex using blocking antibodies (@PDL1, @TIM3 or @Galectin 9) ameliorated balance between IL-12 and IL-10 secretion by DCs, but also increased T cell expansion, induced a cytokine profile to a resistant pattern with increased IFN-g and reduced IL-10. In treated cultures, blockade strategies cited above also increased surface markers of memory like cells (CD49d, CD11a, CD45RO). In vertical transmitted dogs with canine leishmaniasis, clinical score correlates with number of CD14+PDL1+ cells in several tissues as liver, spleen and bone marrow. Data suggests that L. infantum parasites may benefit from pro-exhaustion crosstalk between myeloid cells and T cells to evade immune mechanisms of infection control.

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NUTRITIONAL SUPPLEMENTATION COST-EFFECTIVELY DECREASES TUBERCULOSIS INCIDENCE AND MORTALITY IN INDIA: THE RATION OPTIMIZATION TO IMPEDE TUERCULOSIS MODEL

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Undernutrition is the leading risk factor for tuberculosis (TB) in India and is associated with increased TB incidence, severity, and mortality. Through mathematical modeling, we wanted to explore if nutritional interventions could be used cost-effectively to end India's TB epidemic. Using available cost and epidemiologic data, we developed a cohort model which simulated TB disease progression and mortality among undernourished individuals. We simulated an augmented rations intervention wherein undernourished Indian adults would receive 2600Kcal/d diet until they attained a BMI of 20kg/m^2 from the existing government ration system and compared it to a continuation of current subsidies. The model calculated costs and outcomes including TB cases, TB deaths, attainment of BMI >20kg/m^2, and quality-adjusted life years (QALYs). Over 5 years, augmented rations could avert 78% of TB cases, prevent 88% of TB deaths among undernourished Indians, and yield a ten-fold higher resolution of undernutrition compared to the status quo. Additionally, we found that augmented rations would be highly cost-effective, as compared to usual rations, with an estimated incremental cost-effectiveness ratio of $460 which is considerably lower than the per-capita GDP of India ($2104). Most of the cost-effectiveness was driven by the collateral benefit of reducing undernutrition. Our findings were robust to deterministic and probabilistic sensitivity analyses. We conclude that a robust nutritional intervention would be highly cost-effective in reducing TB incidence and mortality while reducing widespread undernutrition in India.

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DIETARY INTAKE AND PNEUMOCOCCAL VACCINE RESPONSE AMONG CHILDREN (5-7 YEARS) IN MSAMBWENI DIVISION, KWALE COUNTY, KENYA

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Vaccine and sufficient food availability are key factors for reducing pneumonia outbreaks in sub-Saharan Africa. In this study, the 10-valent pneumococcal conjugate vaccine (Synflorix® or PCV10) was administered to a child cohort (5-7 years old, n=230) in Msambweni division, Kenya, to determine relationships between dietary intake, nutritional/socioeconomic status of mothers/caregivers, and vaccine response. 7-day food frequency questionnaire(FFQ), dietary diversity score (DDS) and single 24-h dietary recall were used to address participants' dietary assessment and nutritional status. Individual food varieties were recorded and divided into 9 food groups as recommended by Food and Agriculture

Organization. Anthropometric measurements, nasopharyngeal swabs and vaccine administration were performed at the initial visit. Participants were followed after 4-8 weeks with a blood draw for pneumococcal IgG titers assessed by Luminex assay. Chronic malnutrition was prevalent in the cohort (15% stunting, 16% underweight). Unbalanced dietary intake was observed, with mean energy intake 14% below Recommended Dietary Allowances (1822Kcal) for 5-7 years age range.72% of the daily energy derived from carbohydrates, 18% from fats and only 10% from proteins. Poor anthropometric status (stunting/underweight) was associated with low socioeconomic/educational status and mothers/caregivers young age($p<0.001$). Lack of essential micronutrients (vitamins A, E, K) and minerals (calcium, potassium) due to poor consumption of fresh fruits, vegetables and animal source foods (dairy, meat) was observed and correlated with poor vaccine response($p<0.001$). In contrast, children who consumed higher amounts of dietary fiber, vitamin B1, zinc, iron, and magnesium had adequate vaccine response($p<0.05$). Correlation between higher dietary diversity score (DDS), higher Vitamin E, K, Zinc intake and adequate vaccine response was also observed($p<0.03$). Overall, this study highlights ongoing food scarcity and malnutrition in Kenya and demonstrates the links between adequate socioeconomic conditions, nutrition status and vaccine efficacy

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THE GUATEMALA AGRICULTURAL WORKERS AND RESPIRATORY LLLNESS IMPACT STUDY: CLINICAL AND SOCIOECONOMIC IMPACT OF RESPIRATORY ILLNESS IN A COHORT OF GUATEMALAN AGRICULTURAL WORKERS

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The Agricultural Workers and Respiratory Illness (AGRI) study aims to evaluate the clinical and socioeconomic impacts of respiratory illness (influenza, RSV, and COVID-19) among Guatemalan agricultural workers and the potential benefits of work-based vaccination. In the study, a continuously enrolled prospective cohort of banana plantation workers undergoes influenza-like illness (ILI) surveillance using 3 strategies: 1) self-reporting to study personnel at weekly site visits or by phone, 2) sentinel surveillance at worker-health posts, and 3) active screening of workers with absenteeism. Workers with ILI have influenza and RSV RT-PCR (Roche cobas® Liat) and SARS-CoV-2 antigen testing (SD Biosensor®). Workers with ILI and randomly selected asymptomatic controls complete clinical (Flu-iiQ® inventory, outcomes) and economic (direct/indirect costs, household expenditures) surveys at the time of illness and at 7- and 28-day follow up. The study also collects company-reported absenteeism and productivity data (units of production, wages) and collects annual and ILI-related blood samples for serology. Beginning in June 2020, 1,431 workers were enrolled (80.8%); the cohort is mostly male (84.1%) and young (31.3 years, SD=8.7); 69.2% work in the fields and mean monthly income is $355.7 (SD=$128.0). Obesity (10.6%) and kidney disease (3.4%) are the most common self-reported comorbidities. The first 30 workers with ILI (7 SARS-CoV-2, 1 RSV, 0 influenza) had greater Flu-iiQ severity scores at day 7 (2.15 vs 0.44, p=0.02) and 28 (2.28 vs 0.45, p=0.07) than controls (n=236). The first 7 workers with COVID-19 had higher Flu-iiQ severity scores compared to SARS-CoV-2-negative ILI (O-ILI) at day 0 (28.0 vs 12.95, p=0.02), day 7 (3.67 vs 1.31, p=0.15), and day 28 (4.0 vs 0.76, p=0.27). COVID-19 cases had more work absenteeism (p=0.0002) and lost income ($177.8 vs $16.4, p=0.02) than O-ILI at day 7. Per self-report, the worker was usually the index ILI case within their household (93.1%) but site of acquisition (workplace or other) could not be determined. In summary, our preliminary data suggest ILI and COVID-19 placed a significant clinical and economic burden on Guatemalan agricultural workers.

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"COUGHING? IT MIGHT BE TB": ADDRESSING COUGH-RELATED STIGMA IN THE TIME OF COVID-19 THROUGH SOCIAL AND BEHAVIOR CHANGE IN NIGERIA

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Nigeria experienced a noticeable decline in uptake of Tuberculosis (TB) services at the start of the COVID-19 pandemic. Lockdown measures restricted clients' ability to go to health facilities for testing and treatment, similarity in TB and COVID-19 symptoms delayed access to care as patients were screened for COVID-19 before considering TB, and cough-related stigma deterred health-seeking behavior. To mitigate the effects of COVID-19, the USAID-funded Breakthrough ACTION-Nigeria (BA-N) project, the National TB and Leprosy Control Program and TB stakeholders collaborated to develop a national "Check Am O!" social and behavior change (SBC) campaign to address TB in the context of COVID-19. The campaign targeted anyone with any form of cough lasting more than 2 weeks. The main objectives were to increase knowledge of the difference between TB and COVID-19 symptoms, and testing for TB if presenting with symptoms suggestive of TB. Strategic communication approaches included radio spots, animated videos on television and social media, posters, stickers, brochures and a provider job aid. All materials encouraged the audience to call the toll-free national TB hotline for more information. The campaign went on air in December 2020, and formally launched for World TB Day in March 2021. BA-N aired 13,614 radio spots and 2,944 television spots between December 7, 2020 and March 31, 2021. Preliminary data from the April 2021 omnibus survey showed that 71% of adult men and women had been exposed to the campaign. The National TB Hotline received 22,028 calls in the October to December 2020 quarter and 209,125 calls in the January to March 2021 quarter, suggesting the campaign was effective in increasing calls to the hotline, a proxy indicator for behavior change. Additional data on associated knowledge, attitudes and practices and national TB service delivery statistics before and after campaign implementation will be shared in the final presentation. This was the first national SBC campaign to address TB in the context of COVID-19. The campaign's success suggests that this approach may be useful for adaptation in other countries.

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IT'S A FAMILY AFFAIR: HIGH RATES OF INTRA-HOUSEHOLD TRANSMISSION OF SARS-COV-2 IN A HYBRID CLUSTER - COHORT STUDY IN CENTRAL NEW YORK

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Transmission of SARS-CoV-2 within households is a major driver of the global pandemic. In order to assess risk of infection of household associates, we are conducting a community-based cluster study of households in central New York, which began in June 2020. Recently confirmed SARS-CoV-2 infections were identified from Upstate University Hospital medical records. Eligible households were briefed and consented by phone. Enrolled 'initiating' cases and their household 'associates' underwent phone interview, PCR testing at enrollment, and collection of serum at enrollment and roughly 6 weeks later. SARS-CoV-2 infection

status was determined by PCR, IgM positivity, IgG seroconversion, or self-report of a positive test within two weeks of enrollment. Mixed effects logistic regression, accounting for clustering by household, was conducted to analyze risk factors of infection among associates. Through March 2021, we enrolled 70 symptomatic initiating cases and 113 household associates (1-4 associates per initiating case). The median age of participants was 34 years (1-78 years). 55.1% identified as female, 69.9% as non-LatinX white, and 40.0% as essential workers. At enrollment, 48 (42.5%) associates were confirmed to have recent SARS-CoV-2 infection. By week 6, an additional 5 (7.7%) associates were found positive by IgG seroconversion. Among associates infected at enrollment, 14 (29.2%) had an asymptomatic infection, and 11 (22.9%) reported CDC 'warning signs' of shortness of breath, chest pain, or confusion. The odds of infection in adults was 2.56 times that of children. Essential workers (OR=5.54) and individuals of LantinX ethnicity (OR=2.32) were also more likely to be infected. In a multivariate analysis controlling for age, race, presence of comorbid medical conditions, and essential worker status, essential workers remained more likely to be infected (OR = 5.12). We found a very high risk for SARS-CoV-2 infection among household associates with numerous cases captured by serologic testing alone. Essential workers are at particularly high risk and should practice extreme caution if working during the quarantine period.

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INCREASED COVID-19 DISEASE SEVERITY IN HOSPITALIZED AMERICAN INDIAN/ALASKA NATIVE PATIENTS

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In the US, the American Indian/Alaska Native (AI/AN) population has been disproportionately affected by coronavirus disease 2019 (COVID-19) cases, hospitalizations, and deaths. However, there have been limited reports describing relationships between patient risk factors and the COVID-19 disease course in the AI/AN population. The identification of attributes contributing to disease severity is key for improving clinical outcomes. In this study, hospitalized COVID-19 patients (n=332) admitted between 23 April 2020 and 2 February 2021 were recruited from the University of New Mexico Hospital. Patient medical histories and hospital course information were obtained via medical chart reviews and interviews with the patient or legally authorized representative. Data analysis was conducted using Fisher's Exact Test for proportional data and one-way ANOVA for mean data. Patients were stratified by self-reported ancestry: AI/AN (n=121), Hispanic (n=135), non-Hispanic White (n=63), and Other (n=13; not included in statistical analyses due to sample size). On average, the patients were symptomatic for 6.2±5.2 days prior to hospitalization, with no significant difference between groups. AI/ANs were more likely to present at hospital with cough (P=0.004) and increased oxygen requirements (P=1.7x10^{-4}). Established risk factors for COVID-19 severity (e.g., age, BMI, respiratory disease) were either comparable or less prevalent in the AI/AN patients. During the hospital course, AI/ANs had a higher proportion of ICU admissions/deaths (P=0.036), increased oxygen requirements (P=0.022), and longer hospitalization times (P=0.001). These findings suggest that despite the lack of underlying conditions and a similar symptomatic time period prior to hospitalization, the AI/AN group had more severe disease outcomes than the Hispanic and non-Hispanic White groups. Further research should investigate other potential factors contributing to disease severity and the development of specialized care options to alleviate the escalated disease burden on the AI/AN population.

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CROSS-REACTIVITY OF PRE-COVID-19 PANDEMIC SERA FROM A MALARIA-ENDEMIC AREA ON TWO SARS-COV-2 SEROLOGICAL ASSAYS

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Accurate SARS-CoV-2 serological assays are critical for COVID-19 serosurveillance. However, prior studies have indicated possible cross-reactivity of some assays, including in malaria-endemic areas. We tested 213 pre-pandemic samples from Nigeria using two SARS-CoV-2 serological assays: the Abbott Architect IgG and the Euroimmun NCP IgG assay, both targeting the SARS-CoV-2 nucleocapsid protein (NCP). Pre-pandemic samples from 2018 were previously tested for malaria antigens and antibodies to malaria and other endemic pathogens using a Luminex bead-based multiplex assay. To assess antibody binding strength, an avidity assay was performed on these samples and on plasma from 32 SARS-CoV-2 RT-PCR-positive persons. Thirteen (6.1%) of 212 samples run on Abbott and 38 (17.8%) of 213 run on Euroimmun were seropositive for SARS-CoV-2. Anti-*Plasmodium* IgG levels were significantly higher among -false-positives for both Abbott and Euroimmun for five of nine malaria antibodies: PfCSP, pmmsp1, pomsp-1, glurp (Euroimmun only), and Pfama1 (Euroimmun only), but not for pfmsp1, pvmsp1, hrp2 or lsa1. No association was found with active *P. falciparum* infection. An avidity assay at various concentrations of urea wash in the Euroimmun assay reduced loosely-bound IgG: of 37 positive/borderline pre-pandemic samples, 46%, 86%, 89%, and 97% became negative using 2M, 4M, 5M, and 8M urea washes, respectively. The wash reduced antibody avidity in samples from SARS-CoV-2 patients taken ≤28 days of RT-PCR confirmation; thereafter, avidity increased for all urea concentrations except 8M, which decreased avidity on SARS-CoV-2-positive samples to <30%. This validation found moderate cross-reactivity on two SARS-CoV-2 NCP-based serological assays in samples from a malaria-endemic setting. A single urea wash appeared to alleviate cross-reactivity but needs further validation and assessment of optimal concentration. SARS-CoV-2 serological assays targeting NCP should be interpreted with caution in malaria- endemic settings; use of a urea wash, multi-assay testing algorithms, or multi-antigen-target assays should be considered in such settings.

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REVIEWING THE EVIDENCE FOR AND AGAINST SELECTION OF SPECIFIC PYRETHROIDS FOR PROGRAMMATIC PURPOSES

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Pyrethroids are present in all WHO prequalified insecticide treated nets (ITNs) and are still used for indoor residual spraying (IRS). Pyrethroid resistance is widespread in malaria vectors, and differential mortality in discriminating dose assays to different pyrethroids is often observed during susceptibility monitoring in the lab and field. However, there is uncertainty about whether the monitoring methods can reliably identify differential resistance phenotypes. Therefore, when differential mortality is observed, it is unclear if this should be interpreted as an indication of differential levels of susceptibility within the pyrethroid class, or if this should be interpreted in another way (e.g. inherent variability in mortality results; differently calibrated discriminating doses). Considering this, when 'differential susceptibility' is observed there is a question over whether countries can target or preferentially use specific pyrethroid insecticides as an effective resistance management strategy. We reviewed evidence from (i) molecular information, insecticide resistance patterns and testing results (ii) in laboratory colonies and (iii) from field data, and (iv) lessons learned from behavioural assays, to address these questions. Where one pyrethroid is seen to be consistently more effective in resistance screening, preferably coupled with additional, more direct evidence of superior performance, the pyrethroid with the greater killing capacity should probably be deployed, if costs and other considerations, most notably formulation or delivery method (e.g. net type), are comparable. However, the evidence suggests that in areas where pyrethroid resistance exists, different results in insecticide susceptibility assays are not necessarily indicative of a true or operationally relevant difference in potential performance of the specific pyrethroids currently in common use (deltamethrin, permethrin, α-cypermethrin and λ-cyhalothrin). It is not advisable to use rotation between these pyrethroids as an insecticide resistance management strategy.

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EFFICACY OF A SPATIAL REPELLENT FOR CONTROL OF AEDES-BORNE VIRUS TRANSMISSION: A CLUSTER RANDOMIZED TRIAL IN IQUITOS, PERU

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Over half the world's population is at risk for viruses transmitted by *Aedes* mosquitoes, such as, dengue and Zika. The primary vector, *Aedes aegypti*, thrives in urban environments. Despite decades of effort, cases and the geographic range of *Aedes*-borne viruses (ABV) continue to expand. To date, there are no rigorously proven vector control interventions that prevent ABV diseases. Spatial repellents, a new option, are designed to decrease human exposure to ABV by releasing active ingredients into the air that disrupt mosquito-human contact. A parallel, cluster-randomized controlled trial was conducted in Iquitos, Peru to quantify the impact of a transfluthrin-based spatial repellent on ABV infection. From 2,907 households across 26 clusters (13 per arm), 1,578 participants were assessed for seroconversion (primary endpoint) by survival analysis. Incidence of acute disease was calculated among 16,683 participants (secondary endpoint). Bi-monthly adult mosquito collections were conducted to compare *Ae. aegypti* abundance, blood-fed capture rate and parity status through mixed effect difference-in-difference analyses. The spatial repellent significantly reduced ABV infection by 34.1% (95% CI 6.9%, ∞); p = 0.0236, z=1.98). *Aedes aegypti* abundance and blood-fed capture rates were significantly reduced by 28.6% (95% CI 24.1%, ∞); z=-9.11) and 12.4% (95% CI 4.2%, ∞); z=-2.43), respectively. Our trial provides the first conclusive evidence of significant protective efficacy by any chemical vector control intervention, in this case a spatial repellent, to reduce the risk of ABV transmission. Results support vector control as a beneficial component to ABV disease prevention.

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INVESTIGATING BIRD-DELIVERED IVERMECTIN AS A NOVEL URBAN WEST NILE VIRUS CONTROL STRATEGY

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Current mosquito control strategies have limited ability to target *Culex* mosquitoes involved in transmission of West Nile virus (WNV) without disseminating insecticides over large areas. The endectocide ivermectin (IVM) offers a potential alternative, due to its recently described mosquitocidal properties and ability to deliver it via birds that are frequently bitten by WNV vectors. We hypothesized that treating birds that are common bloodmeal sources for *Culex* mosquitoes with IVM will reduce WNV transmission because few mosquitoes will survive long enough to take a subsequent bloodmeal. To assess the efficacy and feasibility of this strategy, we conducted a randomized field trial in backyard chickens and then developed a mathematical model informed by birdfeeder usage and nocturnal roosting habits of common backyard birds. First, we placed 48 chickens into 4 treated and 4 untreated control flocks in backyard coops across Davis, California, administering IVM daily in feed to treated flocks (Jul-Sep 2019). We assessed entomological indices weekly around each coop, monitored serum IVM levels, and tested for WNV antibodies in all chickens. WNV seroconversions were reduced in treated vs. untreated flocks, indicating reduced WNV transmission intensity at treated coops. We also observed a reduction in parous mosquitoes near treated flocks, paired with increased mortality of wild *Culex* following a bloodmeal on a treated vs. untreated chicken, suggesting a change in population structure, and subsequently viral dynamics, due to IVM. To estimate the potential for IVM-based control in wild birds, we developed a spatially-implicit mathematical model of WNV transmission in a neighborhood with IVM-treated birdfeeders. Parameters for bird movement were based on our telemetry of 27 birds in Fort Collins, Colorado (Aug-Sep 2020). Using the model, we estimated up to 84% reduction in infectious mosquito-days and 61% fewer bird infections with IVM-treated feeders. Future work is

needed to refine estimated IVM-induced mortality in wild mosquitoes. Our work provides the foundation for designing future lab and field studies of IVM for local WNV control.

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GENE EXPRESSION TEMPORAL ANALYSIS OF PYRETHROID RESPONSE IN CALIFORNIAN AEDES AEGYPTI

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Aedes aegypti, the vector of Zika and dengue, first established in California in 2013 and have since spread to 22 counties across the state. These mosquitoes arrived with established resistance to pyrethroids, the primary class of insecticides used for public health applications. Pyrethroids act on the insect voltage gated sodium channels causing prolonged depolarization and death, and resistance can be developed through mutations at the target site or through over-expression of detoxifying enzymes like cytochrome P450s and glutathione-S-transferases (GSTs). However, there are over 100 genes coding for P450 enzymes alone in *Ae. aegypti*. To identify putative resistance mediating enzymes as well as the overall genetic response to insecticide exposure, mosquitoes were exposed to permethrin for 1 hour via CDC Bottle Bioassay. They were then placed in a cage and collected 6, 10, and 24 hours post exposure. The transcriptional profiling of the response to pyrethroid exposure identified 20 cytochrome P450s and 6 GSTs that demonstrated significant increases in transcript abundance suggesting their involvement in reducing the toxic effects of pyrethroid exposure. Enzymes associated with oxidation-reduction reactions are overrepresented across time points suggesting the response to insecticidal challenge results in a significant amount of oxidative stress. Another significant feature identified in this analysis revealed upregulation of genes coding for enzymes required for fundamental metabolic activities including glycolysis, the pentose phosphate shunt as well as lipid metabolism. Overall, these findings reveal that this strain of mosquitoes have a strong genetic response to insecticide exposure. In addition, this response appears to require activation of other fundamental metabolic pathways to reduce the associated oxidative stress and to maintain the demand for energy generated by these activities. These results provide evidence of metabolic resistance in this population, new enzymatic targets for investigation, and generates new questions to pursue as to the resistance profile of these invasive Californian populations.

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MAPPING THE FREQUENCY OF GENETIC MUTATIONS CONFERRING INSECTICIDE TARGET SITE RESISTANCE IN AFRICAN MALARIA VECTOR SPECIES

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Resistance in malaria vectors to pyrethroid insecticides, the most widely used class of insecticides for malaria vector control, is a concerning threat to the efficacy of vector control tools including long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). Target site resistance is an important genetic mechanism of resistance to pyrethroids that is caused by mutations in the *Vgsc* gene that regulates the voltage-gated sodium channel, the target site of pyrethroid insecticides in mosquitoes. Understanding the geographic distribution of target site resistance mechanisms, and how this is changing over time across different malaria vector species, can inform strategic deployment of vector control tools. Further, geospatial analyses can quantify relationships between phenotypic pyrethroid resistance in field malaria vector populations and the prevalence of target site resistance mechanisms. Here we develop a Bayesian statistical spatiotemporal model ensemble to interpret species-specific trends in the frequency of two target site resistance mutations in the *Vgsc* gene, 995S and 995F, in the three vector species *An. gambiae*, *An. coluzzii,* and *An. arabiensis* over the period 2005-2017. The models are informed by 2418 observations of the frequency of each mutation in field sampled mosquitoes collected from 27 countries spanning western and eastern regions of Africa. For nine selected countries, we develop annual predictive maps which reveal geographically structured patterns of spread of each mutation at regional and continental scales. The results show associations, as well as stark differences, in spread trajectories of the two mutations across the three vector species. We also find that our mapped *Vgsc* allele frequencies are a significant partial predictor of the prevalence of phenotypic resistance to the pyrethroid deltamethrin in *An. gambiae* complex mosquito populations. The results demonstrate how patterns of phenotypic resistance seen in field vector populations are linked to both vector species composition and target site resistance mechanisms.

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INSECTICIDE SUSCEPTIBILITY OF ANOPHELES GAMBIAE S.L. POPULATION IN HEALTH DISTRICTS AFTER DEPLOYMENT OF NEW GENERATION NETS IN BURKINA FASO

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Long-Lasting Insecticide-treated Nets and Indoor Residual Spraying are the common tools used in vector control that have contributed to reducing malaria transmission in Sub-Saharan Africa. However, the rise and spread of insecticide resistance is a threat to the continued success of these tools. With the advent of new generation insecticide-treated nets (ITN), Burkina Faso has deployed multiple ITN types since 2019, including PBO-pyrethroid, Interceptor G2, and standard pyrethroid-only nets, guided by local resistance patterns and malaria burden. The current study aimed at monitoring the insecticide resistance intensity and mechanisms in the net deployment areas after the 2019 mass distribution campaign. Here, we are undertaking three-years (2019 - 2021) of insecticide resistance monitoring in seven villages from four distinct deployment areas which received a different type of ITN. Thus, experimental hut trials (EHT) at two villages known for high insecticide resistance level and different vector species composition) and World Health Organization (WHO) bioassay tube tests (using deltamethrin and alphacypermethrin) are performed. Screening for the variation in the expression of putative metabolic resistance genes and frequency of target site mutation are underway. No variation in mortality was observed considering the result from the WHO standard tube test results between the two years. *Anopheles gambiae* complex member's overall mortality rate was ~18% after exposure to the diagnostic dose (DD) and ~76% after exposure to 10 times the DD of both insecticides. Preexposure to the synergist PBO showed 10 - 72% increase in the mortality, varying by study villages, indicating an involvement of cytochrome P450 and esterase in the resistance among other mechanisms. However, the preliminary results indicated 18 -50% decrease in the mortality rate of the local vector to all type of tested nets (standard and new generation) in the EHT between 2019 and 2020. Results indicate variation in the mortality rate between sites and decrease in susceptibility of the local vector to all types of nets tested including new generation net in huts trials.

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VECTRON™ T500 (BROFLANILIDE), A NEW INSECTICIDE FOR INDOOR RESIDUAL SPRAYING PROVIDES IMPROVED AND PROLONGED CONTROL OF PYRETHROID-RESISTANT MALARIA VECTORS IN BENIN

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The rotational use of insecticides with different modes of action for indoor residual spraying (IRS) is recommended for improving malaria vector control and managing insecticide resistance. Insecticides with new chemistries are urgently needed and broflanilide is a newly discovered insecticide with a novel mode of action. We investigated the efficacy of a wettable powder (WP) formulation of broflanilide (50%) for IRS on mud and cement wall substrates in experimental hut against pyrethroid-resistant malaria vectors in Benin, in comparison with pirimiphos-methyl CS (Actellic 300CS). There was no evidence of cross-resistance to pyrethroids in CDC bottle bioassays. At application rate of 100 mg/m^2 of VECTRON™ T500 in experimental huts in Covè, Benin, the mortality of wild pyrethroid-resistant *An. gambiae* sl entering the huts over 12 months was 53% to 63%, which was mostly similar to pirimiphos-methyl CS (53%), regardless of wall substrates. Initial experimental hut mortality rates with VECTRON™ T500 were steady over 11 months. Monthly, *in situ* wall cone bioassay mortality of susceptible and pyrethroid resistant mosquitoes remained >80% for 16 months with VECTRON™ T500, irrespective of the substrate and for 7-8 months with Actellic CS. The results of this hut study show that VECTRON T500 is non-inferior to Actellic 300CS against pyrethroid-resistant mosquito vectors and could thus be a crucial addition to the current portfolio of IRS insecticides.

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SAFETY OF SINGLE DOSE MOXIDECTIN COMBINATION THERAPY FOR BANCROFTIAN FILARIASIS

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In sub-Saharan Africa, the World Health Organization (WHO) recommends 5-7 years of mass drug administration (MDA) for elimination of lymphatic filariasis (LF) with a combination of ivermectin (IVM) and albendazole (ALB), and a combination of IVM plus ALB plus diethylcarbamazine (DEC; IDA) in areas of the world without onchocerciasis or loiasis. Moxidectin (Mox) has recently been approved for treatment of onchocerciasis in people 12 years and older. We are now conducting the first clinical trial of moxidectin for LF. This Phase III, randomized, open-label, masked-observer superiority trial in Côte d'Ivoire compares Mox+ALB (MoxA) with IVM+ALB (IA) and Mox+DEC+ALB (MoxDA) with IDA for treating LF. In a separate abstract we have described the preliminary efficacy results. Here we describe its safety and tolerability. We enrolled 141 non-pregnant, non-breast feeding adults 18-70 years old with no chronic medical problems, 99 with *Wuchereria bancrofti* microfilariae (Mf) levels >40Mf/mL and 42 with Mf levels <40Mf/mL. The first 73 participants were treated and monitored closely in an inpatient setting with active adverse event (AE) monitoring, including daily tests of liver and kidney function for 72 hours post-treatment, and again at day 7. The subsequent 68 participants were treated in their village and followed for AEs at 24 and 48 hours. There were no serious AEs. Seventy-one percent of participants experienced at least 1 AE; 85% of all AEs were mild (grade I). The most common AEs were creatinine elevations (20%), increased respiratory rate (20%), muscle and joint pain (18%), hypertension (17%), AST elevation (16%) and headache (15%). There was no difference in the rate (χ^2 p = 0.59) or severity (Wilcoxon p = 0.21) of AEs across treatment groups, and rates of AEs did not differ between those with higher (>40) vs lower (<40) Mf counts (χ^2 p = 0.75). These first data suggest that Mox is safe for use in combination with ALB and/or DEC for LF elimination. Given the established safety of Mox and ALB for onchocerciasis, MoxA may also be useful for areas co-endemic for LF and onchocerciasis.

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MOXIDECTIN EFFICACY IN SINGLE DOSE COMBINATION THERAPIES WITH ALBENDAZOLE AND DIETHYLCARBAMAZINE FOR TREATMENT OF LYMPHATIC FILARIASIS IN CÔTE D'IVOIRE: PRELIMINARY RESULTS FROM A RANDOMIZED CONTROLLED TRIAL

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Lymphatic filariasis (LF) remains endemic in much of Africa despite many years of annual mass drug administration of ivermectin (IVM) and albendazole (ALB). Moxidectin (Mox) is a macrocyclic lactone closely related to IVM but with a much longer half-life and has proven more effective for sustained suppression of onchocerciasis microfilardermia compared to IVM. To determine whether Mox combination therapy might also be superior for LF, we are conducting a phase III, randomized, open-label superiority trial comparing Mox+ALB (MoxA) against annual IVM+ALB (IA), the current standard mass drug administration (MDA) used in areas of sub-Saharan Africa not coendemic for loiasis. This is the first study to evaluate Mox efficacy against *Wuchereria bancrofti*. We are concurrently comparing Mox + diethylcarbamazine (DEC) + ALB (MoxDA) against IVM+DEC+ALB (IDA), which is used for MDA in areas outside sub-Saharan Africa unlikely to meet 2030 LF elimination goals. The primary study endpoints are Mf clearance at 12 (IA vs. MoxA) or 24 (IDA vs. MoxDA) months post-treatment, as measured by 1ml filtration of night venous blood. Enrolment for the study began in August, 2020, and to date we have 6-month efficacy data for 44 adult participants infected with >40 Mf/) per mL of blood at baseline (MoxA n=10, MoxDA n=11, IDA n=10 and, IA n=13). To date, none of the participants in the MoxA (baseline Mf range 52 - 549) or MoxDA group (baseline Mf range 83 - 1633) had detectable blood Mf at 6 months post-treatment. In contrast, eight (62%) of the IA recipients (baseline Mf range 73 - 1091) and four (40%) of the IDA recipients (baseline Mf range 61 - 946) were microfilaremic (Mf ranges 0 - 153 and 0 - 27), at 6 months (p=0.001 Kruskal-Wallis equality of populations rank test). These preliminary data strongly suggest that combination therapies with MoxA and MoxDA are superior to IA and IDA in clearing *W. bancrofti* Mf at 6-months. Study enrolment is ongoing, and 6-month data from additional participants, as well as 12-month data from the participants reported here should be available by August 2021.

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TRANSCRIPTOMIC ANALYSIS REVEALS ACTIVATION OF CONSERVED IMMUNE PATHWAYS IN THE HUMAN SKIN RESPONSE TO THE BITES OF DIVERGENT VECTOR SPECIES OF GLOBAL HEALTH IMPORTANCE

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Arthropod vectors such as mosquitoes and sand flies deliver infectious pathogens into the skin of humans while taking a blood meal. Pathogens are co-delivered with vector saliva containing a complex mixture of immunomodulatory components, including salivary proteins and microbiota. In animal models, vector bites and saliva are critical for establishing infection and exacerbating disease pathogenesis. However, the mechanisms by which vector bites do this in humans remain poorly understood. To address this, we conducted the BITE Study, the first clinical study to perform global profiling of the human skin immune response to vector bites. Healthy human volunteers were exposed to uninfected bites of one of three vectors of global health importance – the mosquitoes *Aedes aegypti* and *Anopheles gambiae*, or the New World sand fly *Lutzomyia longipalpis*. Skin biopsies were collected from bite sites at 4 hrs and 48 hrs after vector exposure. The skin transcriptome from the bite sites was determined by RNA-seq and compared to unbitten skin as a negative control. Despite these vector species being separated by over 200 million years of evolutionary divergence, upstream regulator analysis revealed remarkable conservation of the skin transcriptomic response to vector bites, including prominent activation of TNFα, TLR9, and interferon/STAT1 signaling, even in the absence of pathogens. In agreement with histologic findings, computational deconvolution of the bulk RNA-seq data highlighted a transient early influx of neutrophils and monocytes at 4 hrs post-bite, followed by later recruitment of monocytes, T cells, and dendritic cells to bite sites by 48 hrs. Current work is focused on applying digital spatial profiling to determine the architecture and organization of cutaneous immunity to vector bites. Our discovery of a conserved vector bite response in humans underscores the potential for developing vaccines against vector-borne pathogens by targeting mediators of early immune events in the skin.

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HUMORAL IMMUNITY AGAINST AEDES AEGYPTI SALIVA ASSOCIATED WITH DEVELOPMENT OF INAPPARENT DENGUE: A LONGITUDINAL OBSERVATIONAL COHORT IN CAMBODIA

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Defining dengue burden and the *Aedes aegypti* vector determinants that exacerbate transmission and disease outcome are critical for Cambodian public health authorities. Characterization of human immunity to *Aedes* mosquito saliva is required to expand the armamentarium of control measures for sudden and emerging mosquito-borne epidemics. We established the first prospective cohort to understand *Aedes aegypti* saliva-specific humoral immunity in the context of dengue virus infection. Children aged 2 to 9 years old enrolled in a community-based cohort called PAGODAS (Pediatric Assessment Group of Dengue and Aedes Saliva) in Cambodia. They were followed semi-annually for antibodies to dengue virus and *Ae. aegypti* salivary gland homogenate using enzyme-linked immunosorbent assays and dengue-specific neutralization titers. Children presented with fever at any time to undergo dengue rapid testing and confirmatory polymerase chain reaction. From July 13 to August 30, 2018, we enrolled 771 children and follow them to present day. At baseline, 22% (173/770) of children had evidence of neutralizing antibodies to one or more serotypes of dengue. Of the 597 dengue-naïve children in August 2018 to August 2020, 51 children had symptomatic PCR-confirmed dengue while 148 dengue-naïve children had clinically inapparent dengue defined by neutralization assays. Individuals with higher antibodies to *Ae. aegypti* salivary gland homogenate were 1.5x more likely to seroconvert for any dengue serotype on PRNT50 (HR 1.47 95% CI 1.05–2.06; p=0.02), primarily driven by individuals with inapparent dengue (HR 1.64 95% CI 1.12–2.40; p=0.01). High levels of humoral immunity to *Ae. aegypti* saliva are associated with future development of dengue infection, specifically inapparent dengue, in dengue-naïve Cambodian children. This provides the rationale to explore how anti-vector saliva antibodies may influence development of clinical disease in humans.

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SAFETY AND IMMUNOGENICITY OF AGS-V PLUS, A MOSQUITO SALIVA PEPTIDE VACCINE AGAINST ARBOVIRAL DISEASES

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Mosquito-borne diseases cause ~360 million cases and >600,000 deaths annually, although the true burden is likely underestimated. A vaccine efficacious against multiple mosquito-borne diseases could significantly impact public health. AGS-v PLUS is a vaccine containing five mosquito salivary peptides, aiming to induce an immune response that blocks mosquito-borne pathogen transmission. A Phase 1 randomized, double-blinded, placebo-controlled clinical trial was conducted to determine safety and immunogenicity of AGS-v PLUS. Five dosing groups received injections on days 1 and 22 as follows: Group 1, two doses non-adjuvanted AGS-v PLUS; Group 2, ISA-51-adjuvanted AGS-v PLUS and saline placebo; Group 3, two doses ISA-51-adjuvanted AGS-v PLUS; Group 4, two doses Alhydrogel-adjuvanted AGS-v PLUS; and Group 5, and two doses saline placebo. Primary objectives were to determine safety and tolerability, and to measure immune responses. 51 participants were enrolled, 11 in Group 3 and 10 in the other groups. No participant experienced any treatment-emergent or serious adverse event. 134 solicited symptoms were reported: 20 local and 35 systemic in 25 (49%) participants after dose 1, and 23 local and 56 systemic in 23 (45%) participants after dose 2. All were considered mild/moderate except for one severe fever that occurred in a placebo recipient. The most common solicited local symptom was pain, occurring in 15 (29%) and 11 (22%) of participants after dose 1 and 2, respectively. The most common solicited systemic symptoms were headache, malaise, and fatigue, occurring in 9 (18%), 5 (10%) and 7 (14%) participants after dose 1, and 9 (18%), 11 (22%) and 11 (22%) participants after dose 2, respectively. Solicited symptoms after placebo injections appear similar to those after vaccine, except that

pain was less common in the placebo group. Compared with placebo, all groups had significantly higher interferon-gamma and IgG responses to AGS-v PLUS pooled peptides. In this first-in-human study, AGS-v PLUS was well-tolerated and generated strong immune responses. Further testing to determine expanded safety, tolerability, and efficacy is warranted.

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RISK-STRATIFYING YELLOW FEVER PATIENTS AT THE TIME OF ADMISSION

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The course of yellow fever (YF) is poorly understood. During the 2017-2018 outbreaks in Minas Gerais, Brazil, a cohort of PCR-confirmed YF patients (n=300) was followed longitudinally at Hospital Eduardo de Menezes (HEM), Belo Horizonte with the goal of characterizing the clinical progression of YF and identifying risk factors for mortality in YF inpatients. We analyzed available demographic, clinical, sonographic and laboratory exposure factors predictive of a fatal outcome (n=120) using univariate analysis of relative risk, with continuous variables dichotomized on optimal cutoffs identified on ROC curves. For patients who had sufficient serum studies drawn on admission (n=285), we generated a classifier based on laboratory results using random forests, an ensemble learning method of classification, which we trained on a randomly selected half of the dataset. Older age (p=0.04), prior history of pulmonary disease (RR 2.2 [1.6, 3.1]), clinical signs of acute liver failure including neurologic findings (somnolence (RR 1.7 [1.3, 2.3]), coma (2.8 [2.3, 3.3]), confusion (2.0 [1.5, 2.6]), seizures (2.3 [1.9, 3.0]), and asterixis (1.9 [1.0, 3.3])), bleeding diatheses (epistaxis (1.6 [1.1, 2.8]), gingival bleeding (2.2 [1.7, 2.8), melena (1.8 [1.2, 2.7]), hematemesis (2.1 [1.6, 2.7]), and petechiae (2.0 [1.5, 2.6])), jaundice (1.8 [1.4, 2.4]), dyspnea (1.7 [1.3, 2.2]) or oliguria (2.6 [2.1, 3.2]), and sonographic evidence of hepatomegaly (RR 2.0 [1.2, 3.4]) or loss of renal corticomedullary differentiation (2.5 [1.7, 3.5]) were all predictive of mortality. In our random forests classifier, the three most important variables by structural and classification accuracy measures were international normalized ratio (mean decrease in accuracy 0.051) , aspartate aminotransferase (0.028), and creatinine (0.017), which are typical markers of acute liver failure. The resulting classifier distinguished between fatal and nonfatal cases with AUC=0.77. These results suggest that YF patients may be effectively risk-stratified on the basis of data available at the time of admission.

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PERI-URBAN TRANSMISSION OF DENGUE IN THE CAMBODIAN 2019 EPIDEMIC: A PHYLOGEOGRAPHIC APPROACH TO UNDERSTAND ARBOVIRAL OUTBREAKS

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Understanding transmission dynamics of dengue (DENV) outbreaks is critical to mitigation of pathogen spread. In 2019, Cambodia suffered its worst DENV epidemic in recorded history with a significant portion of the burden in peri-urban and rural areas. We collected sera from 105 DENV-positive Cambodian patients, extracted viral RNA, prepared and sequenced cDNA libraries on Illumina platforms. We first used a de novo assembly method within the open-source IDseq platform to identify the closest pre-existing phylogenetic sequence in GenBank and constructed 105 full-length consensus genomes mapping to that reference with Nextflow (51 DENV1 genomes, 52 DENV2 genomes, 3 DENV4 genomes). We compiled resulting sequences into Bayesian phylogenetic time trees via BEAST for each serotype. With Bayesian coalescent methods, we estimated changes in effective population size and reproduction number through time for DENV-1 and DENV-2. The proportion of sequence pairs within the same 'transmission chain' were identified as those with a least common ancestor within the past six months and compared them across geographic distance, age, gender, and infection severity. Our results indicate that both the effective population size and effective reproduction number had correlated increases through time for DENV1, and more substantially for DENV2. DENV1 sequences showed identity on extremely local scales whereas DENV2 transmission was over a greater geographical space. Transmission chains were more tightly associated in those under age 10 years old. Infection severity had a significant effect on sequence affinity with higher fevers (>38.5°C) and lower white blood cell counts generating more tightly-related transmission chains. In conclusion, genetic diversity of peri-urban transmission of DENV is dependent on spatial distance, but over a larger geographic scale than has been previously described in urban environments. More intensely focal transmission resulted from lack of homotypic immunity to the epidemic DENV1 and geographically restricted lifestyles of younger participants as well as those with clinically severe infections.

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FLUOROQUINOLONE RESISTANT ESCHERICHIA COLI RECOVERED AT THE INTERFACE BETWEEN HUMANS, POULTRY AND THEIR SHARED ENVIRONMENT- A POTENTIAL PUBLIC HEALTH RISK

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Commensal *Escherichia coli* residing in the guts of humans and animals are reservoirs of multidrug resistance genes, including quinolone resistance genes. This study aimed to characterize fluoroquinolone resistance in *E. coli* recovered from poultry workers, chickens, and farm/market environments in Abuja, Nigeria. Of 110 *E. coli* isolates, quinolone-resistance phenotypes were observed in 68.2% (n=75) isolates. Plasmid-mediated quinolone resistance (PMQR) genes were detected in 63.6% (n=70) isolates, mainly from chickens 34.3% (24/70). Among PMQR genes, *qnrS1* was the most detected in 80% (56/70) of isolates followed

by qnrB19 in 20% (14/70) of isolates. We detected *aac(6)-Ib-cr* gene responsible for reduction in the activity of ciprofloxacin in two (2.9%) PMQR-positive *E. coli* isolates recovered from chickens. Most (15/22) isolates with ciprofloxacin-resistance and Nalidixic acid-resistance (19/75) showed double mutations in the quinolone-resistance determining regions of *gyrA*, with single or double mutations in *parC*, and single mutation in *parE*. The most prevalent amino-acid substitutions observed were S(83)L+D(87)N in *gyrA* (46.5%, n=20), S(80)I in *parC* (51.2%, n=22) and S(458)A in *parE* (14%, n=6). Plasmid-mediated colistin resistance (PMCR) gene *mcr-1.1* (n=2), and genes encoding extended-spectrum beta lactamase (ESBL) - *bla*CTX-M-15 and *bla*CTX-M-65 (n=2), were detected in PMQR-positive isolates. PMQR genes were prevalent in *E. coli* isolates recovered from healthy humans, chickens and the poultry farm/market environments. PMCR genes (*mcr-1.1*) occurred in PMQR-positive isolates recovered from manure and drinking water originating from poultry farm/market environments. It was found that the gene encoding ESBL coexisted with *qnrS* positive isolates of human and avian origin. Horizontal transfer of PMQR genes among *E. coli* isolates in the human-poultry-environment interface has public health implications for the spread of antimicrobial resistance. Relevant government agencies should enforce regulations to restrict the use of critically important antimicrobials in poultry production.

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SURVEILLANCE FOR ZOONOTIC INFLUENZA A VIRUSES IN LIVE BIRD MARKETS, NORTHERN VIETNAM

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Highly pathogenic avian influenza (AI) virus outbreaks pose a serious threat to both poultry and human health. Such outbreaks have the ability to cross over and infect neighboring countries via live bird markets (LBMs) and perhaps become enzootic among local aquatic birds and poultry. We employed a One Health approach to examine live bird markets (LBMs) in northern Vietnam for evidence of zoonotic influenza A viruses. Environmental, animal, and animal worker samples were collected from four LBMs across four provinces (Hanoi, Lang Son, Lao Cai, and Quang Ninh) in northern Vietnam from January 2019 - January 2021. At each sampling visit, up to three NIOSH two-stage aerosol samplers were positioned 0.5 m from the ground on tripods and connected to SKC AirCheck TOUCH pumps run at 3.5 L per min for up to 4 hrs. Up to ten oropharyngeal (OP) and fifteen cage swabs were collected from poultry near the NIOSH samplers. Market poultry workers permitted nasal wash sample collections and completed questionnaires. All samples were screened for influenza A virus using real-time reverse transcription polymerase chain reaction (qRT-PCR). Positive samples were used for egg inoculation to assess the infectivity of the detected viruses. A total of 3,114 LBM samples were collected. Among the 2,896 samples examined by qRT-PCR thus far, 892 (30.8%) tested positive for influenza A virus, including 202 (40.5%) of the bioaerosol samples, 374 (36.3%) of the poultry cage swabs, 288 (41.9%) of the poultry OP swabs, and 28 (4.1%) of the human nasal washes. Among the 2,692 samples examined with egg culture thus far, 527 (19.6%) yielded viable influenza A virus, including 43 (9.1%) of the bioaerosol samples, 260 (26.1%) of the poultry cage swabs, 224 (33.5%) of the poultry OP swabs, and 0 human nasal washes. Poultry cages and bioaerosols at LBMs are environmental sources of infectious influenza A viruses that may pose an occupational risk for zoonotic infection as evidenced by virus-positive human nasal washes. Additionally, bioaerosol sampling appears to be a sufficient influenza virus surveillance tool that could potentially replace more invasive bird swab sampling.

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DETECTION AND MOLECULAR CHARACTERIZATION OF ZOONOTIC AND PANDEMIC SWINE INFLUENZA VIRUS IN JOS: IMPLICATION FOR PUBLIC HEALTH

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Pig is a mixing vessel for Influenza A viruses and have been implicated in pandemics. Human and animal influenza are inextricably linked therefore pigs are important for the generation of novel influenza viruses with pandemic potentials. This study aimed to detect, isolate, characterize and determine the phylogeny of Influenza A virus in Jos and its environs in Nigeria. Eighty six nasal / tracheal swabs and 55 oropharyngeal swabs were collected from apparently healthy pigs and pig handlers respectively and were screened by PCR and virus isolation. Six pig samples and four human samples were positive with CT value below 35. Next Generation Sequence of the amplicons from one positive virus was carried out and local alignment tool was used to compare gene sequences obtained with others in the GeneBank. Nucleotide sequence and construction of Phylogenetic trees of the eight influenza gene segments were done using MEGA 7 software and the Neighbour- Joining method with 1,000 boostrap replicates. The Nigerian A/swine/Nigeria/19RS1081-19/2017_H1N1- PA is closely related to human isolate MH637504 A/Baltimore/P0258/2018(H1N1). The study showed the detection and characterization of pandemic 2009 influenza A H1N1 from pigs and presents the second full genome sequence in Nigeria and the first in Plateau State. Genomic sequence data obtained has been deposited in the GeneBank with accession number MN54844-PB1, MN540843-PA, MN540838-HA, MN540841-NP, MN540841-NA, MN540839-MP, MN540842-NS. Inter specie transmission from human to pigs appears persistent in Nigeria and can be a source of epidemic and pandemic. This further justifies One Health approach in the control of influenza. We therefore advocate regular surveillance, molecular characterization as part of the preparedness to prevent or mitigate the next pandemic.

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PLAGUE INFECTION IN URBAN SMALL MAMMALS AND FLEAS IN MADAGASCAR

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Plague is a zoonotic disease caused by *Yersinia pestis* and usually transmitted to humans via bites from rodent fleas. Madagascar is the most affected country in the world and plague is mainly occurred in rural areas but rarely in urban cities. In 2017, an outbreak of urban pneumonic plague was recorded in Antananarivo and Toamasina with a few cases of bubonic plague. To better understand the situation of plague in the urban areas we conducted surveillance on reservoirs and their fleas to identify human risk exposure to plague and refine plague prevention measures. Between January and May 2019, we captured small mammals using live traps in 22 markets belonged to 5 urban areas of Antananarivo, Antsirabe, Fianarantsoa, Toamasina and Mahajanga. Serum and spleen samples were collected from small mammals captured to detect IgG anti-F1 *Y. pestis* antibodies by ELISA and for testing *Y. pestis* F1 antigen using rapid diagnostic test (RDT). Fleas were collected from small mammals and identified to determine the flea index (FI) as the average number of fleas per host and *Y. pestis* infected fleas. Overall, 652 small mammals belonging to three rodent and one shrew species were trapped. *Yersinia pestis* F1 antigen were detected in 31 (4.8%) small mammals including *R. norvegicus* (n=15, 3.3%), *R. rattus* (n=9, 6%), *S. murinus* (n=6, 17%) and *M. musculus* (n=1, 14.3%). Animals positive were trapped in eight

markets of Mahajanga, Toamasina and Fianarantsoa. *Rattus norvegicus* is the predominant rodent in 4 urban areas except Fianarantsoa where *R. rattus* were found in abundance. No animals were positive for IgG antibodies; this may indicate an early stage of plague infection in the RDT positive individual or an evolution of plague resistance. A total of 1,822 *X. cheopis* fleas were collected from small mammals and no flea were positive to *Y. pestis* infection. The FI ranged from 0.2 to 7.5 and above the threshold risk (1) in 14 markets. Our findings point towards the importance of the urban markets in the maintaining of plague in enzootic cycle among rodent population. Both in Central Highlands and in coastal areas, rodent survey should be conducted regularly to identify plague risk indicators.

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SCOPE AND OPPORTUNITIES FOR A ONE HEALTH APPROACH FOR GUINEA WORM CONTROL

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Guinea worm disease (GWD) is a neglected tropical disease caused by the nematode *Dracunculus medinensis*. The disease was targeted for eradication several decades ago because of its limited geographical distribution, predictable seasonality, straightforward diagnosis, and exclusive infection of humans. A growing body of evidence now suggests that GWD can affect both humans and animal populations, challenging this last attribute. Accordingly, we endeavored to review publicly available epidemiological data that could support the utility of a One Health approach for GWD control in the six countries that have reported human GWD cases since 2015 – Angola, Cameroon, Chad, Ethiopia, Mali, and South Sudan. Our results demonstrate that while human GWD cases have dramatically declined overall, recent years have witnessed a gradual increase in human case counts, a rapidly growing number of reported animal infections, and cases in new geographies. A majority of the documented animal Guinea worm infections have occurred in dogs, but infections have also been reported in cats, baboons, and a donkey. Given that molecular evidence has demonstrated humans and dogs share exposure to the same populations of worms, it has been hypothesized that the observed increase in animal cases may be driving the increase in human GWD cases – or at a minimum, sustaining the risk of human infection. While this shift likely reflects changes in surveillance and not novel transmission patterns in animal species, it underscores the importance of embracing new approaches if the goal of eradicating GWD is to be achieved. There is a compelling case for controlling GWD using a framework grounded in a One Health approach. This framework should find its foundation in improving disease surveillance, pinpointing the dominant routes of infection, and elucidating the disease burden in animals; determining transmission risk factors among animals and from animals to humans; and identifying practical ways to foster horizontal and multidisciplinary approaches to surveillance, diagnosis, and intervention.

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URBANIZATION SHAPES THE ABUNDANCE AND DIVERSITY OF RODENT-BORNE PATHOGENS IN MALAYSIAN BORNEO: IMPLICATIONS FOR ZOONOTIC DISEASE RISK

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Urbanization a widespread and significant process of global change that modifies the landscape rapidly, extensively, and often permanently. Although this process results in the destruction and fragmentation of natural habitats, it also creates new ecological niches and an abundance of resources in cities, allowing some species to thrive. Rodents are amongst the most successful urban mammals, with several species distributed globally in cities and many others persisting locally in urban and suburban areas. However, rodents are also known reservoirs of many significant human diseases, including leptospirosis, Lyme disease, and viruses that cause hemorrhagic fevers. Our work seeks to understand how the ecological and environmental context of cities influences zoonotic disease dynamics and human disease risk, using rodent-borne diseases as a model system. In this study, we use a combination of ecological and evolutionary approaches to explore how the community composition of rodents, their ectoparasites, and their pathogens vary across an urban-rural gradient in Sarawak, Malaysia. We find that although the microbial response to urbanization is heterogeneous overall, there is clear evidence that urbanization promotes the persistence and spread of a subset of zoonotic pathogens and viruses, including globally significant zoonotic diseases like leptospirosis. We hypothesize that differences in the response of pathogens to urbanization may be driven by differences in transmission dynamics and replication strategy, which suggests a potential route through which disease risk can be mitigated in urbanizing regions across the globe. Finally, we explore how features of the urban environment influence human disease risk and discuss how a One Health-based approach to urban pest management can be used to ensure the continued and healthy coexistence of people and rodents in city environments.

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EMERGENCE OF ZOONOSES: WOULD GOLD MINING BE A CONTRIBUTING FACTOR?

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The emergence of pathogens has increased significantly since 1940, particularly of zoonotic origin (60% of emerging diseases), mainly from wildlife (72%). Anthropization and disruption of ecosystems favor the crossing of inter-species barriers, particularly in areas of high biodiversity such as the Amazon. Would people practicing gold pmining, especially unregulated, be particularly affected by zoonoses?Capitalizing on a study among malaria on illegal gold mines in French Guiana, a complete clinical examination and a biological sampling were carried out on consenting adults working on these mines. The examination recorded the dermatological signs of leprosy and leishmaniasis and history of vaccination against yellow fever. Biological tests were performed for neutralizing antibodies (Ab) against yellow fever, phase I and II IgG against Q fever, and Microscopic Agglutination Test against leptospirosis. Between October and December 2019, 380 individuals were included. The seroprevalence of leptospirosis was 28.1% (CI95%=23.6-33.0). This high seroprevalence is consistent with a wild cycle of leptospirosis in the Amazon where many mammals are known to be leptospires carriers. The prevalence of Q fever was 2.9% (CI95%=1.2-4.6), which is lower than the prevalence on the coast, which weakens the hypothesis of a deep forest or terrestrial reservoir of *Coxiella burnettii*, a reservoir still unknown in the region. The majority of participants reported being vaccinated against yellow fever (91.8%) and 97.9% had seroneutralizing Ab. The risk of a yellow fever epidemic, as in Brazil in 2016-2019, seems therefore unlikely. Ten percent (0.7-1.3) of the participants had lesions of cutaneous leishmaniasis. Five persons had typical lesions of leprosy thus an estimated prevalence of 78.9/10,000 py. All came from the Brazilian state of Maranhão, where the prevalence in 2019 was 17 times lower

at 4.5/10,000 py. These unique data shed new light on the transmission cycles of zoonoses that are still poorly understood in the region. In the era of One Health, special attention must be paid to these vulnerable populations in direct contact with biodiversity.

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DURABILITY OF SEROPOSITIVITY IN PATIENTS WITH NEUROCYSTICERCOSIS AFTER LEAVING AN ENDEMIC AREA

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Seroepidemiological surveys play an important role in establishing the prevalence of neurocysticercosis (NCC), a leading cause of epilepsy in many developing countries. However, these antibody-based techniques can skew estimations of morbidity due to persisting seropositivity following disease cure. Using a serological assay of recombinant and synthetic *Taenia solium* antigens, we identified the rates of seropositivity following cure in patients with various forms of NCC. In an optimized ELISA format, we measured IgG to the previously described antigens T24H, GP50, and a truncated Ts8 isoform. These assays produced ROC-based signal/noise (S/N) cutoffs with 96.34% sensitivity and 95.92% specificity when tested with 70 NCC positive and 49 NCC negative samples, with GP50 reactivity driving assay performance. In longitudinal analyses of serum collected from 64 patients over a course of up to 10 years following disease cure, patients with extra-parenchymal (intraventricular and basilar subarachnoid/racemose) NCC maintained the highest levels of seroreactivity post-cure compared with parenchymal NCC. At 5 years post-cure, 50% and 84.21% of ventricular and subarachnoid patients, respectively, continued to be seropositive compared to only 30% of patients with parenchymal disease. Notably, 60% of patients cured of subarachnoid NCC continued to exhibit seropositivity even 10 years post-cure. Correlation analysis showed that GP50 S/N and time to/since cure were inversely correlated (R -0.66, $p<0.0001$), suggesting that degree of seroreactivity is dependent on both burden of disease and duration of cure. In patients with calcified NCC, we found that seropositivity post-cure was correlated with the number of calcifications (R 0.62, P=0.0016), indicating that these calcifications may help maintain seroreactivity. Our findings show that seroreactivity in patients with NCC varies based on disease type and can persist for many years following cure in a population that is not being re-exposed to *T. solium* antigens. These data will be important for designing future seroepidemiology studies to ensure accurate estimations of disease burden.

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CROSS-SECTIONAL STUDY OF POPULATION SCREENING FOR URINARY ANTIGENS TO DETECT SUBARACHNOID NEUROCYSTICERCOSIS IN A COMMUNITY SETTING

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Subarachnoid neurocysticercosis (SANCC) is the most morbid form of *Taenia solium* infection. SANCC is diagnosed usually at late stages when treatment options are limited, and patients experience frequent adverse treatment complications due to advanced disease. We validated population screening to detect cysticercosis antigens (cyst-Ag) in urine to identify SANCC in earlier asymptomatic stages. Urine collection is practical, non-invasive, and readily accepted in Peru. Our objective was to identify cases of SANCC in a population-based setting using a two-step screening process of urine based antigen tests followed by neuroimaging. We conducted a cross-sectional study in 2018 to screen for SANCC in residents of Tumbes, Peru, (n=8115). We collected first morning urine from participants at their homes and calculated optical density ratios (ODR) for cyst-Ag in these samples using ELISA and monoclonal antibody set B158/B60. We offered all participants with an ODR≥3 a non-contrast MRI of the brain to identify intracranial *T. solium* cysts, and a clinical evaluation for neurologic symptoms consistent with neurocysticercosis (NCC). Of 8315 individuals screened, 81 (0.97%) had an ODR≥3 and were offered MRI. Seventy-four obtained MRI and 17 had SANCC; 13 (76.4%) reported no symptoms. Another 7 had non-subarachnoid forms of viable NCC. Globally, the prevalence of SANCC was 2 per 1000 persons, but there were significant regional differences with higher prevalence of urinary cyst-Ag and SANCC in regions with greater immigration. In these regions, the positive predictive value (PPV) of the urine screen was 32.5% for SANCC and 42.0% for all NCC. Population urine screening in a *T. solium* endemic region identified cases of asymptomatic SANCC with an acceptable PPV. An ongoing observational cohort and planned clinical trial will help determine whether early medical intervention of asymptomatic SANCC is safe, and whether this approach merits further evaluation of efficacy to improve clinical prognosis.

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CYSTIHUMAN: A MODEL OF HUMAN NEUROCYSTICERCOSIS

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Cysticercosis is a neglected tropical disease that was deemed "eradicable" by the World Health Organization. Neurocysticercosis (NCC) is a major cause of epilepsy in developing countries, also causes intracranial hypertension and hydrocephalus, and can lead to death. Simulation models can help identify interventions likely to bring higher benefit to cost ratios before they are implemented at a large scale and high cost. CystiHuman, the first human NCC model, was developed to allow for cost-effectiveness analyses that focus on the actual impact of the disease rather than using proxies as is the case with transmission models. It also allows the comparison of a broader range of interventions (e.g., treatment of NCC symptoms) than usual models. CystiHuman is an agent-based model that projects NCC and associated pathologies. It uses the output of another model, CystiAgent, which projects the evolution of pig cysticercosis and human taeniasis. It includes a model of human cyst stage and location, and of symptom and treatment likelihood. CystiHuman accounts for delays in the appearance of human symptoms to help make time-dependent predictions of changes in the human disease. *R*CystiHuman was used so far to model the situation at baseline in three endemic villages in Peru. It gives reasonable results for the disease and its clinical presentation, and reproduces patterns of increase in NCC prevalence with age. Initial simulations suggest that NCC prevalence increases rapidly after a peak in taeniasis but decreases very slowly after this peak has passed. In conclusion, it is possible to develop and calibrate a model of human NCC and obtain reasonable results. Initial outcomes

suggest that short-term interventions may not be well suited to achieve significant changes in human NCC. Further work is needed to validate the model and develop projections of the economic and health benefits/costs of different interventions.

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THE ROLE OF BENZIMIDAZOLE TREATMENT IN THE CLINICAL MANAGEMENT OF ALVEOLAR ECHINOCOCCOSIS IN GERMANY - BENEFITS AND CHALLENGES

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Alveolar echinococcosis (AE) is a progressive parasitic disease caused by the larval stage of Echinococcus multilocularis (EM). Benzimidazoles are the only approved pharmaceutical treatment options, yet drug intolerance and non-response occur frequently and hamper further clinical management. This retrospective cohort study included 275 patients. Descriptive statistics included demographics, stage of disease, initial symptoms, treatment options, and complications. A regression model revealed risk and protective factors for adverse outcomes. Additionally, a cross-sectional study evaluated the absorption of albendazole depending on nutrition and possible side effects. Median age at diagnosis was 55 years, 55% were female. When diagnosis was established, more than 60% reported no specific symptoms, yet 62% presented in an advanced stage. AE was inoperable in 57% and hence treated with benzimidazoles (BMZ). Within this group, 7% suffered from absolute BMZ intolerance resulting in hepatotoxicity. A total 9% received a structured treatment interruption (STI). A follow-up period of 803 cumulative patient-years was monitored. While 26% of patients were cured, 71% were stable and 3% suffered from progressive disease. Risk factors for adverse outcomes included advanced stage, vascular and biliary complications, BMZ toxicity and relapse after STI. Surgical treatment, high albendazole serum level and a long-term BMZ treatment were identified as protective factors. Using decreasing serum levels of Echinococcus antibodies and IgE as surrogate markers for treatment response, BMZ treatment proved to be effective. In contrast to the drug information, the absorption of albendazole does not necessarily require 40g of solid fat. Side-effects often reported included gastrointestinal discomfort and diarrhoea. BMZ allowed for a long-term stable disease, especially if well-tolerated with sufficient serum levels. However, cases with non-response to BMZ or BMZ toxicity resulted in adverse outcomes with complex clinical management. In carefully selected patients, a STI can present a valuable alternative to life-long BMZ treatment.

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NOVEL HUMAN INFECTION WITH AN UNKNOWN SPECIES OF DRACUNCULUS, VIETNAM.

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With the recent detection of *Dracunculus medinensis* (Guinea worm) in Angola, there has been an increased concern that dracunculiasis may be present in countries not previously endemic for Guinea worm. In 2020, a 23-year-old male living in northern Vietnam with no history of foreign travel was hospitalized, and five large nematodes were recovered from abscesses on the arms and legs. These nematodes measured 30—60 cm in length and were grossly consistent with *Dracunculus* sp. A segment of one worm was sent to the reference laboratory at the US Centers for Disease Control and Prevention (CDC), where the species of the worm was evaluated using microscopy and molecular tools. Microscopically, the segment of worm possessed a finely striated cuticle and L1 larvae measuring an average of 339 µm long were present. The larval measurements excluded *D. medinensis* as the causative species and were instead consistent with a few described species of *Dracunculus* mostly reported from reptile hosts. Sequencing of the entire 18s gene placed this specimen in a clade separate from *D. medinensis* and more closely aligned with *D. lutrae* and *D. insignis.* Species could not be further resolved due to the lack of additional morphologic features and missing sequence data for most members of the genus. The only prior report of *Dracunculus* spp. in Vietnam was *D. houdemeri* collected from checkered keelback snakes (*Fowlea piscator*). While the larvae of *D. houdemeri* are consistent with the measurements in this specimen, no DNA sequence data are available for confirmation. Surveillance for similar cases among animals and humans in the region is warranted to better understand possible transmission routes. Additionally, this study highlights the importance of effective public health surveillance systems demonstrated in this collaborative work between public health authorities from Vietnam, WHO and US-CDC.

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RPACAN3990, A NOVEL, HIGHLY SENSITIVE RECOMBINASE POLYMERASE ASSAY WITH A VISUALIZABLE READOUT

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Angiostrongylus cantonensis (Ac) is one of the leading causes of eosinophilic meningitis worldwide. A field deployable molecular detection method could enhance both environmental surveillance and clinical diagnosis of this emerging pathogen. Accordingly, RPAcan3990, a recombinase polymerase assay (RPA) was developed to target a region predicted to be highly repeated in the Ac genome. The assay was then adapted to produce a visually interpretable fluorescent readout using an orange camera lens filter and a blue light. Using Ac genomic DNA, the limit of detection was found to be 1fg/µl by both fluorometer measurement and visually. All clinical samples tested known to be positive for Ac from various areas of the globe were positive by RPAcan3990. Clinical samples known to be positive by other etiologies of eosinophilic meningitis (i.e. *Toxocara sp*) were negative in the RPAcan3990 assay. The optimal incubation temperature range for the reaction was found to be between 35-40 ºC. The assay successfully detected 1fg/µl of Ac genomic DNA after incubation at human body temperature (in a shirt pocket). In conclusion, these data suggest RPAcan3990 is potentially a point of contact molecular assay capable of detecting Ac by efficiently producing visually interpretable results with minimal instrumentation.

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EXPLORING THE GENETIC DIFFERENCES AMONG POPULATIONS OF TRICHURIS TRICHIURA FROM LAOS, TANZANIA AND COTE D'IVOIRE WITH DIFFERING RESPONSES TO ALBENDAZOLE-IVERMECTIN COMBINATION TREATMENT

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Trichuris trichiura, one of the most important Soil Transmitted Helminth (STH) species, infects over 500 million people globally with a major socio-economic impact in developing countries. STH Mass Drug Administration (MDA) programs use either albendazole or mebendazole. However, these benzimidazole drugs have very low efficacy against *T. trichiura,* with cure rates and egg reduction rates typically <30% and <50%, respectively. Recent trials of albendazole-ivermectin combination treatments revealed high efficacy at two geographical sites with egg reduction rates above 98% in Pemba, Tanzania and Lao PDR. In contrast, there was much lower efficacy at a third site in Cote d'Ivoire with an egg reduction rate below 70%. To explore whether this difference in efficacy could be due to genetic differences in the parasite populations, we have undertaken Illumina short-read deep amplicon sequencing of multiple loci on 29,

37 and 22 *T. trichiura* PCR positive fecal samples from Pemba, Tanzania, Lao PDR and Cote d'Ivoire, respectively. Phylogenetic analysis of the ITS-2 rDNA locus revealed that the Amplicon Sequence Variants (ASVs) from all samples from Pemba, Tanzania and Lao PDR clustered together whereas those from Cote d'Ivoire clustered separately. Primers targeting the mitochondrial *nad1*, *nad4* and the major β-tubulin gene generated ASVs mapping to the appropriate reference sequences from all samples from Pemba, Tanzania and Lao PDR but not from any samples from Cote d'Ivoire. There were no potential candidate benzimidazole resistance mutations at codons 167, 198 and 200 in the β-tubulin gene in any of the mapped ASVs from Pemba, Tanzania and Lao PDR. Overall, these results suggest that the *T. trichiura* population from Cote d'Ivoire is genetically divergent to those from Pemba, Tanzania and Lao PDR and this has the potential to underlie the differences in ivermectin-albendazole efficacy. Further sequence analysis of additional genetic loci is underway.

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INVESTIGATIONS OF THE ROLE OF DRUG RESISTANCE IN RELAPSING BABESIA MICROTI INFECTIONS

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Guidelines for the treatment of babesiosis rely on combination therapy, with most patients receiving azithromycin and atovaquone during their treatment course. Atovaquone is the key anti-infective in all recommended treatment regimens for relapsing babesiosis. However, a low barrier to the development of atovaquone resistance is well defined for a variety of pathogens *in vitro* and *in vivo*. Several reports have demonstrated the emergence of mutations in the gene encoding target of atovaquone, *cytb*, in samples of relapsing babesiosis. Our expanded survey of relapsing babesiosis samples has demonstrated additional mutations in *cytb* with several repeatedly mutated sites including amino acid changes near the conserved PEWY region, and amino acid changes at site 134, which was previously identified as key to atovaquone resistance in other organisms and an *in vivo* model of *B microti* infection. In addition, we have surveyed for mutations in *rpl4*, the gene that encodes that target protein for azithromycin. We repeatedly see amino acid changes at position 86, exclusively in samples of relapsing disease. OF the six relapsing cases tested 100% (6/6) had mutations in *cytb* and 50% (3/6) were found to have *rpl4* mutations. We also surveyed 20 non relapsing cases and found no mutations in these samples. Our data highlights that relapsing disease is often but not exclusively associated with mutations in the targets for atovaquone and azithromycin and we are working to define the role these mutations play in the successful treatment of these complicates babesiosis patients.

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DEVELOPMENT OF A LATERAL FLOW ASSAY FOR NEUROLOGICAL TOXOPLASMOSIS

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Opportunistic neurological infections in persons living with HIV (PLHIV) are challenging to diagnose. CT and MRIs with a clinical suspicion can guide treatment, but definitive diagnosis remains elusive for many patients. *Toxoplasma gondii (T. gondii)* is known to cause Toxoplasmic Encephalitis in PLHIV. Though difficult to diagnose, it has up to 90% clinical response rate to treatment. Innovations in *T.gondii* diagnostics, such as our team's urine-based diagnostic western blot utilizing hydrogel nanoparticle antigen concentration, require laboratory equipment and skilled personnel, limiting its range. To expand access to the diagnostic assay, we initiated structural and functional studies of the antigen of interest, GRA1, to allows for transition to a lateral flow assay. We have tested 8 antibody clones for their ability to form a sandwich around GRA1, resulting in an antibody pair with detection to 62.5pg/ml of recombinant antigen. Despite this high sensitivity in recombinant antigen, formation of the sandwich is not observed in urine from *T.gondii*-infected patients who are positive for GRA1 via western blot. Immunoprecipitations into mass spectrometry of *T. gondii* identified SAG2, a surface antigen, as a binding partner to GRA1. This interaction has been confirmed by western blotting. Circular dichroism studies suggest that GRA1 has a 96% alpha helical structure that refolds in the presence of calcium or increasing pH. Enzymatic digestion studies suggest that GRA1 undergoes N-linked glycosylation on the 30th amino acid. This glycan is estimated to be one third the size of the total GRA1 structure and may be obstructing antibody binding. Despite being a known secretory protein, GRA-1's function and structure is currently unknown; our data sheds light on GRA1's signaling role and structural modifications, which impact design of lateral flow immunodiagnostics. Moving forward, we will use a mass spectrometry based protein-protein interaction identification technique to map the binding sites of the 8 antibody clones as well as SAG2 to GRA1. This data will assess if the antibodies are impeded in their binding by the glycan or binding partners.

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CATALYSIS OF DRUG DISCOVERY RESEARCH AGAINST NEGLECTED TROPICAL DISEASES (NTDS) AT THE UNIVERSITY OF YAOUNDE 1: IMPACT OF THE BIOVENTURE FOR GLOBAL HEALTH

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Our vision at the Antimicrobial and Biocontrol Agents Unit (AmBcAU), University of Yaounde 1 is to improve the health and well-being of people burdened by infectious diseases of poverty through research and innovation towards the elimination of specific neglected tropical diseases (NTDs). Within the last 10 years, we have partnered with African and extra-African public and private institutions to pursue our drug discovery research. We have successfully implemented a middle-high throughput drug discovery platform in a conducive research environment to perform routine phenotypic *in vitro*/*in vivo* screening of natural products and libraries of small molecules against the causative agents of malaria, leishmaniasis, amoebiasis, Human African trypanosomiasis, Buruli ulcer, toxoplasmosis and resistant bacterial infections. Among the major contributors to this achievement, the impact of the Bio-Venture for Global Health (BVGH) through WIPO Re:Search, a global consortium that drives innovative public-private partnerships to address unmet medical needs for neglected infectious diseases and build research and development capacity in low- and middle-income countries is paramount. Indeed, this consortium has initiated and nurtured strong international partnerships between University of Yaounde 1 and institutions such as University of South Florida, FL, USA, that provides support in natural products isolation and structure elucidation; Merck Healthcare KGaA, Germany, Johnson and Johnson's Janssen pharmaceuticals, USA; Eisai Co., Ltd., Japan and Pfizer all providing target-based chemical libraries of inhibitors of critical metabolic pathways in eucaryotic pathogens; and the Guangzhou Institutes of Biomedicine and Health (GIBH), that collaborates on drug discovery against Buruli ulcer. To date, we have achieved significant data on hit inhibitors of the targeted pathogens to support further drug development against the diseases of interest.

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ARGONAUTE MEDIATED GENE SILENCING IN CRYPTOSPORIDIUM INFECTION

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The World Health Organization reports diarrhea kills around 760,000 children under five every year. *Cryptosporidium* is a leading cause of moderate-to-severe diarrhea in children. Nitazoxanide is the only FDA-approved medication available for cryptosporidiosis treatment, but it has limited efficacy in malnourished children and is ineffective in immunocompromised individuals. Therefore, more effective treatment options are urgently needed. Recently we developed a unique technology to silence genes in this pathogen using antisense RNA-argonaute (Ago) complexes. We have demonstrated that Ago complexes block expression of essential genes involved in *Cryptosporidium* infection. In addition, we treated orally mice with labeled complexes to show the feasibility to deliver RNA-Ago on intestinal cells of infected mice. Overall, our results show the possibility to conduct In vivo silencing to study gene function in Cryptosporidium. Therefore Ago-RNA complexes could be used to inhibit selected genes and reduce Cryptosporidium infection.

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SINGLE ADMINISTRATION OF A BIODEGRADABLE MICRONEEDLE CAN SUBSTITUTE FOR REPEATED APPLICATION OF EYEDROPS IN THE TREATMENT OF ACANTHAMOEBA KERATITIS

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Infectious keratitis (i.e., an infection of the cornea) is one of the main causes of corneal opacities and the 4th cause of blindness globally. *Acanthamoba* keratitis is one of rare etiologies and is known to be associated with contact lens usage, but, in developing countries, it can account up to 2% of microbiology-proven cases mostly without association with contact lens. The treatment relies on topical antibiotics, biguanides. To achieve and maintain the required therapeutic concentration in the cornea where the tear fluid continuously rinses the surface, the antibiotics must be frequently applied, even while the patient is sleeping. However, the inevitably poor compliance decreases drug bioavailability. In this study, a single microneedle (MN) is injected into the cornea to substitute for the repeated application of eyedrops in the treatment of *Acanthamoba* keratitis. After comparing the mechanical integrity and drug release profiles of three different drug tips, the drug tip with the "high" drug concentration that releases 12.5 ng drug within 3 days is applied to a cornea to evaluate the transferability and *in vivo* drug release. In the treatment of infectious keratitis with repeated application of eyedrops for six consecutive days, a single MN injection is substituted for the initial 3 days of eyedrop applications. The progression remains similarly attenuated after 3 days without eyedrops, and comparable efficacy is achieved on day 6 when combined with delayed eyedrop treatment from day 3. Thus, the single administration of a biodegradable MN can substitute for the repeated application of eyedrops in the treatment of *Acanthamoba* keratitis.

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RELATIONSHIP OF GALECTIN-3 BIOMARKER FOR CLINICAL OUTCOMES IN A CHAGAS DISEASE COHORT FROM A BRAZILIAN ENDEMIC REGION

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Chagas cardiomyopathy evolves through inflammation, cardiac remodeling, fibrosis. Represents a leading cause of infectious myocarditis worldwide, with an estimate 12.000 deaths per year, by 2010. Galectin-3 (GAL-3) can translate fibrogenesis potential and stratify who will evolve towards cardiac disease spectrum and earlier death. We evaluated GAL-3 levels are associated with cardiomyopathy onset, progression, or all-cause death among a cohort subset of no benznidazole-experienced patients from a vectorial controlled transmission and highly endemic Brazilian region. We described baseline sociodemographic and clinical data, Gal-3, NT-proBNP, serology titles, ECG minor or major abnormalities, and functional class. The median follow-up time was 2.5 years, and we reassessed changes in: NT- proBNP, ECG, NYHA, and all-cause mortality. We ran multivariate-adjusted Cox regression with Gal-3. We studied 1313 patients, mean age 59.8 (± 12.5) years, mostly female (n=884; 67%), frequent comorbidities (hypertension, 64.5%; dyslipidemia, 41.2%; diabetes, 10.1%); NYHA I-II, 57.2%. ECG abnormalities at baseline were frequent: minor, 23.3%, and major, 60.1%. Overall, Gal-3>25.9 ng/mL was associated with deaths and more disease severity markers. One hundred (8.2%) patients died. Gal-3 was higher among deceased 19.9 (±8.7) ng/mL, survivor's levels, 17.5 (±6.2) ng/mL; p<0.001. For higher Gal-3 stratum, HR 2.07 [CI95%: 1.21-3.52]; for composite higher Gal-3 and major-ECG, HR 6.85 [CI95%: 3.10-15.10]; while for Gal-3>25.9 ng/mL, and abnormal age-adjusted NT-proBNP, HR 7.93 [CI95%: 3.88-16.21]. Worsening ECG, age-adjusted NT-proBNP, NYHA wasn't associated with Gal-3>25.9 ng/mL. Among no benznidazole experienced *T.cruzi* infected patients, Gal-3 >25.9ng/mL depicted higher death rates. When associated with major abnormalities in ECG or abnormal NT-proBNP, even higher HRs weren't related to representing disease evolution.

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ELEVATED PEDIATRIC CHAGAS DISEASE BURDEN COMPLICATED BY CONCOMITANT GASTROINTESTINAL PARASITES AND MALNUTRITION IN EL SALVADOR

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The eradication of *Rhodnius prolixus* from Central America was heralded as a victory for controlling *Trypanosoma cruzi* transmission. While public health officials believed this milestone achievement would effectively stop vector transmission, case reports of acute vector transmission began amassing within a few years. Our investigation employed a cross-sectional serosurvey of children either presenting with fever for clinical care or children living in homes with known triatomine presence in the state of Sonsonate, El Salvador. Over the 2018 calendar year, we identified a 2.3%

Chagas disease seroprevalence among children with hotspot clustering in Nahuizalco. Positive serology was significantly associated with dogs in the home, older participant age, and a higher number of children in the home by multivariate regression. Concomitant gastrointestinal parasitic infection was noted in a subset of studied children; 60% having at least one gastrointestinal parasite and 15% having two or more concomitant infections. Concomitant parasitic infection was statistically associated with an overall higher parasitic load detected by qPCR. Lastly, we identified a four-fold higher burden of stunting in our cohort compared to the national average, with four-fifths of mothers reporting severe food insecurity. Our study highlights that pediatric polyparasitism and malnutrition are common comorbidities in El Salvador, and a systems-based approach is warranted when treating Chagas disease seropositive children.

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ELEVATED SARS-COV-2 VIREMIA IN THE AMERICAN INDIAN/ ALASKAN NATIVE POPULATION: RELATIONSHIP WITH INCREASED COVID-19 DISEASE SEVERITY

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There are limited data describing COVID-19 disease severity in disproportionately affected minority groups, particularly American Indians/ Alaska Natives (AIs/ANs). This prospective observational study (15 May-20 October 2020) at the University of New Mexico Hospital determined the relationship between SARS-CoV-2 viral burden and clinical outcomes in patients (n=94) of different ancestries. SARS-CoV-2 viral loads in the upper respiratory tract (URT) and peripheral blood (viremia) were quantified on days 0, 1, 2, 3, 6, 9, and 14 in hospitalized COVID-19 patients with non-severe and severe disease. The cohort was stratified into AI/AN and all other races/ethnicities combined (non-AI/AN). Among the 94 patients, 45.7% identified as AI/AN. Baseline characteristics (i.e., comorbidities and laboratory values) at admission were similar between the AI/AN and non-AI/AN groups. Individuals of AI/AN descent had more severe disease (odds ratio [OR]=7.46, [95% CI, 2.20-25.29], P=0.001, adjusted for age, sex, remdesivir, and steroids). Viral loads in the URT were comparable between ancestral groups with non-severe and severe disease. However, viremia was higher in the AI/AN group with severe disease on days 0, 1, 2, and cumulatively across 14 days (P=0.009). AI/AN ancestry was associated with a greater risk of having viremia (OR=4.73, [1.67-13.43, P=0.004, adjusted with identical covariates). SARS-CoV-2 viremia during hospitalization was associated severe disease (OR=9.19, [3.33-25.34], P=1.84x10-5, adjusted with identical covariates). In summary, individuals of AI/AN descent had more severe disease, characterized by elevated blood viral loads, despite comparable comorbidities. Viremia during hospitalization was associated with severe disease and was more common in the AI/AN group. These findings may explain, at least in part, the disproportionate disease burden of COVID-19 witnessed in this population. It will be important to define the biological/molecular basis of these virological findings in future studies to design interventions for improved clinical outcomes.

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TRANSCRIPTOMICS REVEALS NOVEL DIFFERENTIAL GENE EXPRESSION PATTERNS IN HOSPITALIZED AMERICAN INDIAN/ALASKA NATIVE PATIENTS WITH COVID-19: POTENTIAL INSIGHTS INTO ENHANCED DISEASE SEVERITY

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Race and ethnicity data in the US indicate that minority groups suffer disproportionately from COVID-19 cases, hospitalization rates, and mortality. Throughout the pandemic, American Indian/Alaska Native (AI/AN) populations have had the highest hospitalization rates and second highest proportion of cases and mortality. In New Mexico, the AI/AN population has experienced the highest number of COVID-19 cases, hospitalization rates, and deaths. However, the underlying molecular characteristics of COVID-19 pathogenesis in AI/AN remains unresolved. To identify molecular pathways for targeting by immunotherapy, we are conducting a prospective observational study (15 May 2020 to date) in hospitalized COVID-19 patients. Daily clinical and laboratory measures are obtained, along with peripheral blood samples (days 0, 1, 2, 3, 6, 9, and 14). To date, 336 hospitalized patients have been enrolled. Transcriptomics by next-generation sequencing was performed on a subset of temporally collected samples from patients stratified into AI/AN (n=26) and all other races/ethnicities combined (non-AI/AN, n=13). Enrichment analysis was performed using MetaCore™ for significantly (P<0.05) dysregulated genes ($\log_2$). The AI/AN group had elevated Furin on days 2 and 6 which can facilitate conversion of the SARS-CoV-2 spike protein into S1 for binding to ACE2 and subsequent endocytosis. In addition, antiviral cytokines (IFN-α, IFNL3, and IFNL4) were downregulated early in the infection, whereas IFN-γ was upregulated. Early infection was also characterized by reduced expression of HLA-DMB, HLA-DRB1, and MHC class II which can lead to diminished antigen presentation. An increase of P-selectin expression was observed until day 6, at which time, PAI1 was upregulated. The combination of these two events can enhance platelet-leukocyte adhesion, thrombosis, and acute respiratory distress syndrome in COVID-19. Collectively, these results provide novel insight into the molecular basis of enhanced disease severity in the AI/AN population and identify potential targets for future therapeutics.

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AN ADENO-ASSOCIATED VIRUS (AAV)-BASED GENE DELIVERY FOR IMMEDIATE PROTECTION AGAINST PANDEMIC CORONAVIRUSES

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The emergence of novel coronaviruses from zoonotic transfer as well as the variants of concern (VOC) from the COVID-19 pandemic continue to pose major challenges for society. Although multiple vaccines against

SARS-CoV-2 have been deployed at a record pace to combat the current pandemic, development times and safety testing for traditional vaccines hinder rapid response to new outbreaks. Passive immunization by adeno-associated virus (AAV) is a plug-and-play system which can provide immediate protection against future pandemics. As a proof of concept, we delivered the potent neutralizing Ab LCA60 via AAV in a humanized mouse challenge model for Middle East Respiratory Syndrome Coronavirus (MERS-CoV). Vaccinated mice showed reduction in lung viral titer and gross lung pathology against MERS-CoV m35c4 challenge one week after AAV administration. In response to COVID-19, we used the same AAV system to deliver REGN10933 and tested efficacy in a mouse adapted SARS-CoV-2 model. Similar protection was seen in vaccinated mice suggesting broad applicability against different CoVs. Currently, we are developing an AAV-based pan-sarbecovirus therapeutic by delivering a broadly neutralizing antibody, ADG2, to mice followed by challenge with SARS-CoV-1 MA15, SARS-CoV-2 MA10 and other pre-pandemic CoVs. Our proof-of concept experiments demonstrate the significant potential for AAV-based passive immunization strategies to provide a quick countermeasure for future pandemic/pre-pandemic viruses, especially for first responders prior to the development of new vaccines.

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ASSOCIATION BETWEEN HYPERINFLATION (DEFINED BY RIB COUNT) ON CHEST RADIOGRAPHS WITH VIRAL ETIOLOGY AMONG CHILDREN WITH SEVERE PNEUMONIA IN LOW-INCOME AND MIDDLE-INCOME COUNTRIES (LMICS)

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The Pneumonia Etiology Research for Child Health (PERCH) study, a case-control study of severe and very severe pneumonia in children in low-income and middle-income countries (LMICs), used a novel analytic method which incorporated sensitivities and specificities of multiple specimens and laboratory test results to estimate probabilities of viral or bacterial pneumonia etiology. As hyperinflation has been associated with viral infections, using these PERCH probability estimations in combination with rib counts on chest radiographs may be useful for predicting pneumonia etiology. In this study, chest radiographs of HIV-uninfected cases aged 1-23 months enrolled into PERCH in Mali, Zambia, and Gambia with either a high probability of viral (HPrV) etiology (≥90%) (n=105) or high probability of bacterial (HPrB) etiology (≥90%) (n=82) were selected for rib counts by a pediatric pulmonologist blinded to etiology results. The number of visible anterior and posterior ribs on both the right and left sides were recorded. Definitions of hyperinflation were created using varying definitions of anterior and posterior rib counts, with odds ratio (OR), sensitivity, specificity, and predictive values for HPrV vs. HPrB cases calculated for each definition. Definition 1 (the presence of ≥6 anterior or ≥9 posterior ribs on both the right and left hemithorax) produced the highest odds ratio for HPrV etiology (OR 3.73, 95% CI: 1.53-9.10). For HPrV cases, presence of ≥6 anterior ribs on both the right and left hemithorax maximized the sum of sensitivity and specificity (84% and 34%, respectively), followed by Definition 1 (sensitivity=91.7%, specificity=25.3%). While hyperinflation has previously been associated with viral etiology, no definition of hyperinflation in this study resulted in both high sensitivity and specificity, nor high positive and negative predictive values for distinguishing probable viral from bacterial infection. Our findings suggest that with further research, rib counts may be a useful research tool for aiding in viral pneumonia identification.

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FOOD INSECURITY IN INDIA: THE CORRELATION WITH UNDERNUTRITION AND ANTHROPOMETRY AMONG HOUSEHOLD CONTACTS OF TUBERCULOSIS CASES

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India accounts for more than one quarter of the world's tuberculosis (TB) cases. Undernutrition is widely prevalent in India and is the leading risk factor for TB; the COVID-19 pandemic and its impact on food security are likely exacerbating this situation. The TB Learning the Impact of Nutrition Study (TB LION) examines how undernutrition and food supplementation alter immune responses to *Mycobacterium tuberculosis* infection and risk of progression to TB disease among household contacts (HHC) of TB cases in Puducherry and Tamil Nadu, India. We assessed the prevalence of food insecurity (based on household food insecurity assessment survey [HFIAS] scores), malnutrition based on body mass index (BMI), triceps skinfold thickness (as a measure of body fat), and their correlation in HHC using Pearson correlation and Chi-square tests. Between July 2019 - April 20, 2021, we enrolled 356 participants; 145 (41%) male and the median age was 35 years (range 18-59). By reporting severe food insecurity conditions in the home and/or reporting insecurity more frequently on HFIAS, 165 (66.8%) participants were categorized as food secure, 38 (15.4%) mildly food insecure, 9 (3.6%) moderately food insecure, and 35 (14.2%) severely food insecure. Overall, 51 (14.3%) were undernourished (BMI <18.5 kg.m^2) and 305 (85.7%) well nourished (BMI ≥ 18.5 kg.m^2), and the median triceps skinfold thickness was 20.7 mm (range 4-20.4). We found a significant, negative correlation between HFIAS and BMI ($r = -0.18$, $p = 0.003$), between HFIAS and triceps skinfold ($r = -0.22$, $p=0.0004$), and a significant, positive correlation between BMI and triceps skinfold ($r=0.66$, $p <0.0001$). Although only 14.3% of HHCs were malnourished, rates of food insecurity were far higher and the two are strongly associated. Although anthropometry is typically used to assess nutritional status and associated TB risk, these findings underscore the fragile nature of access to food and the need for a broader assessment of and response to undernutrition. Moreover, sustained mild food insecurity and negative health effects, are likely with the interruption of food access from COVID-19.

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DEVELOPMENT AND PERFORMANCE OF AN EMERGENCY DEPARTMENT-BASED SYNDROMIC SURVEILLANCE PLATFORM IN PUERTO RICO TO PREDICT INCREASES IN COVID-19 COMMUNITY TRANSMISSION

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Syndromic illness surveillance platforms integrating data from multiple emergency departments (EDs) have been utilized to monitor a broad range of health trends, including seasonal respiratory illnesses, and, more recently, indicators of COVID-19. Puerto Rico, other territories, and international settings in which electronic medical record (EMR) technologies are being implemented can benefit from the development of syndromic surveillance platforms to identify emerging issues in real-time.

Due to delays in diagnostic test results and reporting, syndromic indicators may more quickly predict or indicate increases in COVID-19 transmission. We collaborated with two tertiary care hospitals and one community outpatient emergency room in Puerto Rico to extract data on ED visits and monitor syndromic and diagnostic indicators of COVID-19 transmission. Data were obtained for visits starting March 1, 2020 and are updated on an ongoing weekly basis. Demographics, triage, chief complaint, rapid test, and International Classification of Diseases, 10th revision (ICD-10) diagnosis code data were used to construct dashboards to visualize trends in respiratory indicators. Our syndromic indicators identified week-to-week increases in COVID-19-like illness, shortness of breath, cough, and rapid test percent positive one to two weeks faster than published data on COVID-19 testing from the territorial health department. Concordance in syndromic and diagnostic trends, once information was complete, indicate this platform is well-calibrated to monitor COVID-19. Due to the breadth of information available and immediacy of ED visit data, syndromic surveillance platforms can not only be used to monitor COVID-19 closer to real-time than other surveillance approaches, but also create resilient infrastructures through which other emerging health issues can be identified and monitored. The COVID-19 trends observed in Puerto Rico have been useful for situational awareness for clinicians at participating institutions, and, more broadly, for informing public health decision making and pandemic response efforts.

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COVID-19 INFLUENCE ON ANTENATAL CARE ATTENDANCE IN NTCHEU AND NKHATA BAY DISTRICTS, MALAWI

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While major gains have been made over the last two decades to control malaria, the rapid emergence and spread of COVID-19 created substantial global disruptions impacting routine health services, including antenatal care (ANC) attendance in sub-Saharan Africa. This is a serious threat to the health of pregnant women and their unborn babies, as lack of routine ANC means women will not have access to vital, life-saving services, including malaria prevention. Project data from a study assessing the uptake of preventive health services in pregnant women showed a 14% decline in average monthly ANC attendance at the beginning of the COVID-19 pandemic (April–June 2020) compared to January 2019 – March 2020 in 20 study health facilities (HF) in Ntcheu and Nkhata Bay Districts, Malawi. To examine the extent to which knowledge and perceived risk of COVID-19 infection disrupted the uptake of ANC, in-depth interviews were conducted during July-August 2020 with 68 randomly selected women aged 16–49 years who were pregnant or delivered in the previous 12 months. Data were coded and analyzed using NVivo 12. Most respondents knew at least three symptoms of COVID-19 and were aware of at least two actions to prevent infection; however, COVID-19 was perceived as a greater risk over malaria. Country stay-at-home policies, lack of access to masks, HF closures, poor quality of care, and fear of SARS-CoV-2 infection at HF were cited as barriers to continued ANC attendance. Noted motivations for continued ANC attendance included desire to care for one's own health and unborn child, positive perceptions of HF staff, and access to information on strategies to mitigate COVID-19 infection. This study provides insights on the extent to which COVID-19 risk perception influenced ANC attendance during the early phase of the COVID-19 pandemic. Demand generating activities highlighting HF actions to prevent COVID-19 transmission, reiterating the importance of regular ANC attendance during pregnancy, and communicating the high risk for poor outcomes due to malaria in absence of preventive care may be considered to promote and maintain ANC attendance.

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EVOLUTION OF PFS47 AND THE ADAPTATION OF ANCESTRAL PLASMODIUM FALCIPARUM TO VECTORS OF HUMAN MALARIA

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Previous studies indicate that *Plasmodium falciparum* originated when *Plasmodium praefalciparum*, a gorilla malaria parasite, was transmitted to humans by sylvan anopheline mosquitoes. Eventually, the parasite adapted to vector species like *An. gambiae* in Africa and *An. dirus* in Asia, allowing it to spread to human populations beyond tropical Africa. We explored the evolutionary genetics of *Pfs47,* to assess whether the mosquito immune system was a barrier for adaptation of ancestral *P. falciparum* to new vectors of human malaria. We have shown that *Pfs47*, a protein on the surface of the parasite, allows *P. falciparum* to evade the mosquito immune system by interacting with compatible *Pfs47*-receptors in the mosquito gut. Analysis of 4,971 *Pfs47* gene sequences from different continents revealed signatures of selection, and a strong geographic population structure between and within continents, consistent with natural selection of *Pfs47* haplotypes by different vector species. Interestingly, we detected more ancestral *Pfs47* haplotypes in Asia/Oceania, suggesting that ancestral *P. falciparum* readily adapted to Asian vectors. Furthermore, transformed *P. falciparum* carrying *Pfs47* orthologues of *P. praefalciparum* or *P. reichenowi,* two ape malaria parasites adapted to African sylvan vectors*,* were more effective at evading the immune system of *An. dirus* (Asian vector) than of *An. gambiae*. Consistent with these results, phylogenetic analysis showed that the *Pfs47*-receptors of African sylvan malaria vectors, such as *An. moucheti* and *An. marshallii*, are evolutionary more closely related to *Asian* vectors than to *An. gambiae*. Our results suggest that ancestral *Pfs47* haplotypes had compatibility to Asian vectors and facilitated an early adaptation of *P. falciparum* to that continent, likely occurring prior to adaptation to *An. gambiae.* Thus, we hypothesize that Asian *P. falciparum* may harbor ancestral variants in other genes. In depth genetic analysis of *Pfs47* revealed unexpected aspects of the evolutionary history of *P. falciparum* that appear to be determined by its mosquito vectors.

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ENGINEERING A BIODEGRADABLE TRANSGENE IN THE DENGUE MOSQUITO AEDES AEGYPTI

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Aedes aegypti is a critical vector for transmitting Zika, dengue, chikungunya, and yellow fever viruses to humans. Conventional methods to prevent mosquito-transmitted diseases have been insufficient, and as a consequence promising genetics-based vector control approaches are being developed. Less clear is how such transgenes can be removed from the environment, a concern that is particularly relevant for self-propagating gene drive transgenes that can rapidly spread into a population in a super-Mendelian manner. Here, we lay the groundwork for a transgene removal system based on a eukaryotic DNA repair mechanism called single strand annealing (SSA), which requires two molecular features to remove a target sequence: 1) two parallelly identical sequences, named

direct repeats (DRs) and 2) an intervening sequence where a targeted DNA double strand break (DSB) can be induced. We hypothesized that if a transgene is engineered with flanking DRs, then it can be deleted via SSA-based DNA repair when required. A transgene we refer to as the SSA-based rescuer strain (*kmo*^{RG}) was engineered to have DRs in the *Ae. aegypti kynurenine 3-monooxygenase* (*kmo*) gene flanking the intervening cargo genes, *DsRED* and *EGFP*. Targeted induction of DSBs in the *DsRED* transgene successfully triggered complete elimination of the entire cargo from the *kmo*^{RG} strain and restored the wild-type *kmo* gene and thereby normal eye pigmentation. This work provides the proof-of-principle underlying an SSA-based transgene removal system, which could be leveraged to more efficiently remove transgenic material following field evaluations of genetic control approaches involving vector mosquito populations.

1393

AN IMPROVED GENOME ASSEMBLY OF THE WEST NILE VIRUS CULEX QUINQUEFASCIATUS REVEALED NEW INSIGHTS INTO CHROMOSOME AND GENOME EVOLUTION IN MOSQUITOES

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Mosquitoes of two subfamilies Anophelinae and Culicinae have different genome sizes and chromosome arrangements. However, the details of these differences are not completely understood. In this study, chromosome and genome evolution in mosquitoes was investigated using highly improved chromosome-scale assemblies for four species from the major branches of the mosquito evolution, including recently reported *Culex quinquefasciatus* genome. The quality of this genome was improved using Oxford Nanopore Technology sequencing, physical and Bio-Nano optical mapping, and Hi-C scaffolding. Phylogenetic analysis, conducted in this study clarified the divergence time between Culicinae and Anophelinae as ~135 Mya while between *Cx. quinquefasciatus* and *Ae. aegypti* ~69 Mya. In addition to the previously detected chromosomal rearrangements, we identified a pericentric inversion in sex-determining homomorphic chromosome 1 between *Cx. quinquefasciatus* and *Ae. aegypti*. Comparison of the rate of the chromosomal evolution in different species indicated that, similarly to the X chromosome in *An. coluzzii*, the sex-determining chromosome 1 has significantly higher rate of evolution in *Cx. quinquefasciatus* but not in *Aedes aegypti*. Our study supports the previous observations that genome evolution in Culicinae mosquitoes is associated with significant expansion of transposable elements in their genome. Concentrated only in the pericentromeric and some internal regions in the genomes of *Anopheles* species, they spread along the arms toward the telomeres in *Cx. quinquefasciatus* and became evenly distributed in the *Ae. aegypti* genome. We hypothesized that overrepresentation of the repeats in the genome of *Ae. aegypti* is related to the low recombination rates during meiosis and a dramatic decrease in the population sizes in this mosquito.

1394

EFFECTS OF DISRUPTED CIRCADIAN CLOCK ONAEDES AEGYPTI BEHAVIORS

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Aedes aegypti displays strong circadian patterns in blood feeding, mating, and endogenous gene expressions. Much of its behavior is believed to be under the control of an endogenous clock, which consists of a series of transcriptional and translational negative feedback loops. This results in cyclical gene expression and circadian behavioral activity with high activity peaks towards the later part of the light phase. Furthermore, in this species blood-feeding reduces the expression of circadian clock genes. Female mosquitoes stop responding to host cues after blood-feeding, and it has been hypothesized that clock gene suppression is the mechanism through which this is achieved. To examine the connection between the endogenous clock and various *Aedes aegypti* behaviors, we used CRISPR/Cas9 system-mediated gene knockout of *AeCycle* to disable the endogenous circadian clock. We show that CYCLE does not form a functional dimer with CLOCK and that the endogenous clock is disabled. This reduces hatching rates, adult survival and female response to host odor. Furthermore, mating success (after 1 hour) of both *AeCyc*$^{-/-}$ male and female is significantly lower than in the WT. This demonstrates the extent to which these essential behaviors are under endogenous clock control. These data are also consistent with the suggestion that clock gene suppression after blood feeding is one of the mechanisms through which females stop responding to hosts. Surprisingly however, the propensity to blood feed in *AeCyc*$^{-/-}$ females is significantly higher than in WT females, indicating that blood feeding itself may not be under endogenous clock control.

1395

PRINCIPLES OF 3D GENOME ORGANIZATION IN MALARIA MOSQUITOES

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Chromosomes are hierarchically folded within cell nuclei into territories, domains and subdomains, but the functional importance and evolutionary dynamics of these hierarchies are poorly defined. In disease vectors, nuclear architecture may modulate gene expression underlying epidemiologically relevant traits. Of the malaria triad members (human, *Plasmodium*, *Anopheles*), only mosquitoes are lacking studies of the 3D genome organization. Here, we comprehensively profiled spatial genome organizations of five *Anopheles* mosquito species and showed how different levels of chromatin architecture influence each other. The principles of the 3D chromosome folding were determined using Hi-C - a technology that exploits *in vivo* chromatin proximity information. Patterns observed on Hi-C maps were associated with cytological structures, epigenetic profiles, and gene expression levels. Topologically associated domains (TADs) found in A-compartments correspond to genomic regions with high levels of gene expression, while TADs in B-compartments correspond to regions with low levels of gene expression. Evolutionary analysis revealed conservation of chromatin architecture within synteny blocks and enrichment of synteny breakpoints in regions with increased genomic insulation, associated with gene-rich environments. At the level of individual loci, we identified specific, extremely long-ranged looping interactions, conserved for ~100 million years. These long-distance chromatin contacts can span a distance up to 31 Mb. The anchors of the largest loops are not associated with the clustering of active genes and also display low levels of H3K27me3 enrichment, which indicates that they do not correspond to Polycomb-mediated loops. Our data suggest that large loops found in *Anopheles* are formed by other, yet unknown, mechanisms. Overall, our results provide a new framework for understanding of how genomes are organized and function within the nuclear space in disease vectors. An improved understanding of the 3D mosquito genome will inform efforts focused on the control of malaria mosquitoes through genetic approaches.

1396

USE OF A NOVEL HIGH-THROUGHPUT INVERTEBRATE AUTOMATED PHENOTYPING PLATFORM ASSAY TO SHOW THAT GAL4-UAS MEDIATED OVEREXPRESSION OF SINGLE GENES IN ANOPHELES GAMBIAE LARVAE CAN CONFER RESISTANCE TO MULTIPLE INSECTICIDES

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Insecticide resistance is a looming threat to malaria and arbovirus control programmes targeting the mosquito vector. Integrated control programmes which include control of larval stages are becoming more important for *Anopheles* control with increasing urbanisation in malaria endemic areas and remains crucial in *Aedes* control. However, the existing WHO recommended mortality-based larval resistance assay is low-throughput and subject to investigator bias. To address these issues, a novel assay was developed using the Invertebrate Automated Phenotyping Platform (INVAPP) and analysis algorithm (Vectorgon) which provides automated quantification of larval motility after insecticide exposure. The INVAPP-Vectorgon assay has been used to: screen large compound libraries for novel compounds; carry out dose response assays; and investigate resistance phenotypes (where a suitable comparator strain is available). Additionally, this assay has been developed into smartphone technology which has many potential field applications. This assay has been demonstrated to work well to predict resistance phenotypes in *Aedes aegypti* and *An. gambiae* wild type and in transgenic *An. gambiae* first instar larvae. These results highlight the potential of this novel assay for use both in the laboratory and in the field.

1397

AN AMPLICON SEQUENCING PANEL ENABLES LOW-COST POPULATION GENETICS STUDIES IN AEDES AEGYPTI

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Aedes aegypti is the principal vector of dengue virus, a pathogen that is estimated to cause 96m cases of dengue fever a year and 20,000 deaths. Understanding this vector at the population level is a priority for researchers, however in contrast to large scale population genetic studies that have been completed in malaria vectors using whole-genome sequencing, examples of this kind of work in *Ae. aegypti* are smaller in scale. This is in large part due to the size of the genome; 1.3gb genome of *Ae. aegypti* is more than five times the size of *Anopheles gambiae*, and a 10-20X coverage genome will cost ~$200-300usd per sample. Moreover, 65% of this genome consists of repeats and transposable elements, making it effectively unusable. This renders population genetics projects involving hundreds or thousands of samples beyond the reach of even the largest institutes. Targeted genotyping a panel of loci across the genome will reduce these costs significantly and still provide high quality information on population diversity and relatedness. We have designed an amplicon panel appropriate for genomic studies in this species and tested it on a panel of globally distributed genetic backgrounds. Our panel of neutral, multiallelic markers is well powered to enable studies of genetic relatedness, delineation, and diversity in this species. At a maximum per-sample cost of ~$12usd, including DNA extraction and sequencing costs, we believe this technology will be transformative to the field.

1398

HYPERLOPIT ANALYSIS REVEALS CRYPTOSPORIDIUM SECRETORY ORGANELLES

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Cryptosporidium infection is a leading cause of child mortality, no vaccine is available and the current drug treatment against this diarrheal pathogen is inefficient. The disease is transmitted through food or water contaminated with oocysts, the chlorine-resistant parasite stage. The parasite infects the epithelial cells of the small intestine in which it replicates intracellularly.Invasion and intracellular development require extensive modifications of the host cell that remain largely unknown at the molecular level. We recently showed that parasite secreted proteins play an important role in this process, however, our knowledge remains limited to a small number of factors from one organelle, the rhoptry. Here we conducted a proteomic experiment, hyperLOPIT, on fractionated Cryptosporidiumsporozoites. We resolved numerous clusters of proteins based on their subcellular locationincluding the ribosome, proteasome, ER, mitosome and nucleus. We distinguished multipleclusters enriched in signal peptide containing proteins including the conserved secretory organelles micronemes, rhoptry and dense granules. These assignments were confirmed by epitope tagging candidates followed by localization studies in sporozoites and infected cells. This allowed us to identify the first four Cryptosporidium dense granules proteins. Interestingly, our dataset identified other clusters enriched in signal peptide containing proteins corresponding to the oocyst wall and the crystalloid body, as well as a previously unknown secretory cluster. The latter was found to accumulate around the parasite nucleus and its content is secreted upon invasion. Altogether, this list of Cryptosporidium secreted proteins will help fill important gaps in our knowledge of the host/parasite interplay.

1399

METABOLIC COMPETITION BETWEEN PHOSPHO-CHOLINE SYNTHESIS AND HISTONE METHYLATION REGULATES SEXUAL COMMITMENT IN PLASMODIUM FALCIPARUM

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P. falciparum parasites must balance persistence in the current host with transmission to the next. The first requires continuous asexual replication in red blood cells while the second requires sexual differentiation into gametocytes that can infect the mosquito vector. The frequency of this developmental decision is determined based on stochastic activation of a heterochromatin-silenced locus encoding the transcription factor AP2-G. Recent work showed that the frequency of ap2-g de-repression is responsive to the serum levels of phospholipid precursors such aslysophosphatidylcholine, however, the regulatory mechanisms linking these lipidprecursors to AP2-G expression remained unknown. Using chemical, metabolomic,and genetic approaches, we found that this response is mediated by competition for the universal methyl donor S-adenosylmethionine (SAM) between de novophospho-choline synthesis and histone methylation. When phospho-cholineprecursors are scarce, increased consumption of SAM for de novo synthesis of phospho-choline results in a decrease in the deposition of silencing histonemethylation marks at the ap2-g locus. This weakened silencing state increases the probability of reaching AP2-G expression levels sufficient to trigger the transcriptional feedback loop that activates and locks in the sexual differentiation expression program. Our findings demonstrate the first

mechanistic link between metabolite utilization and gene expression in malariaparasites and explains the connection between lipid metabolism and sexual differentiation.

1401

TUFT CELL-DERIVED ACETYLCHOLINE REGULATES EPITHELIAL FLUID SECRETION DURING HOMEOSTASIS AND TYPE 2 IMMUNITY

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The intestinal epithelium maintains a barrier against microbiota, pathogens, and environmental insults in part through the secretion of fluid and mucus. During the Type 2 immune response to parasitic helminths, IL-13 signaling drives dramatic epithelial remodeling, resulting in goblet cell hyperplasia and increased mucus production that contribute to the "weep and sweep" required for helminth clearance. Tuft cells, rare chemosensory epithelial cells that initiate this Type 2 response upon sensing of helminths and certain microbial metabolites, also express the enzyme ChAT required for synthesis of acetylcholine (ACh). ACh is a potent inducer of epithelial fluid and mucus secretion but is typically thought to be produced by enteric neurons. We find that at homeostasis the microbial metabolite succinate induces rapid fluid secretion in the distal small intestine dependent on tuft cell-derived ACh. Secretion also requires tuft cell chemosensing and is independent of neuronal involvement. Fluid secretion is enhanced during Type 2 inflammation, consistent with the observed increase in ChAT+ tuft cells. Upon infection with the hookworm Nippostrongylus brasiliensis, tuft-specific ChAT-deficient mice suffer delayed worm clearance despite robust epithelial remodeling. Our findings suggest that upon sensing of luminal signals produced by helminths and microbes, tuft cells stimulate an epithelium-intrinsic effector unit to rapidly respond with fluid secretion. This response is amplified by epithelial remodeling that occurs during the Type 2 response, contributing to anti-helminth immunity.

1402

A KINASE THAT COORDINATES NUCLEAR ABSCISSION IN THE MALARIA PARASITE PLASMODIUM FALCIPARUM

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Apicomplexa, a phylum of parasitic unicellular eukaryotes, exhibits extraordinary diversity in their mechanisms of cellular division. Plasmodium falciparum, the deadliest cause of malaria in humans, undergoes alternative rounds of DNA replication and nuclear division followed by a single round of cytokinesis. This division strategy, known as schizogony, allows a single parasite to form approximately 30 daughter parasites every 48 hours. Despite the biological and therapeutic attractiveness of this division strategy, little is known about the proteins that coordinate this process. Here, we identify a Tousled-like kinase (TLK) encoded by P. falciparum and using super-resolution microscopy we show that TLK has adual localisation, showing both a pericentromeric localisation and localising to the cleavage furrow of dividing nuclei. Inducible knockdown of TLK results in a severe growth defect. Using expansion microscopy, we show that TLK depleted parasites undergo DNA replication but are unable to perform nuclear abscission, resulting in the formation of parasites with multiple genome copies inside a giant conjoined nuclear envelope. Moreover, we show this phenotype is likely caused by perturbations in microtubule formation. These findings begin to uncover molecular detail of the poorly explored biology of apicomplexan cell division and highlight a potential target for therapeutic development.

1403

IDENTIFICATION OF NEW RHOPTRY SECRETION FACTORS IN TOXOPLASMA USING THE CILIATE TETRAHYMENA

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Together with ciliates and dinoflagellates, apicomplexan parasites belong to the Alveolata superphylum. Apicomplexa possess secretory organelles called rhoptries, that undergo regulated exocytosis during invasion. Upon injection into the host cell, rhoptry proteins support invasion, vacuole formation, and subversion of host immune function. Combining decades-old pioneering ultrastructural observations in Apicomplexa with data on regulated secretion from the ciliate Paramecium tetraurelia, we recently showed that rhoptry exocytosis involves a "rosette" of 8 particles embedded in the plasma membrane. The rosette is conserved in Ciliata and its formation requires conserved Alveolata "Nd" proteins. These findings point to the existence of an Alveolata-conserved mechanism for organellar membrane fusion events, and provide proof of concept that we can decipher rhoptry exocytosis machinery using a PAN-Alveolata approach. Here we extend our ciliate analogy to uncover new rhoptry secretion factors. We used the transcriptional profiles of Nd genes of the ciliate Tetrahymena thermophila to select genes that are co-regulated, and also conserved in Apicomplexa. In this way we identified two uncharacterized Tetrahymena proteins that contribute to the exocytosis of secretory organelles. They have similar architecture and show homology with Plasmodium Cysteine Repeat Modular Proteins (CRMPs), a family of proteins essential for Plasmodium transmission from the mosquito to the host. We will present the functional characterization of the two Toxoplasma CRMPs, including their dynamic location during invasion. While they are not required for rosette formation, we show that they are essential for rhoptry secretion, suggesting a function distinct from that of previously characterized exocytic "Nd" factors.

1404

ACTIN ORGANIZATION AND DYNAMICS IN MOTILE TOXOPLASMA GONDII PARASITES

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Apicomplexan parasite gliding is powered by a thin layer of filamentous (F)-actin and specialized myosinmotors that lie just beneath the parasite plasma membrane. How is this actomyosin network organized to give rise to coherent traction forces and move the cell forward? Using single-molecule imaging, we tracked individual actin filaments and myosin A light chain (MLC1) complexes in living extracellular Toxoplasma gondii tachyzoites. These measurements revealed that actin filaments can undergo rapid directional movement while myosin is largely immobile, consistent with anchorage to the inner membrane complex. F-actin transport direction was strikingly heterogeneous, suggestiveof a dynamic or self-organized system, rather than uniformly following the fixed polarity of underlying microtubules as previously believed. To understand whether actin polarity and velocity patterns could arise from filament-filament and filament-motor interactions alone, we developed a continuum model for actin self-organization constrained by the geometry of the parasite. In the absence of filament turnover, this model predicted the emergence of actin patches that recirculate up and down the cell, a "cyclosis" that we observed experimentally for drug-stabilized actin bundles in live parasites. The addition of actin turnover and polymerization at the parasite apical end enabled the emergence of a second steady-state mode, in which

actin polarity and transport is largely rearward. These results suggest that polarized actin dynamics govern the transition between experimentally observed bidirectional (patch or pendulum) and unidirectional (helical, circular, twirling) gliding modes. In summary, this work combines experiment and theory to present a framework for actomyosin self-organization during gliding motility.

1405

THE IMPACT OF ANTIGEN DOSE ON THE GENERATION, FUNCTION AND LONGEVITY OF LEISHMANIA-SPECIFIC CD4+ MEMORY T CELLS

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There is currently no approved vaccine against human cutaneous leishmaniasis(CL). This is due in part to insufficient knowledge regarding the immunodominant Leishmania antigens, the nature of T cells that respond to them and the factors that regulate the generation, function and longevity of different subsets of memory T cells against these antigens. Antigen dose is known to influence the magnitude and quality of the host immune response. Specifically, antigen dose has been shown to regulate the differentiation of naïve CD4+ T cells intoTh1 effector cells that mediate resistance toCL. However, the influence of antigen dose on the magnitude and quality (cytokine production and function) of Leishmania-specific CD4+ T cells memory response is unknown. Recently, we identified Leishmania phosphoenolpyruvate carboxykinase (PEPCK) as protective antigen and showed that PEPCK335-351 peptide is the dominant CD4+ T cell epitope. We generated PEPCK specific TCR transgenic (PEG) mice and found that >90% of their CD4+ T cells express PEPCK-specific TCR. We found that PEG CD4+ T cells specifically respond to PEPCK in vitro and in vivo. Upon adoptive transfer into congenic mice followed by immunization with different doses (0.05, 0.5 or 5 nmol) of PEPCK 335-351 peptide, PEG cells proliferated robustly and produced IFN-© in a dose-dependent manner. Interestingly, high dose (5 nmol) PEPCK peptide induced higher frequency of polyfunctional (IFN-©+IL-2+TNF+) cytokine producing PEG cells and this was associatedwith higherexpression of CD44, CCR7 and CD62L, markers that characterize central memory (Tcm) phenotype. Moreover, PEG cells in mice immunized with high dose PEPCK peptide showed higher IFN-© recall response following secondary in vivo peptide or L. major challenge. This higher IFN-© response was associated with better protection against virulent L. major challenge. Collectively, these findings show that antigen dose influences the generation, magnitude and function of Leishmania specific CD4+ memory T cells.

1406

PD-1 LIMITS TREG IMMUNE SUPPRESSION DURING ACUTE TOXOPLASMOSIS

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While much is known about the factors that promote the development of diverse Treg cell responses, less is known about the pathways that constrain Treg cell activities. The studies presented here reveal that at homeostasis there is a population of effector Treg cells that express PD-1, and that blockade of PD-L1 or loss of PD-1 results in increased Treg cell activity. In response to infection with the parasite T. gondii, the early production of IFN-γ results in widespread upregulation of PD-L1. Moreover, blockade of PD-L1, whole body deletion of PD-1, or lineage-specific deletion of PD-1 inFoxp3$^+$ cells prevented the loss of the effector Treg cells but resulted in reduced pathogen specific CD4$^+$ T cell responses during infection. Thus, at homeostasis basal PD-L1 expression constrains and tunes the pool of Treg cells, but during infection the upregulation of PD-L1 provides a mechanism to contract the Treg cell population required to maximize the development of pathogen specific CD4$^+$ T cell responses.

1407

GUT MICROBIOTA COMPOSITION MODULATES THE SPLENIC GERMINAL CENTERS DURING PLASMODIUM INFECTIONS THROUGH IMMUNE DISTRACTION

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The gut microbiota has been shown to play a role in both human and rodent Plasmodium infections. Our lab recently demonstrated that gut microbiota composition can modulate the severity of malaria using mice from different vendors, susceptible (Tac) and hypersusceptible mice (CR). We found gut microbiota-dependent numbers of germinal center (GC) B cells and parasite-specific antibody titers between the two vendors. These findings were inversely correlated with parasite burdens of the mice. To further characterize the gut impact on infection, we harvested the Peyer Patches (PP), MLN, ILN and spleens from the mice before and on various days. We found differences between Tac and CR PP's before infection, with increased numbers of neutrophils, γδ Tcells, T follicular helper cells, and GC B cells in CR mice, suggesting an active immune response at this site. Accordingly, flow cytometry analysis of IgA+ fecal bacteria confirms that CR mice have higher basal IgA+ bound bacteria, and both vendors have a proportional increase of IgA bound bacteria from day 8-10 post Plasmodium infection. As the infection progresses, CR starts to form splenic germinal centers, but these mice are not able to maintain splenic GCs against Plasmodium while also maintaining an active immune response in the gut, correlating to a lower ratio between splenic TFhand TFr cells in CR animals when compared to Tac. These results provide a mechanistic insight into the impact of the gut microbiota on the extra-gastrointestinal tract germinal center response and will be an important factor to consider in the development of optimal malaria treatments.

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