Innovative tools are needed to complement the existing approach for malaria elimination. Gene drive mosquitoes are one potential new technology in the control of malaria vectors. Target Malaria is one of the research projects developing this technology, and in July 2019, the project proceeded to an important step for this evaluation pathway: the small-scale release of non-gene drive sterile male mosquitoes in a village in Burkina Faso. In addition to the entomological and laboratory work to prepare for this important milestone, significant community and stakeholder engagement work was done. This study provides a review of engagement activities relevant to field trials on non-gene drive genetically-modified mosquitoes as well as an assessment framework—using both qualitative and quantitative studies as well as an audit procedure. The latter was implemented to evaluate whether the release activities could proceed with the appropriate level of agreement from the community. This paper shows the importance of this first phase of work to innovate and learn about engagement processes for responsible research in the field of genetic approaches for malaria vector control. The function of these assessments is crucial for the learning agenda. The assessments demonstrated ways to increase understanding and ensure effective progress with field studies and, therefore, the pathway for responsible research. In conclusion, gene driven technology is increasingly considered as a promising approach to control vector borne diseases, in particular malaria. Stakeholders’ involvement in this research process is one of the recurring requirements in international guidance documents. With this paper Target Malaria offers an opportunity to explore the practical achievements and challenges of stakeholder engagement during early phases of a technology evaluation, and in particular how it implemented an assessment framework to learn from its experience.

5001

ACCELERATING MALARIA ELIMINATION IN CAMBODIA: ANALYSIS OF IMPACT OF THE "LAST MILE" INTENSIFICATION PLAN

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The Greater Mekong Subregion countries are preparing to enter the final phase of malaria elimination. To maintain this momentum, more nuanced, and targeted approaches are required. Cambodia’s National Center for Parasitology, Entomology, and Malaria Control (CNM) and partners are implementing an intensification plan called the “last mile” strategy to accelerate progress. This comprises a focalized aggressive set of interventions including house-to-house fever screening (AFS), targeted bed and hammock net distribution, chromoprophylaxis of forest goers (IPPT) and target drug administrative (TDA). Implementation is supported by village and mobile malaria workers and health centres with outreach to remote communities. Analysis of routine malaria data was done to measure the impact of the last mile strategy and guide future plans. From 25th December 2020 to 1st March 2023, last mile had been implemented in 123 villages (population 75,195). Village mean and median malaria annual parasite index (API) during the 12 months prior to implementation were 1.075 and 0.258 (IQR: 0.036), respectively. In the 12 months after the intervention, these were 0.178 and 0 (IQR: 0, 0). The paired difference of the village median APIs was compared using the Wilcoxon signed-rank test giving a p-value of < 0.0001. To compare the declining rates, an interrupted time series analysis was performed using segmented regression. Before the last mile implementation, mean and median declining incidence rate ratio were 0.106 and -0.114 (IQR: -0.152, -0.061), respectively, compared to -0.530 and -0.511 (IQR: -0.576, -0.511) after the intervention, p < 0.0001. Overall cases in these villages decreased by 52% from 1906 in 2020 to 863 in 2022, despite the number of malaria tests performed remaining steady. The reduction in cases was even higher among mobile populations at 59%. Although this initial result suggests the last mile intervention significantly reduced malaria cases in implementation villages, implementation is ongoing and the final analysis will be presented. Lessons learned and implications for other countries aiming for elimination will be discussed.
Malaria Case-Based Surveillance is pivotal in the detection of asymptomatic cases that are missed at health facilities and provides a better understanding of risk factors in transmission foci.

Enhanced Active Case Detection to Eliminate Malaria in Yala Province, Thailand


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Thailand’s malaria elimination strategy includes several active case detection (ACD) methods, both proactive and reactive. In Yala Province, where there is intermittent civil unrest, malaria cases declined from 1,592 in fiscal year 2015 (FY15) to 57 in FY22. In FY16, following a successful pilot project among military personnel, local authorities expanded ACD to include civilian populations, relying on community health workers to navigate the unique context. This study used routine surveillance data to examine ACD yield (i.e., confirmed cases among total screened) and trends among all 821,334 blood draws and 14,855 confirmed cases in Yala from FY15-FY22. T-tests were run for mean differences, and spatial trends were assessed using intercept-only generalized linear regression models. Over the study period, ACD screenings represented 58.3% of all blood draws in Yala (compared to 74.1% nationally). Among 478,708 ACD blood draws, 1,328 cases were detected, representing a yield of 0.28% (range 0.01%-0.92%, compared to 0.07% nationally). Among 478,708 ACD blood draws, 1,328 cases were detected, representing a yield of 0.28% (range 0.01%-0.92%, compared to 0.07% nationally). In FY18, local authorities replaced other proactive methods with special case detection (SCD), which used a small team to screen every village member, in an effort to reduce costs and optimize ACD. As a result, SCD screenings increased from 1,769 in FY17 to 15,546 in FY18; however, SCD yield significantly decreased from 0.73% in FY17 to 0.10% in FY18 and down to 0.01% by FY22 (p < 0.05). Yield for reactive case detection was higher in the first 6 years compared to the last two years (0.39% compared to 0.004%, p < 0.05), consistent with declining malaria incidence. Geospatial results showed that subdistricts with higher ACD yield clustered in the center of the province. Although ACD yield in Yala is higher than elsewhere in Thailand, these results show that there is an opportunity to further refine targeting and methods to make efficient use of resources. Epidemiological data could help quantitatively define high-risk populations and areas. Thailand plans to use these results and further analyses to develop standard operating procedures to accelerate malaria elimination among these at-risk populations in Yala.
PREGNANT WOMEN EXCLUSION IN CLINICAL TRIALS FOR MALARIA, TUBERCULOSIS, AND COVID-19: A REVIEW OF TRIAL REGISTRY DATA

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5007

A PROGRAM EVALUATION OF REACTIVE FOCAL DRUG ADMINISTRATION IN NORTHERN SENEGAL

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5006

TRIAL REGISTRY DATA

PREGNANT WOMEN EXCLUSION IN CLINICAL TRIALS FOR
findings.

the use of incomplete routine surveillance data may have influenced these incidence rates compared to the standard intervention package; however, remained high, nor were monthly declines in incidence rates (IRR = 0.97, 1.24) from roll-out through September 2018, when intervention coverage between rFDA and comparison groups (IRR = 0.77, 95% BCI = 0.48, 1.00) were similar across groups (IRR = 0.97, 95% BCI = 0.93, 1.01). RFDA was not associated with sustained, lower incidence rates compared to the standard intervention package; however, the use of incomplete routine surveillance data may have influenced these findings.

NODDING SYNDROME CLINICAL CHARACTERISTICS, RISKS FACTORS, ACCESS TO TREATMENT, AND PERCEPTIONS IN GREATER MUNDRI AREA, SOUTH SUDAN

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5008

Nodding syndrome (NS) is a neurodegenerative disease of unknown etiology, affecting poor people in Tanzania, South Sudan, Uganda, the Democratic Republic of Congo, Cameroon, and the Central African Republic. It presents with head nodding and other seizures; often associated with debilitating complications, including impaired cognitive and physical development and delayed sexual maturity. Previous attempts to identify a potential cause have focused on infections, nutrition, toxins, autoimmune, hormonal and metabolic derangements, and genetic factors, but all were inconclusive. Control of onchocerciasis and its vector, the blackfly, by community-directed treatment with ivermectin and larviciding of rivers were proposed to prevent new cases. However, certain communities in the affected areas believe that NS is transmitted from person-to-person and thus can be prevented by isolation of cases from other family members or peers. To further unravel the many unknowns of NS, we conducted a house-to-house survey and case-control studies in Mundri County, South Sudan to investigate the clinical characteristics, risk factors, access to treatment and perceptions about NS. In total, 224 cases with median age of seizure onset 10 years were identified. Head nodding was more common in 22.3%, and head nodding plus other types of seizures in 77.7% cases. Wasting, stunted growth, delayed sexual development and speech and behavioural abnormalities were observed in 23.6%, 22.2%, 17.3%, 19.4% and 5.6% cases, respectively. The consumption of rat-meat, but not other bushmeat was associated with an increased risk of NS (OR 9.31, CI 1.27–406.51). Cases were more likely to have taken ivermectin in the last 5 years (OR 2.40, 95% CI 1.33–4.43), and were less likely to share a bedroom with other children (OR 0.06, 95% CI 0.02–0.16) or adults (OR 0.27, 95% CI 0.13 0.56). In conclusion, rat-meat consumption is an unlikely risk factor for NS, and ivermectin intake was more common among NS cases than controls. Importantly, we documented that children with NS are stigmatized because of the misconception that NS is transmitted through direct contact.

DIABETES-ASSOCIATED MAJOR LIMB AMPUTATION IN SOLOMON ISLANDS: EPIDEMIOLOGICAL CHARACTERISTICS AND CLINICAL MANAGEMENT

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Solomon Islands is classified as a UN “Least Developed Country” and faces the 9th highest prevalence of diabetes globally. Limited resources, poor infrastructure, and challenging geography make medical and surgical delivery difficult. This retrospective study aims to describe the characteristics and clinical management of patients undergoing diabetes-associated major limb amputation. It is the first study on major limb amputation in Solomon Islands. Demographic and clinical data was abstracted from charts belonging to patients with diabetic ulcers who underwent major limb amputation at four surgical centers. Summary statistics were gathered from this dataset. Over a 5-year period (2018-2023), 338 adults underwent major limb amputation secondary to diabetes-associated infections. Of these, 285 patients had medical records available for data abstraction. The median age for these patients was 55 years (range: 18-83), 50.5% (N=144) were male. The most common known cause of initial ulceration was general trauma (N=30). The second most common cause was rat bites (N=15). The median time from initial presentation to amputation was 3.5 days (75% CI: 2.5-4.5). The mean clinical classification score was 5.50 ± 0.75 (N=278) for patients experienced delay in accessing amputation. Late presentation was also common with 33% (N=93) waited 30 or days before seeking medical attention. Blood sugar levels were controlled in only 1.4% (N=4) of patients during hospital stay. The mean wait time between recommendation of amputation and operation was 7 days (range: 0-86). Among all patients who underwent major limb amputation, 12% (N=41) died prior to discharge. In conclusion, major limb amputation is a costly and radical procedure which contributes to severe disability in the local context. Earlier presentation, effective diagnosis of foot/limb infection, aggressive management of infection, and earlier access to surgical care are likely to prevent limb loss. This research shows the importance of investing in diabetes prevention to prevent downstream complications. Future research should investigate locally modified and sustainable methods to improve limb salvage and preventative foot care.

5010

LEVERAGING PARTICIPATORY MAPPING AND FINE-SCALE GEOSPATIAL ANALYSES TO OPTIMIZE COMMUNITY-BASED HEALTHCARE PROGRAMS AND POLICIES

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In rural communities, where geography is often the main barrier to access primary healthcare, community health workers (CHWs) provide essential primary health services. Sophisticated geospatial data and analyses are transforming health systems; however, these approaches are rarely developed to serve community health programs and policies. As an integral part of the public health system, there is a critical need to develop evidence-based decision making tools (e.g. data dashboards, hotspot mapping, and precision health) at local scales relevant for community health programs. A set of high-resolution geospatial data, analyses and decision-making tools are being integrated into community health programs in Ifanadiana, a rural district of Madagascar. The backbone of this initiative was the collection of high-quality geospatial data for the district via participatory mapping in OpenStreetMap, resulting in the mapping of over 100,000 buildings and 20,000 km of footpaths. This data enabled us to accurately estimate field-derived travel times to primary care facilities for each household in the district and identify geographic distance as a major barrier to primary care use. We then demonstrated that geographic barriers persist at the community health level and we developed a spatial algorithm to optimize the location of community health sites within each CHW catchment, the results of which are embedded in an online data dashboard. Finally, we are using this dataset to optimize the planning and implementation of proactive CHW programs, estimating optimal routes for CHWs to visit every household in a catchment, contributing towards universal access to healthcare. All of these geospatial analyses are publicly available as e-health tools to guide programs. Our work highlights how high-resolution geospatial analyses can be integrated into existing community health programs to address spatial inequalities and barriers to achieving universal health coverage. Scale-up of these tools could contribute to optimizing community health systems globally, in line with the 2018 guidelines from the World Health Organization.
THE EFFECT OF COMMUNITY-BASED PACKAGE OF INTERVENTIONS ON IMPROVING INSTITUTIONAL DELIVERY CARE SERVICES UTILIZATION IN ARBA MINCH HDSS, SOUTHERN ETHIOPIA: A CLUSTER-RANDOMIZED CONTROLLED TRIAL

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Regular utilization of maternal healthcare services reduces maternal morbidity and mortality. However, evidence shows that women do not use the existing services, especially institutional deliveries, with a substantial inequity between urban and rural areas. This study evaluated the effect of a package of community-based interventions on the improved institutional birth rate in rural Ethiopia. We conducted this cluster-randomized controlled trial (NCT05385380) from 2019 to 2021 at the Arba Minch Health and Demographic Surveillance System site. We randomly assigned six kebele clusters to the intervention and four to the control arm. We used a package of interventions, which included providing information on safe motherhood via videos or audiocassettes with a birth preparedness card for pregnant females, training for community volunteers and health extension workers, and improving maternity waiting home services. Women in the control arm received routine services only. We used generalized mixed-effects logistic regression models to evaluate the effect of the intervention on the outcome variables. We enrolled 727 pregnant females across the 10 clusters, with a 617 (84.9%) successful follow-up rate. The proportion of institutional deliveries in the intervention arm was increased by 16.1% from 36.4% (174/478) at the baseline to 52.5% (224/427) at the end line. The adjusted odds ratio [AOR] for McNemar’s Test = 1.5; 95% confidence interval [CI]: 1.1 to 2; p < 0.001). However, the control arm had a 10.3% decrease in institutional deliveries from 164/294 to 150/190. In addition, pregnant females who received the intervention were twice as large (AOR 2.8; 95% CI: 1.2, 6.4) likely to give birth at a health institution than those in the control arm. This study demonstrates that an integrated community-based intervention package can increase utilization of institutional delivery care services in rural Ethiopia. Therefore, we recommend that functioning maternity waiting homes and audio-video-supported education for pregnant women be part of routine maternal healthcare to achieve the “leave no one behind commitment” in maternal health.

IMPACT OF A MOBILE OBSTETRIC REFERRAL EMERGENCY SYSTEM (MORES) ON REDUCING CARE DELAYS IN RURAL LIBERIA

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Maternal mortality disproportionately affects low- and middle-income countries, including Liberia. Due especially to late presentation with infectious conditions, maternal mortality can be reduced with obstetric triage systems which are unavailable in Liberia. Mobile health interventions are a promising but underutilized method to reduce care delays for such patients. This study assessed the impact of a Mobile Obstetric Referral Emergency System (MORES) in rural Liberia. Front-line health providers working at 18 rural health facilities and 2 hospitals received training on obstetric triage and MORES. Data on 300 patients at three time points: 0, 6-months, and 12-months were collected to assess care delays and clinical outcomes. MORES usability data were recorded through WhatsApp messages and provider interviews. Baseline and midline results (N=200) are reported with endline data being collected. Results demonstrated a significant decrease in time from decision to incision for Cesarean section (n=60) from 482 minutes pre-training to 186 minutes post-training (M=296 min, 4.33 hours reduced). In total, 43 providers referred 359 patients with thousands of messages through MORES. Messages included infectious diagnoses, transfer rationale, time from rural facility to hospital, and patient outcomes. MORES was highly usable and acceptable and demonstrated components of health systems strengthening, including decreased transfer times, improved inter-professional dynamics, feedback mechanisms, exposing hidden delays, and establishing a preliminary electronic health record with follow-up for high-risk patients. MORES demonstrated high acceptability and improved clinical metrics, particularly reduced delay to C-section. Capacity building included patient tracking in a setting where electronic health records are unavailable, and where formal follow-up care is not standardized. Future studies should evaluate scalability throughout Liberia and mixed methods analysis of patient perceptions.
Test-treat-track (TTT) approach to eradicate malaria relies on the detection of Histidine-Rich Proteins 2/3 (HRP2/3) through rapid diagnostic tests (RDTs), followed by treatment with artemisinin-combination therapies (ACT). However, data suggest that in the Horn of Africa alone, HRP2/3 deletions resulted in up to 50% of to be missed by RDTs. Recent reports of kelch13 mutations suggest resistance conferring single nucleotide polymorphisms are widespread. Our group was the first to document kelch13 R622I in P. falciparum malaria. The completed study results will be presented at ASTMH.

Sequencing of kelch13 was assessed to identify kelch13 mutations using qualitative PCR and quantitative by digital droplet PCR (ddPCR). Analysis of the remaining samples will confirm whether there is significant proportion 9/42 (21%) of infections contained either polyclonal infections or partial deletions (21%) of infections contained other polyclonal infections or partial deletions by ddPCR. A significant proportion 9/42 (21%) of samples contained HRP2/3 deletions combined with kelch13 mutations (P = 0.0673). Further analysis of the remaining samples will confirm whether there is significant correlation between the deletions in HRP2/3 and the kelch13 mutations. Our study raises the spectre of co-selection of drug and diagnostic resistance in P. falciparum malaria. The completed study results will be presented at ASTMH.

FOR MALARIA DETECTION AND SPECIATION

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In clinical settings, patients are often misdiagnosed and do not receive correct timely treatment. Rapid multiplexed tests comprising variants of the infectious diseases can be pivotal in identifying the infections and enhance the physician's ability to diagnose use appropriate treatment and avoid misusage of drugs. Point-of-care tests (POCTs) with rapid and accurate diagnosis are needed. Loop mediated amplification (LAMP) significantly reduces detection time with a simplified sample preparation step and a rapid amplification step under isothermal conditions. For widespread usage, POCTs must be simple to perform with minimal technical expertise biosafety and provide diagnosis within 30 minutes. We have developed a microfluidic cartridge based POCT for malaria diagnosis. The cartridge is constructed with a multilayered architecture forming channels, reservoirs and integrating a porous lateral flow (LF) strip loaded with dry LAMP reagents in a closed system. The LF strip acts as a filter as well as provide passive flow of assay fluids (sample, buffer solutions). The cartridges are loaded on a companion battery-powered reader device comprising a heater for isothermal amplification. A combination of LEDs, optical filter and light-intensity sensors performs a real-time visual fluorescence readout of the LAMP products. A complementary smartphone app provides wireless control over the device components as well as recording the real-time LAMP data to improve diagnosis accuracy. This POCT platform dubbed “MicroLAMP” is coupled with a multiplexed panel for detection and differentiation of malaria parasites using a minimally invasive finger prick blood sample. The POCT tool is broadly applicable to multiple infectious diseases at a low cost.
an introduced thymidine kinase from *Herpes simplex* virus has been successfully used in DNA labelling studies using BrdU and EdU, this approach may not be feasible for analysis different *Plasmodium* species, multiple laboratory lines and field isolates. To overcome this, we have designed and synthesised new thymidine-based probes that overcome the need for an endogenous thymidine kinase enzyme. Using CuAAC with a fluorescent azide and flow cytometry, we have shown that these DNA Detect™ probes robustly label replicating asexual intraerythrocytic *P. falciparum* parasites, while EdU as a control failed to label parasites. The DNA Detect™ chemical probes are synthetically accessible and thus have broad applicability as tools to further understand the biology of different *Plasmodium* species, including laboratory lines and clinical isolates.

**USE OF MINIMALLY INVASIVE TISSUE SAMPLING (MITS) TO DETERMINE THE CONTRIBUTION OF MALARIA INFECTIONS TO MORTALITY IN CHILDREN UNDER 5 YEARS OF AGE IN THE CHAMPS NETWORK**

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Background Malaria remains a major killer of children globally, but accurately quantifying endemic country estimates is difficult as current tools lack sensitivity. Such estimates are needed to prioritize targeting of commodities and interventions.

Methods Seven sites in Africa and Asia participating in Child Health and Mortality Prevention Surveillance (CHAMPS) collected comprehensive data from stillbirths and children <5 years of age within their catchment areas. Minimally invasive tissue sampling (MITS) was performed on those enrolled within 24 hours of death. Underlying, intermediate, and immediate causes of death (CoD) were assigned by local expert panels, utilizing sociodemographic, clinical, laboratory, and verbal autopsy data according to standardized protocols. The expert panel also determined if the death could have been prevented with existing, recommended measures. Analyses were conducted in 4 sites with high malaria endemicity (Mozambique, Kenya, Sierra Leone and Maldives) to describe factors associated with malaria-related deaths, estimate malaria-specific mortality, and assess the proportion of preventable deaths in 1 to <60 month-olds.

Findings Between 2016-2022, MITS was used to determine CoD for 773 infant and child deaths. Malaria played a significant role in the causal pathway in 237 (30.7%), 75.7% of which were aged 12–60 months. Of all malaria deaths, 24.9% occurred in the community, 16.0% were medically unattended, and 98.7% were determined to be preventable. *P. falciparum* was the sole infecting pathogen in 164 (69.2%) of the malaria-related deaths: Bacterial co-infections were in the causal pathway of 24.5%, viral co-infections in 13.1%, and malnutrition in 30.5% of the cases. Interpretation/Malaria remains a significant cause of childhood deaths in the malaria-endemic sites of CHAMPS despite available treatments and prevention measures. Importantly, one in 4 malaria deaths also had a bacterial co-infection, supporting the use of antibiotics in severe malaria patients.

**DECONSTRUCTING TRANSMISSION OF VISCERAL LEISHMANIASIS THROUGH ANALYSIS OF BLOOD FED SAND FLIES**

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Visceral leishmaniasis (VL) is a vector-borne neglected parasitic disease transmitted by sand fly bites. VL causes up to 90,000 new cases per year and has a fatality rate of 95% without treatment. In India, VL is nearing elimination, yet disease outbreaks continue to occur. With many features of VL transmission not well understood, partly due to disease fecundity that is influenced by local environment, sand fly behavior, and human density and activity, in-depth analysis of field collected blood fed female sand flies can offer valuable insights into the dynamics of VL transmission. In addition to understanding host preference, we hypothesize that linking the source of the sand fly blood meal to *Leishmania* infection status and burden will shed light on the directional aspects of parasite transmission and point to potential infection reservoirs in a natural setting. To achieve this, we are developing tools using single sand flies experimentally fed on blood of various animals, spiked or not with *Leishmania donovani*. We dried the dissected midgut of individual blood fed female sand flies onto Whatman 903 protein saver card filters, used for specimen preservation in the field, and optimized DNA recovery to an average of 200-300ng per specimen; enough to run several concurrent assays. Using a sensitive Taq-Man probe-based

**ALTERED IL-7/IL-7R SIGNALING IN CD4+ T CELLS FROM PATIENTS WITH ACTIVE VISCERAL LEISHMANIASIS**

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CD4+ T cells play an important role in controlling *L. donovani* infection, through IFN-γ, required for activation of macrophages and killing of intracellular parasites. However, CD4+ T cell effector functions are hampered in visceral leishmaniasis (VL) patients. In a recent study that defined a transcriptional signature for CD4+ T cells from active VL patients, we found that expression of the IL-7 receptor (IL-7R, CD127) was downregulated, compared to CD4+ T cells from endemic controls (ECs). Since IL-7/IL-7R signaling is critical for the survival and homeostatic maintenance of CD4+ T cells, we investigated the role of this signaling pathway in active VL patients, relative to ECs. CD4+ T cells were enriched from peripheral blood collected from VL and EC subjects and expression of IL7 and IL-7R mRNA was measured by real time qPCR. IL-7 signaling potential and surface expression of CD127 and CD132 on CD4+ T cell and various cell subsets was analyzed by multicolor flow cytometry. Plasma levels of soluble IL-7 and IL-7R were measured by ELISA. In line with previous findings, we found reduced IL7R mRNA expression in CD4+ T cells as well as reduced soluble IL-7R mRNA expression in VL patients. However, plasma levels of soluble IL-7 were higher in VL patients. Interestingly, expression of the IL-7R was higher on VL CD4 T cells as compared to EC, with activated CD38+ CD4+ T cells showing higher surface expression of IL-7R (CD127 and CD132), compared to CD38- CD4+ T cells in active VL patients. CD4+ T cells from VL patients had higher signaling potential after stimulation with recombinant IL-7 compared to EC, as measured by phosphorylation of STAT5. Thus, despite reduced IL7R mRNA expression in CD4+ T cell from VL patients, surface expression of IL-7R was higher and increased phosphorylated STAT5 was seen following exposure to IL-7. Thus, despite lower IL-7mRNA expression, IL-7 signaling appears to be functional and even enhanced in VL CD4 cells and cannot explain the impaired effector function of VL CD4 T cell. The enhanced plasma IL-7 may have caused reduced IL7R transcription by CD4+ T cells as a part of homeostatic feedback mechanism.
dPCR targeting kinetoplast DNA, we successfully detected and quantified *Leishmania* parasites from single infected guts. We also obtained strong amplification from single flies fed on human, goat, cow, pig, and dog blood using a multiplex PCR for blood meal analysis based on the mitochondrial cytochrome c oxidase subunit one gene, and we are currently testing others. After completing laboratory optimization, we will validate these tools in VL outbreak villages in India. This toolbox can be adapted to specific *leishmaniasis* foci for greater understanding of sand fly behavior in a field setting. Using established techniques to address epidemiologically relevant questions can enhance our understanding of *leishmaniasis* transmission within human communities and improve policy-making decisions towards better vector-control programs.

**5022**

**NEUTROPHILS IN PATHOGENESIS OF POST KALA-AZAR DERMAL LEISHMANIASIS (PKDL), FRIEND OR FOE?**

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Post Kala-azar Dermal Leishmaniasis, a sequel of apparently cured visceral Leishmaniasis, presents in South Asia with papulonodular (polymorphic) or hypomelanotic lesions (macular). Neutrophils are the first line of defense in VL and facilitate disease establishment by serving as ‘trogan horses’. However, knowledge regarding a biological role, if any, for neutrophils in PKDL is limited. This study aimed to delineate the status of neutrophils at the dermal lesions of PKDL, and their possible functionalities. Accordingly, the presence of lesional CD66b+ neutrophils along with their functional status was assessed by immunofluorescence/ immunohistochemistry in terms of activation (CD66b+/CD64+), degranulation (CD66b+/MPO+), release of neutrophil elastase (NE) and matrix metalloprotease 9 (MMP9) along with their transcriptomic profile using the Visium (10X) platform. The levels of circulating neutrophil chemo-attractants CXCL8, CXCL1/2/5, CCL2 and 20, along with cytokines, IL-6, IFN-γ, IL-4, IL-10, TNF-α, IL-17 and IL-23 as markers for inflammation were evaluated by a multiplex assay. As compared to skin from healthy individuals, PKDL cases demonstrated an increased infiltration of activated neutrophils (increased CD66+, MPO, NE). There was an increase in plasma levels of neutrophil chemo-attractants, along with pro-inflammatory and regulatory cytokines. Levels of MMP9 were elevated in both circulation and at lesional sites, with concomitant collagen I degradation. However, the tissue damage was moderate, perhaps owing to the concomitant increase in expression of TIMP1. The immune responses constituted a mixed Th1 (IFN-γ, IL-6), Th2 (IL-4) and Treg (IL-10) profile, with a conspicuous absence of the Th17 phenotype. The increased levels of CXCL8 and CXCL5 correlated with the proportion of infiltrated neutrophils; furthermore, this homing was IL-10 dependant. Taken together, in PKDL, a chronic dermatosis, the presence of activated neutrophils at lesional sites suggested its permissive role(s) in modulating the lesional landscape and facilitating disease progression.

**5023**

**ALTERED PROFILE OF CD4+ T CELLS CHEMOKINE RECEPTOR EXPRESSION DURING VISCERAL LEISHMANIASIS**

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Robust Th1 cell responses, which activate macrophages to kill intracellular parasites, are required to control *Leishmania* infection. Yet, VL patients do not control the infection despite expansion of CD4+ T cells and increased IFNγ expression in the spleen. Chemokines &/or chemokine receptors are involved in cellular migration & are critical in the inflammatory response. In a recent study that defined a transcriptional signature for CD4+ T cells from active VL patients, we found several differentially expressed chemokine receptor genes compared to CD4+ T cells of healthy endemic controls (HEC). Since CD4+ T cell plays crucial roles in parasite clearance, there is need to understand the role of altered chemokine receptor expression on CD4+ T cells & their different subsets. In this study, we validated the gene expression & surface protein expression of differentially expressed chemokine receptors found in human VL subjects compared to HEC by real-time dPCR and multicolor flow cytometry, respectively. We found elevated mRNA & surface protein expression of CCR5, while reduced CCR4 & CCR6 expression in VL patients CD4+ T cells. CCR5 was upregulated on Th1 cell subsets indicating the expansion of CCR5+ Th1 cells in peripheral blood that may be responsible for Th1 cells trafficking towards the infected tissue. CCR4 expression was reduced on regulatory T cells (Treg) & central memory T cells (Tcm) suggesting reduced frequencies of these cells in peripheral blood during VL. Our results suggest that VL patients possess unique chemokine receptor expression on their surface compared to healthy subjects. The implications of these finding have on VL pathogenesis & how the knowledge of these changes can provide direction for the development of new and improved therapeutic approach against VL is under investigation.

**5024**

**A POTENTIAL ROLE FOR ADIPOCYTES IN VISCERAL LEISHMANIASIS**

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Leishmaniasis is a chronic parasitic disease in which parasites are found in host macrophages throughout the reticuloendothelial organs. Recent data indicate that many cells not thought of traditionally as immune cells contribute to the cytokine/chemokine environment locally where the parasite survives, and to the systemic immune responses. Adipocytes are versatile cells whose functions in systemic energy homeostasis and as reservoir for excess energy are well documented. Adipocytes are present in subcutaneous tissues near the parasite inoculum, but their potential involvement in immunoregulation of leishmaniasis is unknown. To determine whether and how adipocytes interact with the *Leishmania* species, we differentiated human preadipocytes into adipocytes and incubated them with *L. infantum*, *L. major* or *L. braziliensis* promastigotes. We found that the *Leishmania* spp. parasites were taken up by human adipocytes and transformed morphologically to the intracellular amastigote form, but this occurred differently between the species. *L. infantum* was taken up by 20.0% of adipocytes, whereas *L. major* or *L. braziliensis* were taken up by 11.5 or 4.8% of adipocytes, respectively. None of the parasite species replicated intracellularly over 48 hrs. RT-qPCR for candidate immunoregulatory transcripts revealed up-regulation of the IL6 and PPARγ but down-regulation of genes involved in lipogenesis such as Adiponecin, Lipoprotein lipase and Leptin receptor. There were quantitative, but no apparent qualitative differences between the species. These data suggest a potential role for adipocytes in *Leishmania* spp. infection, although the roles may differ between different *Leishmania* species.

**5025**

**IMMUNE MODULATION INDUCED BY LEISHMANIA EUKARYOTIC INITIATION FACTOR BEFORE LEISHMANIA INFANTUM INFECTION OF THP1 DERIVED MACROPHAGES**

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Leishmaniases are a complex group of neglected infectious diseases of poverty, and serious public health problems. Development of novel control strategies remains a research priority. *Leishmania* Eukaryotic Initiation Factor (eIF) antigen is a natural Th1 type natural adjuvant that stimulates
CATALASE IS DETRIMENTAL FOR LEISHMANIA VIRULENCE
(WITH NOTES ON EVOLUTION OF CATALASES IN TRYpanosomatidae)

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Catalase is one of the most abundant enzymes on Earth. It decomposes hydrogen peroxide, thus protecting cells from dangerous reactive oxygen species. The catalase-encoding gene is conspicuously absent from the genome of most representatives of the family Trypanosomatidae. The exceptions are monoxenous relatives of Leishmania spp., and representatives of the genera Blastocritidia, Obscuromonas, and Vickermania. In this work, we expressed the Leptomonas seymouri-derived catalase from the Leishmania mexicana beta-tubulin locus using a novel bi-cistronic expression system, which relies on the 2A peptide of Teschovirus A. We demonstrated that catalase-expressing parasites are severely compromised in their ability to develop in insects, to be transmitted and to infect mice, and to cause clinical manifestation in their mammalian host. Taken together, our data support the hypothesis that the presence of catalase is not compatible with the dixenous life cycle of Leishmania, resulting in loss of this gene from the genome during evolution of these parasites. To complement these data, we ablated a catalase-encoding gene from the Leptomonas seymouri genome and established an add-back, where catalase was overexpressed from the 18S rRNA locus of L. seymouri. Our study demonstrated that parasites’ development and infectivity in vivo (in Dyadiscus peruvianus model) depends on the expression level of this enzyme. These studies were further complimented by biochemical characterization of three independently-acquired catalases (of Blastocritidia, Leptomonas, and Vickermania in vitro, which showed that the enzyme of Blastocritidia monstop is cyanide-resistant, an unprecedented feature among all investigated monofunctional catalases.

ACUTE KIDNEY INJURY IN CHILDREN WITH SICKLE CELL ANEMIA IS LINKED TO TUBULOINTERSTITIAL INJURY AND MICROcirculatory dysfunction

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Acute kidney injury (AKI) is common in hospitalized children, including patients with sickle cell anemia (SCA). AKI is a diverse clinical syndrome and the pathophysiology of AKI in children with SCA associated AKI is not well understood. Here we investigated immune pathways associated with AKI in 185 children with SCA hospitalized for a vaso-occlusive pain crisis and 65 children with SCA in steady state as controls. AKI was defined in hospitalized children using the KDIGO definition as ≥1.5-fold change in creatinine within seven days or an absolute change of ≥0.3 mg/dL within 48 hours excluding children with a 1.5-fold change in creatinine from 0.2 mg/dL to 0.3 mg/dL. Using ELISA, we measured serum levels of markers of kidney structure and function (cystatin C, neutrophil gelatinase associated lipocalin [NGAL], renin), tubulointerstitial stress and inflammation (tissue inhibitors of metalloproteinases 1 [TIMP-1], interleukin-18 [IL-18]) and microcirculatory dysfunction (P-selectin, angiopoietin-2 [Angpt-2]), and...
Hypoglycemia, defined as a blood glucose <2.2 mmol/L, is associated with death in pediatric cerebral malaria (CM). The optimal duration of glucose monitoring in CM is unknown. We collected data from 1674 hospitalized Malawian children with CM to evaluate the association between hypoglycemia and death or neurologic disability in survivors. We assessed the optimal duration of routine periodic measurements of blood glucose. Children with hypoglycemia at admission had a 2.87-fold higher odds (95% CI: 1.35-6.09) of death and, if they survived, a 3.21-fold greater odds (95% CI: 1.35-6.09) of sequelae at hospital discharge. If hypoglycemia was detected at 6 hours but not at admission, there was a 7.27-fold higher (95% CI: 1.51-6.86) risk of death and, if they survived, a 3.21-fold greater (95% CI: 1.35-6.09) risk of sequelae at hospital discharge. Children with hypoglycemia at admission had a 2.87-fold higher odds of death in pediatric cerebral malaria (CM). The optimal duration of glucose monitoring in CM is unknown. We collected data from 1674 hospitalized Malawian children with CM to evaluate the association between hypoglycemia and death or neurologic disability in survivors. We assessed the optimal duration of routine periodic measurements of blood glucose. Children with hypoglycemia at admission had a 2.87-fold higher odds (95% CI: 1.35-6.09) of death and, if they survived, a 3.21-fold greater odds (95% CI: 1.35-6.09) of sequelae at hospital discharge. If hypoglycemia was detected at 6 hours but not at admission, there was a 7.27-fold higher odds of death (95% CI: 1.85-8.56). The presence of newly-developed hypoglycemia after admission was not independently associated with mortality (95% CI: 1.85-8.56). The presence of newly-developed hypoglycemia after admission was not independently associated with mortality (95% CI: 1.85-8.56). The presence of newly-developed hypoglycemia after admission was not independently associated with mortality (95% CI: 1.85-8.56).
Fever illnesses are a leading reason for pediatric medical consultations. Most febrile children attending primary care facilities have uncomplicated and self-limited infections, but a small proportion may progress to life-threatening conditions requiring prompt and advanced care. However, early in the course of illness, it is difficult to identify which children are at risk for severe and fatal infections. In resource-constrained settings, this is further complicated by limited health care providers and laboratory services. Here we tested the hypothesis that quantifying plasma biomarkers of immune and endothelial activation at clinical presentation may enhance risk-stratification of febrile children. This study was conducted in the Mozambican pediatric cohort of the FIEBRE study. Between December 2018 and February 2021, febrile children aged 2 months - <15 years were enrolled. We measured plasma levels of Angpt-2, CHI3L1, IL-6, IL-8, sFlt-1, sTNFR1, sTREM-1, suPAR, PCT, and CRP at presentation using Luminex and ELISA. Standard clinical and laboratory parameters were assessed at enrollment, and clinical outcomes were evaluated up to 28 days later. A total of 1,040 children were enrolled and had a plasma sample taken for biomarker measurement. 531 (51.1%) were outpatients and 509 (48.9%) were inpatients. Of these, 19 children died within 28 days. Of the 1,040 children, 763 (73.4%) were “Well”, 11% (19) of cases were “Improved”, 2% (4) were the “Same”, 2% (4) were “Worse”. No severe adverse events occurred. Of the 1,040 children, 763 (73.4%) were “Well”, 11% (19) of cases were “Improved”, 2% (4) were the “Same”, and 2% (5) of cases were “Worse”. No severe adverse events occurred. Findings to date support the operational feasibility and clinical safety of a setting with a nationalized emergency medical service and a high burden of malaria. The TMDS was launched in Ghana on November 16th, 2022, in the low-resource communities of Jamestown and Usshertown in Accra. When children ≤10 years became sick at night, their guardians called MotoMeds. EMTs consulted local on-call physicians as needed. Follow-up phone calls were performed at 10 days. The TMDS model, known as MotoMeds, must be evaluated in settings with distinct healthcare infrastructures and burdens of disease. Our objective was to implement MotoMeds TMDS in Ghana to assess the model’s operational feasibility and clinical safety in a setting with a nationalized emergency medical service and a high burden of malaria.
ERGOLLOGIC EVIDENCE OF MARBURG VIRUSES AND A BUNDIBUGYO VIRUS-LIKE EBOLVIRUS IN MADAGASCAR ROUSETTE BATS

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Barry R. Miller2, Julius J. Lutwama3, Robert M. Kityo4, Ann M. unknown since this virus was first recognized in Uganda in 1959. Building

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Ebolaviruses and marburgviruses are causative agents of viral hemorrhagic fever diseases with a high case fatality in humans. Historically, outbreaks of marburgviruses have occurred in Central and South Africa, connected to mining or cave-tourism activities in caves where the reservoir species, Egyptian rousette bats (EREs; Rousettus aegyptiacus), roost. Recent Marburg virus disease outbreaks in Equatorial Guinea, Guinea, and Ghana have highlighted gaps in our current knowledge of Marburg virus (MARV) distribution and at-risk areas for spillover. On Madagascar, a single species of rousette bat, Madagascan rousette, resides. We conducted serology-based biosurveillance to assess whether ebolaviruses and marburgviruses circulate enzootically in Madagascan rousettes. Serum samples from 559 Madagascan rousettes and two other species of fruit bats were tested by a multiplex microsphere-based immunoassay for immunoglobulin (Ig) G reactivity against soluble envelope glycoprotein (GP) ectodomain trimers of filoviruses. Antigen-antibody complexes were detected via Luminex xMAP-based technologies, with IgG levels reported as a median fluorescence intensity. Seropositivity cutoffs were determined with a three-sigma-rule (99.7%) probability distribution of naïve ERB sera and latent cluster analysis of field-collected Madagascan rousette sera. We detected IgG binding antibodies against MARV (7.6%; 43/559), Ravn (RAV) (13.4%; 74/559), and Bundibugyo virus GP (4.8%; 27/559). Interestingly, 5.3% (31/559) were co-positive for IgG against MARV and RAV. Serological profiles of Madagascan rousettes suggest the presence and co-circulation of uncharacterized ebolaviruses and marburgviruses. Specific serological footprints in Rousettus species native to Madagascar extends our understanding of the host reservoir-pathogen dynamics, implicating the bat genus Rousettus as a reservoir for marburgviruses. Future research is necessary to determine possible evidence of viral chatter between bats and other wildlife or domestic animals, and spillover risk to humans.

EXPOSURE OF EGYPTIAN ROUSETTE BATS (ROUSETTUSS AEGYPTIACUS) AND A LITTLE FREE-TAILED BAT (CHAEREPHON PUMILUS) TO ALPHAVIRUSES INUGANDA

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The reservoir for zoonotic n’nyong-nyong virus (ONNV) has remained unknown since this virus was first recognized in Uganda in 1959. Building on existing evidence for mosquito blood-feeding on various frugivorous bat species in Uganda, and seroprevalence for arboviruses among bats in Uganda, we sought to assess if serum samples collected from bats in Uganda demonstrated evidence of exposure to ONNV or the closely related zoonotic chikungunya virus (CHIKV). In total, 682 serum samples collected from six bat species were tested by plaque reduction neutralization test (PRNT) for neutralizing antibodies against ONNV and CHIKV. Forty out of 303 (13.2%) Egyptian rousette bats from Maranagambo Forest and 1/13 (8%) little free-tailed bats from Banga Nakwiko, Entebbe contained neutralizing antibodies against ONNV. In addition, 2/303 (0.7%) of these Egyptian rousettes contained neutralizing antibodies to CHIKV, and 8/303 (2.6%) contained neutralizing antibodies that were nonspecifically reactive to alphavirus. These data suggest the possibility of enzootic circulation of ONNV and CHIKV in Uganda, although Egyptian rousette bats are unlikely to serve as reservoirs for these viruses given the inconsistent occurrence of antibody-positive bats.
CENCURUT VIRUS: A NOVEL ORTHONAIROVIRUS FROM ASIAN HOUSE SHREWS (SUNCUS MURINUS) IN SINGAPORE

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Orthonairovirus is a genus of viruses in the family Nairoviridae, order Bunyavirales, with a segmented circular RNA genome. They typically infect birds and mammals and are primarily transmitted by ectoparasites such as ticks. Four of nine Orthonairovirus genogroups can infect humans, with Crimean-Congo hemorrhagic fever virus infections displaying case fatality rates up to 40%. Here, we discover and describe a novel Orthonairovirus as Cencurut virus (CENV). CENV was detected in 34 of 57 Asian house shrews (Suncus murinus) sampled in Singapore and in a nymphal Amblyomma helvolum tick collected from an infected shrew. Pairwise comparison of CENV S, M, and L segments had 95.0 to 100% nucleotide and 97.5 to 100% amino acid homology within CENV genomes, suggesting a diverse viral population. Phylogenetic analysis of the individual gene segments showed that CENV is related to Erve, Lamusara, Lamgora, and Thiafora viruses, with only 49.0 to 58.2% nucleotide and 41.7 to 61.1% amino acid homology, which has previously been detected in other shrew species from France, Gabon, and Senegal respectively. The high detection frequency suggests that CENV is endemic among S. murinus populations in Singapore. The discovery of CENV, from a virus family with known zoonotic potential, underlines the importance of surveillance of synanthropic small mammals that are widely distributed across Southeast Asia.

EPIDEMIOLOGY AND GENETIC DIVERSITY OF NOVEL PARAMYXOVIRUSES RELATED TO LANGYA VIRUS IN RODENTS AND SHREWS IN BANGLADESH

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Rodents and shrews live in close proximity to humans and have been identified as critical hosts of zoonotic pathogens. As part of a broad One Health surveillance effort, we conducted surveillance for novel zoonotic viruses in wildlife, domestic animals, and people. Paramyxoviruses (PMV) were one of the priority viral groups targeted for the surveillance. Here, we report discovery of novel PMV in wild rodent and shrews in Bangladesh. We collected oral and rectal swab samples from rodents (n= 1019) and shrews (n= 186) twice a year, in the dry and wet seasons, from three districts in Bangladesh from 2016 to 2018. To detect known and novel paramyxovirus, we tested swab samples using consensus PCR assay targeting RdRp genes of paramyxovirid specific genera primers. Overall, 2.1% (25/1205; 95% CI: 1.3-3.0) animals was positive against PMV. The prevalence of PMV was similar in rodents (2.06%; 21/1019) and shrews (2.15%; 4/186). We detected 4 novel strains of PMV from 25 samples of 06 species of rodents and house shrew. The PMV was more prevalent in wet than dry seasons. We identified landscape, sex, and health conditions significantly associated with PMV shedding in multivariable logistic regression model. Phylogenetic analysis revealed that three PMV strains identified in rodents are genetically related to Jelollision virus. The discovery of one PMV strain genetically related to the novel zoonotic Langya virus in shrews warrants further investigation. However, the whole genome and molecular characterization are required to ascertain the virus pathogenicity and similarity with Langya or a novel strain of PMV. This study discovered that diverse strains of paramyxovirus, including those related to Langya virus, are present in shrews and rodents in Bangladesh. Future studies in Bangladesh should continue to characterize PMV viral diversity, and Langya virus should be included as possible etiologies for humans at high-risk human-animal interfaces that test negative for common pathogens.

INVESTIGATION OF RIFT VALLEY FEVER OUTBREAK ASSOCIATED WITH ‘ABORTION STORMS’ IN MBARARA DISTRICT, UGANDA 2023

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Rift valley fever (RVF) is a zoonotic disease of public health and economic importance. Uganda has reported sporadic acute human cases since 2016 and identified convalescent livestock herds. In January 2023, acute disease with high fevers was reported in cattle among one dairy farm in Mbarara district as well as presenting with classical ‘abortions storms’ that is stereotypically characterized RVF diseases in larger outbreaks in the East-African rift valley. The Mbarara district rapid response team supported by the national rapid response team together with partners responded to these reports and blood samples were collected and taken to the Uganda Virus Research Institute (UVRI) for testing and confirmation. We conducted both human and veterinary investigations following a one health approach where 90 human samples, 42 cattle samples, 5 goat samples, and 4 milk samples from the affected farm were collected and tested by PCR, IgM, and IgG ELISA at the UVRI Viral hemorrhagic fever laboratory. Next Generation Sequencing (NGS) is being conducted and ongoing. To date (20th March 2023), 27.7% (25/90) human cases have been confirmed by PCR and IgM ELISA with 3 deaths, 23.8% (10/40) cattle tested positive by PCR and 69% (29/42) were positive by IgG ELISA whereas 2 sheep tested positive by IgG and 2 milk samples tested positive by PCR. 30 animals had reports of abortions in a space of two months within the one farm, 96% (24/25) of human cases reported contact with livestock, and 80% (20/24) are males with an average age of 37.7. Most human cases are alive (88%) and only 2 had hemorrhagic symptoms with the predominant symptoms being fever and general body weakness. This was the first active infection in cattle and milk samples that has been detected in Uganda associated with a cluster of acute human cases. Animal movement control measures were instituted to limit the spread and provided health education for high-risk groups such as abattoir workers and herdsmen. Recommendations for public health and animal preventive measures included immunization, vector control and health education in the affected communities to mitigate the effects of RVF.

GENETIC DIVERSITY AND AMINO ACIDS VARIATIONS AT VACCINE TARGET SITES IN RABIES VIRUSES COLLECTED FROM DIFFERENT HOST SPECIES IN MAKUENI AND SIAYA COUNTIES, KENYA

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Rabies, a viral disease that causes lethal encephalitis is endemic in Kenya and is transmitted to humans mainly by domestic dogs. Rabies kills an estimated 2000 people annually, despite there being effective vaccines for dogs and humans. This study characterized the genetic diversity of RABV
obtained from brains of suspected rabid animals from Makueni county, Eastern region and Siaya county, Western Kenya and determined variances within the antigenic sites of RABV vaccines currently in use in Kenya. Brain biopsies (165) confirmed positive for rabies with rapid kits were collected between July 2021 and August 2022 from dogs, cats, cows, sheep and goats and re-screened for RABV by qPCR. Whole genome sequences (WGS) and individual nucleoprotein (N) and glycoprotein (G) genes were used for phyloTeny, The amino acid variances in the N and G genes antigenic sites were compared to three RABV vaccine sequences: Pitman-Moore L503 (PM), Challenge Virus Standard (CVS) and the Pasteur vaccine (PV) strains. Of the 165 brain samples, 156 were positive by qPCR and 141 (74 from Makueni and 67 from Siaya) produced useable sequences. Phylogenetic lineages drawn from WGS, individual N and G genes showed two geographical distinct lineages: The Eastern Kenya sequences overwhelmingly (n=69) clustered with the Africa 1b lineage, with only 3 in Africa 1a. In contrast, the Western Kenya sequences (n=64) clustered with Africa 1a with only 3 in Africa 1b. The nearest common ancestor of the Africa 1a traced to Sudan, while the Africa 1b traced to Tanzania. The percent amino acid homologies of the N gene to the RABV vaccines were at least 97.6% for PV, 97.8% for CVS and 98.5% for PM. The homology with the G gene were at least 93.0% for PV; 93.2% for CVS and 92.2% for PM. Our data confirm geographical isolation of RABV in Eastern and Western Kenya. The data suggests limited migration, probably through wildlife carnivore movement or translocation of domestic dogs by humans. The observed amino acid variances RABV vaccines antigenic sites would predict good vaccine efficacy, indicating that the RABV endemicity in Kenya is due limited programmatic vaccine coverage.

## 5041

### A GUT COMMENSAL PROTOZOA REMOTELY TUNES PULMONARY DISEASE SEVERITY

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The microbiome plays a crucial role in regulating the immune system. The bacterial composition of the microbiome, in particular, is known to alter eosinophil responses to various stimuli with certain bacterial profiles having been associated with increased susceptibility to the development of allergies and asthma. However, the precise role of the other microbial kingdoms within the microbiome, such as viruses, fungi, and protozoa, in modulating immune responses and susceptibility to disease is not yet fully understood. Commensal protozoa are an integral component of the vertebrate microbiota, yet they are vastly understudied compared to their pathogenic counterparts. While colonization with commensal protozoa is known to alter the local immune response in the intestine and aid in protection against enteric bacterial pathogens, it is unclear whether these organisms can also influence extra-intestinal immune response. Here, we show that the gut-dwelling commensal protozoan Tritrichomonas musculis (T. mu) can remotely shape the immune landscape in the lungs following colonization. Despite not directly affecting the health of the host, the presence of T. mu in the gut causes accumulation of eosinophils in the lungs and is dependent on the inter-organ migration of intestinal derived inflammatory group 2 innate lymphoid cells (iILC2). This sustained eosinophilia in the lungs also requires interaction between iILC2s, T cells, and B cells, which form a tripartite immune network that provides a specific niche for lung eosinophils. This network exacerbates allergic airway inflammation induced by house dust mite exposure while also limiting early bacterial dissemination. This network exacerbates allergic airway inflammation induced by house dust mite exposure while also limiting early bacterial dissemination. This network exacerbates allergic airway inflammation induced by house dust mite exposure while also limiting early bacterial dissemination.

CD30L EXPRESSION ON CD4+ T CELLS IS REQUIRED FOR THE DEVELOPMENT OF ALLERGEN- AND HELMINTH-DRIVEN TYPE 2 INFLAMMATION IN THE LUNG

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Type 2 immune responses are associated with helminth infections but also drive allergic disorders. Transcriptomic analyses of house dust-mite (HDM)-driven pulmonary type-2 inflammation revealed a marked upregulation in genes associated with the Th2 activation pathways (e.g.IL-4, IL-5, IL-9, IL-13, and IL-33), but also other important inflammatory mediators including TNF/TH28 also known as CD30L. Additionally, single cell RNA sequencing on CD4+–sorted lungs cells showed marked expression of CD30L on lymphoid cells, but most noticeably on allergen-activated CD4+ T cells. To explore the role played by CD30L in Type 2 associated pulmonary inflammation, we used two experimental mouse models, including HDM-intranasal sensitization and Ascaris infection in the lungs. Flow cytometry analysis revealed that the effector memory Th2 cells, defined by TCRβ+CD4+CD44+CD154+IL-13+, from both HDM-allergic and Ascaris-infected lungs showed a marked over-expression of CD30L, when compared with naive cells. Interestingly, repeated infection with Ascaris parasites increased even further the frequency of CD30L+ Th2 effector cells in comparison with primary infection. We next examined the role of CD30L in the CD4+ T cell differentiation in vitro. Naive cells from CD30L
KO mice or from naïve WT mice following CD30L blockade during in vitro differentiation into Th2 cells demonstrated a significant impairment of Th2 cell differentiation (fewer IL-4 producing Th2 cells). Finally, to further examine the functional role of CD30L during the development of a pulmonary type-2 inflammation, our data demonstrated that CD30L KO mice following sensitization to HDM failed to induce an allergen-specific memory Th2 cell response in the lungs when compared with WT mice, based on the marked reduction in the frequency of allergen-specific IL-13 producing memory CD4 T cells. This in turn led to diminished HDM-specific IgG1 and IgE levels, as well as a reduction of eosinophils in the lung tissue. Taken together our preliminary findings suggests that CD30L expression on Th2 cells is required for the development of type-2 inflammatory immune responses in the lung tissue.

5044

HUMAN FITARIAL INFECTION DRIVES A DISTINCT SIGNATURE OF CD8+ T CELL POPULATIONS AT HOMEOSTASIS AND IN RESPONSE TO CYTOMEGALOVIRUS (CMV) IN FILARIAL/CMV COINFECTIONS

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Characterization of the T cell responses in chronic filarial infections has focused largely on CD4+ subsets, with little information of CD8+ T cells. Because CD8+ T cells are central to viral-specific effector responses in most viral infections and because many occur in the context of coincident filarial infections, we sought to understand the nature of CD8+ populations and subpopulations in filarial/viral coinfections. We sought to characterize the heterogeneity and function of CD8+ T cells in filarial/cytomegalovirus (CMV) co-infected subjects through the use of multiparameter (26 color) flow cytometry to profile CD8+ subsets from PBMCs collected from 11 filarial-infected subjects (Fil+) and 6 filarial-uninfected controls (Fil-) at homeostasis and in response to cytomegalovirus (CMV) antigen (CD8 and CD4 megapools consisting of 345 peptides). We used a self-organizing map tool (FlowSOM) to profile CD8+ subsets driven by the filarial infection per se and by CMV. Based on the multidimensional profiling and clustering algorithms using data from PBMCs from all subjects at homeostasis (baseline), the Fil+ group had marked expansion of CD8+CD95+CD107a+PD-1+IFN-g+and CD8+CD45RA+CD107a+GranzymeB+IFN-g+ populations that reflect severe exhaustion and being pro-apoptotic. When stimulated with CMV antigen, the CD8+ T cells from Fil+ subjects expanded a population of CD8+GranzymeB+CD57+ cells compared to Fil- subjects (p<0.03) but failed to induce additional IFN-g and CD107a, both being important for viral control. Additionally, CMV antigen exposure induced a marked increase in the frequency of a memory CD8+CD45RA- subset (CD8+CD45RA-CD107a+IFN-g+CD57+GranzymeB+) whose function remains unknown. Our data suggest that filarial infection is associated with CD8+ T cell exhaustion and apoptosis; further when stimulated with viral antigen these CD8+ subpopulations fail to produce the important cytokines necessary for viral control. These findings are likely important to understand the nature of bystander suppression of viral specific responses induced by chronic filarial infections.

5045

IL-11 REGULATES MUCOSAL RESPONSES IN ACUTE PULMONARY HELMINTH INFECTION

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Larval migration of helminth parasites through the lung drives an early neutrophil-associated inflammation before the establishment of an eosinophil-dominated type-2 immune response. Members of the IL-6 cytokine family, including IL-11, have been described to play a role in acute inflammatory responses. In a mouse model, we observed significantly elevated IL-11 levels in the lung tissue of mice following Ascaris spp. infection (2,196 pg/mL vs 968 pg/mL, p<0.001) at 8 dpi compared to naïve mice. Flow cytometry and confocal imaging demonstrated that lung CD140a+ fibroblasts and EpCAM+ epithelial cells were major sources of IL-11 in the lungs of Ascaris-infected mice. Anti-IL-11 blocking antibodies during Ascaris infection markedly impaired the influx of neutrophils to the lung, whereas administration of rIL-11 not only induced high levels of G-CSF and CXCL1 but also increased neutrophil influx. By using Ascaris-infected IL-11Ra deficient mice, we observed a marked reduction in lung neutrophil influx (20.3 x 105cells vs 51.2 x 105cells, p=0.030) and a decrease of neutrophil-associated mediators (e.g., CXCL1 and G-CSF) in the absence of IL-11 signaling when compared with WT Ascaris-infected animals. To elucidate whether IL-11 production by lung epithelial cells is elicited directly or indirectly, bronchial epithelial cell line (HBE3-K) 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. Moreover, HBE3-K cells stimulated in vitro with different recombinant cytokines, including IL-33, IL-1b, IL-1a, and TGF-β, revealed that TGF-β induces high levels of IL-11 in a dose dependent manner. Similar induction of IL-11 was observed in mouse fibroblast cell line (MM14-Lu) stimulated with TGF-β. Further reported after the first infection, we observed a marked increase of TGF-β levels in vivo (1476.23 pg/mL vs 986.87 pg/mL, p=0.001). Taken together, our data suggests that IL-11 produced in the lung mucosa regulates a neutrophil-dominated inflammation in response to epithelial damage and TGF-β during acute lung helminth infection.

5046

RAPID INDUCTION OF CLINICAL TOLERANCE IN A PLACEBO-CONTROLLED CLINICAL TRIAL INVESTIGATING REPEATED CONTROLLED EXPOSURE TO SCHISTOSOMA MANSONI

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Epidemiological data from endemic settings suggests that (partial) immunity to schistosomiasis develops over time, and is likely enhanced by repeated infections and treatments leading to enhanced or prolonged antigen exposure. Moreover, animal studies have demonstrated that protection can be achieved after repeated immunisation with irradiated cercariae. In this study, we aimed to investigate the protective efficacy and safety of consecutive exposure-treatment cycles with Schistosoma mansoni (Sm) in healthy, schistosoma-naïve participants using the single-sex controlled human Sm infection model. We enrolled 24 participants who were randomised (1:1) to either three (reinfection) or one (infection control) exposures to male cercariae. The infection control group received two mock exposures first. Treatment with praziquantel (or placebo for infection control) was given 8 weeks after the first and second (mock) exposure. All participants were treated with praziquantel 12 weeks after the third exposure. Throughout the study, adverse events were collected as well as serum to measure circulating anodic antigen (CAA) secreted by juvenile and adult worms to determine infection status. All but one participant completed follow-up. The percentage of participants with detectable infection after the final exposure (CAA >1.0 pg/mL) in the reinfection group was 82% (9/11) and 92% (12/13) in the infection control group. There was no difference in the rate of infection. As compared to the second (27%) and third infection (28%). Severe acute schistosomiasis (AS) was observed in both groups after the first infection (2 out of 12 in reinfection group and 2 out of 12 in infection control group), but no AS was reported after the subsequent infections. In conclusion, repeated Sm infection led to clinical tolerance, but did not result in (sterile) protection. Further investigation into the underlying immune response will result in better understanding of immunity to schistosomes.
COMPREHENSIVE ANTIBODY PROFILING IN SCHISTOSOMIASIS REVEALS IMMUNOLOGICAL SIGNATURES OF ACTIVE INFECTION

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Lack of accurate yet accessible diagnostics for neglected tropical diseases such as schistosomiasis is a critical bottleneck in their elimination. Antibody (Ab)-based tests, currently used for serological screening for schistosomiasis cannot distinguish past from current infection. We have developed a multiplexed ‘Ab-omics’ platform for deep biophysical characterization (both Fab & Fc ends) of a broad set of antigen-specific Abs including not only their isotype and subclass but also glycosylation, Fc receptor and complement binding. Machine-learning applied to this high-dimensional data can reveal unique Ab signatures predictive of disease state and outcome. Here, we apply the Ab-omics pipeline to Schistosoma mansoni infection. Sera from patients (n=88, from Minas Gerais, Brazil), previously screened for parasite eggs using the Kato-Katz technique, were characterized with the Ab-omics workflow using multiple S. mansoni antigens (SEA, SM25, MEG, CD63, Calumenin) and other helminth and non-helminth antigens. Antigen-coated barcoded beads were incubated with serum and probed with various fluorescently-labeled isotype and subclass probes, tetramerized Fc receptors and lectins. This revealed that SEA-specific IgG4 titers could discriminate between individuals who were egg+ and those who were egg-. However, higher SEA and CD63-specific IgG4, Calumenin-specific Ab FcγR2A and SM25-specific Ab FcγR3B binding and antigen-specific Ab sialylation were found in egg+ individuals compared to egg- individuals. With a total of 324 measured features (18 Ab Fc characteristics x 18 antigens) from each patient, application of a new interpretable machine-learning approach (Essential Regression) revealed a complex interplay of proinflammatory (FcγA, FcγB) and anti-inflammatory (sialylation) Ab features and functions. Machine-learning (LASSO) based feature selection was able to identify a unique set of biomarkers to differentiate egg+ from egg- individuals (AuROC=0.9). Our findings suggest that a purely Ab-based biomarker can achieve accurate diagnosis of current versus past schistosome infection in endemic areas.

MODELING TO SUPPORT DECISIONS ABOUT THE GEOGRAPHIC AND DEMOGRAPHIC EXTENSION OF SEASONAL MALARIA CHEMOPREVENTION IN BENIN

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Seasonal malaria chemoprevention (SMC) has been implemented in Benin since 2019 and targeted more than 400 000 children under 5 in the northern departments of Alibori and Atacora in 2021. The Benin National Malaria Control Program (NMCP) recently considered an extension of SMC, either demographically - children aged 5 to 10 in the same departments would also receive SMC, or geographically to children under 5 in new departments eligible according to WHO criteria. As neither extension had been tested before, the NMCP turned to modeling to estimate their impact. The model OpenMalaria was calibrated to represent the history of malaria interventions and transmission risk in Benin, as well as the age structure of the population. The future interventions which were already planned (mass net distribution campaigns, SMC in children under 5 in Alibori and Atacora, pilot projects of intermittent preventive treatment in infants) were simulated, together with the two extensions of SMC. The model predicted that the demographic extension of SMC could avert on average 4.6 severe cases per 1000 targeted children between 2024 and 2026, while the geographic extension could on average avert between 13 and 14.3 severe cases per 1000 children under 5, depending on the department. To be less cost-effective than the demographic extension, the geographic extension should thus be three times more expensive, when costs from the 2021 campaign indicate it would cost only 40% more. Numbers of severe cases averted per targeted child were similar between operational zones of departments considered for the geographic extension, probably due to similar transmission risks. These findings led to recommending targeting in priority highly populated zones, as SMC in the three most populated zones could avert as many severe cases as in the six other zones. Modeling allowed not only to choose the geographic over the demographic extension, but also to quantify their comparative impact. Modeling can be used to answer questions from decision-makers when they are closely associated to the process, from the refinement of the modeling question to the choice of epidemiological indicators.
MALARIA PLANNING IN MOZAMBIQUE

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In setting the strategic plan for the period 2023 to 2030, the National Malaria Control Programme in Mozambique incorporated mathematical modelling to estimate the impact of four sub-national intervention plans. A ranking of geographic targets for seasonal malaria chemoprophylaxis (SMC), perennial malaria chemoprophylaxis (PMC), indoor residual spraying (IRIS) and RTS,S vaccine from the 2020 stratification provided scenarios to be modelled. These were called “Business as Usual” (BaU) (insecticide treated nets, IRIS), “Core” (BaU plus SMC and PMC, “Core Plus” (Core with SMC, PMC and the RTS,S vaccine) and “Ideal” (Core plus with more widespread SMC, PMC and the RTS,S vaccine), all with case management for simple and severe malaria. Using the OpenMalaria model, calibrated using historical data and transmission risk in Mozambique, the scenarios were simulated to assess impact on malaria. Modelled outputs included malaria prevalence, incidence, clinical cases and mortality. Each scenario was costed and cost-effectiveness analysis was used to assess what an optimal plan would entail, maximizing the number of cases averted in the under 5’s and in the whole population. Comparing Plasmodium falciparum prevalence in 2030 vs in 2022 at the national level, the reduction in prevalence is 17% for the “Core” package, 19% for the “Core Plus” package and 20% for the “Ideal” package. When taking into consideration the cost of interventions, there exists an optimal plan that averts more cases than the “Ideal” package within the same budget. SMC and PMC appeared to be the most cost-effective interventions, with IRS and RTS,S only optimizing averted malaria cases with very large increments in the budget. Mathematical modelling provides a useful tool to support decision-making, specifically allowing for sub-national stratification. National strategic plans can be developed to optimize the number of malaria cases averted given a fixed budget envelope, and the potential long-term impact can be calculated.

CAUSES OF UNDER-FIVE DEATH USING A PROBABILISTIC MODEL (INTERVA5) IN QUELIMANE DISTRCT, CENTRAL MOZAMBIQUE

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Community-based information on causes of stillbirths and under-five mortality is limited in many sub-Saharan countries. This is the case in Mozambique, where child mortality continues to be high despite a wide implementation and coverage of public health interventions with demonstrated impact. To understand why mortality levels remain high, we examine the underlying causes of death among stillbirths and children under the age of five years in Quelimane district, Central Mozambique. We aimed to include all stillbirths and under-5 deaths that occurred 12 months prior to the baseline census conducted from January to August 2022 in Quelimane district (population= 349,842). The main cause of death were computed using a computer algorithm InterVA-5. Only one cause of death was assigned for each case. The cause was chosen taking in consideration those with highest likelihood. Verbal autopsies were performed on 280 (79.1%) of a total 354 under-5 deaths identified through baseline census. The mean time between the date of death and verbal autopsy collection was 8.3 (SD, 5.36) months. Over half of the deaths (54.6%) occurred at a health facility. Birth asphyxia was the most common cause of death among stillbirths and neonates (under 28 days of life), at 57.7% and 40.8% of deaths respectively, and it was more frequent among males. Diarrheic disease was the main cause of death among infants (1-11 months) at 46.0%. For older children aged 1-4 years, malaria was the main cause of death among females (36.4%) and diarrheic disease was the most common cause in males (30.0%). Congenital malformations played an important role in childhood deaths up to the first year (4.3%). This study provided useful population-based insights on the causes of stillbirths and under-5 death and showed that most deaths occurred at health facilities and were related to preventable causes. Urgent strategic public health action targeting the health sector is needed to reduce facility deaths, including a redoubled attention to antenatal care, especially maximizing and improving the public health strategies currently available in order to save lives.
**IMPORTANCE OF COUNTRY PREPAREDNESS IN HANDLING HEALTH EMERGENCY, THE 2023 EBOLA OUTBREAK IN UGANDA**

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In September 2022, an Ebola outbreak caused by the Sudan ebolavirus occurred in Uganda. The outbreak was declared resolved in January 2023 with a toll of 142 confirmed cases, 55 of whom later died. As no vaccine is available for the Sudan ebolavirus, Uganda response was based on a range of non-pharmaceutical interventions (NPIs), including case isolation, tracing of case contacts, and population lockdowns to reduce the spread of the virus. The aim of the analyses was to evaluate the impact of NPIs on the Sudan ebolavirus outbreak by simulating scenarios with different response effort. We built a spatially-explicit synthetic population of Uganda which included heterogeneity of human contact and movement. This synthetic population was then used to perform an individual-based model (IBM) to represent the spread of Sudan ebolavirus in Uganda from September 2022 onwards. A baseline model was created to reproduce the epidemic curve of the outbreak, including the adopted NPIs and the timing of their implementation. To assess the effectiveness of the outbreak response, the baseline model was compared to a scenario simulating the NPI response with a 5 months delay, and one with a less-controlled outbreak. The IBM results showed that scenarios with a late intervention response and with an out-of-control outbreak would have resulted in an Ebola outbreak like the one reported by countries affected by the West Africa in 2014-2016. The late response scenario projected an outbreak with 778 (95% confidence interval [CI]: 665-901) cases and 303 deaths (95% CI: 259-351), 5.5 times higher than the reported outbreak. The outbreak simulated using the less-controlled scenario resolved with 1,916 (95% CI: 911-4,774) cases and 709 deaths (95% CI: 361-1,862), 12.8 times higher than the reported outbreak. Our results showed how Uganda’s rapid response to the Ebola outbreak was able to drastically reduce the toll of the 2022 Sudan ebolavirus outbreak. Our study highlights the significance of preparedness to manage infectious disease outbreaks, as a crucial factor in mitigating the risk of a significant public health emergency.

**THE GLOBALMIX PROJECT: COMPREHensively PROFILING SOCIAL CONTACT PATTERNS IN RESOURCE POOR COUNTRIES**

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Social mixing data are the cornerstone of understanding the infection transmission and modeling the control of pathogens such as SARS-CoV-2, RSV, influenza, and measles that spread via close contact. However, there is a dearth of data on social contact patterns particularly from low-and-middle-income countries. Comparability is hampered with different study protocols, definition of a contact, tool design and data collection procedures and many modelers rely on European contact data projected onto low-and-middle-income country populations. To address this gap, we aimed to quantify social contact patterns in a total of 8 rural and urban sites of Mozambique, Guatemala, India, and Pakistan through The GlobalMix Study. For each country, 1,250 participants reported, in a paper diary, individuals with whom they had contact over two days, and characteristics of those contacts. In addition, members of 126 households carried wearable proximity sensors over 7 continuous days and concurrently kept a diary of all contacts over two of those days. Each sensor autonomously detects dyadic face-to-face (≤6 feet separation) interactions between household members. We will present contact rates and matrices stratified by standardized definitions of age, sex, relationship, occupation, location, and duration of contacts for each site and country. We will quantify household and overall temporal network characteristics such as degree, number and duration of contacts, node loyalty and cosine similarity, and further compare these to contemporaneous diary data. We will present output from Mozambique and showcase the utility of our data. We report 8.8 (95% CI 8.4-9.2) and 6.3 (6-6.6) mean contacts per person per day in rural and urban areas, respectively. The highest median contacts were reported by 15-19-year-olds, and the highest assortativity by school children aged 10-14 years. These patterns are consistent with other studies in resource poor settings. An overarching value of The GlobalMix Study is to make the tools, methods, and data available to the scientific community for use in mathematical models of infectious disease transmission and control.

**TRANSMISSIBILITY OF LEISHMANIA DONOVANI FROM HUMAN TO SAND FLIES IN AN AREA ENDEMIC FOR VISCERAL LEISHMANIASIS IN INDIA**

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On the Indian sub-continent, visceral leishmaniasis (VL) is a fatal form of leishmaniasis caused by the protozoan parasite Leishmania donovani, and transmitted by the bites of the vector sand fly, Phlebotomus argentipes. To achieve and sustain elimination of VL, the transmission potential of L. donovani exposed individuals from across the infection spectrum needs to be urgently addressed. We conducted direct xenodiagnosis on each of the active VL patients (n=77) and Post Kala-azar Dermal Leishmaniasis (PKDL) patients (n=26), before and after successful treatment, asymptomatic individuals (n=183) and HIV-VL patients (n=14). During xenodiagnosis, 30 - 35 female flies were exposed for 30 min each on the subject's forearm and lower leg, or the forearm only. Blood-engorged female flies were held in an environmental cabinet at 28°C and 85% humidity. At 60 -72 hours post- blood meal, flies were dissected and evaluated for L. donovani infection by microscopy as well as by quantitative polymerase chain reaction (qPCR). A subject was considered positive for infectivity to sand flies if promastigotes were observed in one or more individual flies by microscopic exam, or in one or more of the pools of flies by qPCR analysis. We found that 54.6% (42/77) and 77.9% (60/77) of active VL patients transmitted parasites to at least one fly or pools by microscopy and qPCR, respectively. Transmission of infection correlated with severity of VL disease. None of the drug cured VL patients were found xenodiagnosis positive by microscopy at 30 days post-treatment, although 7.7% (6/77) were still positive by qPCR. Both nodular and macular PKDL patients were infectious to sand flies, with enhanced transmission when the flies were fed on nodular lesions. 92.8% (13/14) HIV-VL co-infected patients transmitted infection to flies. Importantly, none of the 184 asymptomatic subjects were infectious to sand flies. In conclusion, these findings confirm that active VL, PKDL and HIV-VL patients transmit L. donovani to the vector, but that early diagnosis and treatment will effectively remove these individuals as infection reservoirs.

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Molecular Identification of Leishmania in Stained Slides from Patients with Cutaneous Leishmaniasis in Santarém, Pará, Brazil

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Tegumentary leishmaniasis (TL) is an infectious disease caused by the protozoan Leishmania. The correct identification of the species of Leishmania is important epidemiologically and to adequate therapy. For the diagnosis of the disease, parasitological tests are used but they cannot identify species. The use of PCR (polymerase chain reaction) can be performed from different biological samples, such as stained smears stained on slides and previously examined by microscopy. As a target for PCR, the ITS-1 (internal transcribe space-1) region of the ribosomal RNA has an adequate number of polymorphisms for species-level distinction, in addition to conserved regions of diagnostic importance. Our objective was to identify Leishmania species from lesion smears from patients with suspected TL, residing in Santarém, Pará, Brazil. For the procedure, 40 lesion smears stained by Giemsa on microscopy slides, from 40 patients with suspected TL, were analyzed by microscopy (1000X) in the laboratory of the Núcleo Técnico de Vigilância em Saúde (NTVS)/Pará. These smears were subjected to DNA extraction using Qiamp DNA Mini (Qiagen) and the DNA was subjected to PCR, according to the amplification conditions used by Godoy et al. 2020. The amplified (320pb) product was submitted to the Sanger sequencing. Our results showed that from 40 slides examined microscopically, 30 were positive and 10 were negative. Of the 10 negatives on microscopy, 100% (10/10) were negative on PCR. Of the 30 positives on microscopy, 83% (25/30) were positive on PCR-ITS-1. 18 samples from 25 submitted to sequencing showed the following results after alignment: 4 Leishmania (Viannia) laisoni, 4 L. (Leishmania) amazonensis, 3 L. (V.) shawi, 2 L. (V.) guyanensis, 3 L. (V.) braziliensis, 1 L. (V.) naiffi, 1 L. (V.) panamensis and 2 L. panamensis/guyanensis. Molecular investigation using ITS-1 as a target showed a specificity of 100% and a sensitivity of 83%. The sequencing of the ITS-1 intergenic region showed that there is a great diversity of Leishmania Viannia species in Santarém and L. (V.) braziliensis is not the most prevalent species in Santarém, Pará, as seen throughout Brazil.

Usefulness of Anti α-Gal Antibodies as Biomarkers of Therapeutic Response in Chagas Disease

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Understanding community knowledge gaps in neglected tropical diseases is vital for planning context-specific prevention and control measures. Over 3000 cutaneous leishmaniasis (CL) cases are reported in Sri Lanka annually. We aimed to assess knowledge of CL among community in a disease-endemic region in Sri Lanka. We conducted a household survey in Anuradhapura district, Sri Lanka using a multi-stage cluster sampling method. The households’ health-related decision-makers were the primary respondent. We used a semi-structured interviewer-administered questionnaire developed through a community engagement and involvement approach. Our sample included 1555 participants with a 98.4% response rate. Participants’ mean age was 48.2 years (SD=13.8), and 1157 (74.4%) were females. Most had completed 11 years in school (n=634, 40.8%). Participants included housewives (n=637, 41.0%), farmers (n=301, 19.4%), self-employers (n=156, 10.0%) and others (n=461, 29.6%). Among participants, 1250 (80.4%) claimed they had heard of the disease (mostly the local name, ‘sand fly or sand flea disease’) while only 141 (11.3%) knew the term ‘leishmaniasis’. Of those who had heard of the disease, 612 (49.0%) claimed they knew only the name. Among...
the participants who knew more about the disease other than its name, 224 (35.1%) were aware that sand fly is the vector of leishmaniasis. The majority reported red (n=173, 27.2%), long-lasting (n=153, 24.0%), and pimples (n=378, 59.4%) as the main characteristics of early cutaneous leishmaniasis lesions. After excluding people with CL, 475 (77.4%) believed leishmaniasis is curable, and 313 (49.1%) knew they should reach the district hospital for treatments. We identified community knowledge gaps related to the vector, clinical manifestations and treatment facilities for leishmaniasis, and public awareness programs should be tailored to address these identified gaps.

5059

A COST-EFFECTIVE LAMP-PCR FOR SCREENING AND MONITORING CHAGAS DISEASE

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The standard treatment for Chagas disease is efficient during the acute phase but only 1% of patients are treated because of poor early diagnosis. Most currently available PCR-based diagnostic methods are expensive, not portable, and do not consider the genomic variability of the parasite. A more specific, sensitive, and cost-effective tool is urgently needed to improve the early screening for Chagas disease in low-income endemic regions. A loop-mediated isothermal amplification (LAMP-PCR) was designed for targeting a highly conserved region in the HSP70 gene of Trypanosoma cruzi. Backward, forward and loop primers were manually designed across a region of 236 bp. The optimal melting temperature and delta G values were estimated in the OligoAnalyzer tool (IDT). Our LAMP-PCR protocol amplified T. cruzi DNA from discreet typing units (DTUs) I, II, IV, V, and VI. The reaction did not amplify DNA from T. rangeli or Leishmania (L. amazonensis, L. braziliensis) used as controls. The proposed method was able to detect about 28 fg of parasite DNA extracted from infected blood samples at 1X106 epimastigotes/mL, equivalent to less than one parasite. Parasite DNA could be detected in different types of samples including blood coat, anticoagulated blood, and buffy coat. This method is fast, temp stable, and cheap. This portable method can facilitate the screening and monitoring of patients in endemic regions for Chagas disease. Further efforts will be focused on simplifying the DNA extraction protocol, evaluating clinical samples, and scaling the method in endemic regions.

5060

A SET OF DIAGNOSTIC TESTS USEFUL FOR THE DETECTION AND IDENTIFICATION OF LEISHMANIA PARASITES CAUSING CUTANEOUS LEISHMANIASIS

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The new WHO roadmap for NTDs 2021-2030 identified diagnostics among top priorities to achieve the 2030 targets for Leishmaniases control. Simple, rapid and accurate diagnostics are essential to control CL. Leishmania (L.) species identification is essential for diagnosis, patient’s management and an adequate therapeutic strategy. Diagnosis tools applied for the identification of L. parasites depend on laboratory equipment and facilities. The aim of this study was to develop a set of innovative and specific molecular tools for L. species detection and identification adapted for well as poorly equipped laboratories. Using comparative genomic analyses of L. genomes we selected species specific, distinctive targets and designed a range of PCR primer pairs that we assessed for taxa-specific DNA amplification of L. species encountered in MENA region. Four primer pairs were retained. Three of them, showing L. species-specific amplification, were investigated to develop a PCR multiplex-lateral flow chromatography (LF) on a customized microfluidics for DNA-DNA hybridization. This test requires basic equipment such as a thermocycler, Readout for detection and identification is made in 3’ on the LF after a 2h PCR. Another pair was designed for generic amplification of a L. DNA fragment containing species-specific SNPs adequate for PCR

5061

THE PATHWAY TO SUSTAINABLE ELIMINATION OF HUMAN AFRICAN TRYPANOSOMIASIS IN DEMOCRATIC REPUBLIC OF CONGO

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Gambiense HAT (gHAT) has been targeted for elimination as public health problem (PHP) by 2020 and now for elimination of transmission (EoT) by 2030. The Democratic Republic of the Congo (DRC), the most affected country; is inexorably moving towards gHAT elimination countrywide. As the trend of the disease may vary from one region to another, we aim to analyze the disease current results and trend toward gHAT elimination as well as the quality and coverage of gHAT control activities using data archived in the WHO Atlas of HAT from 2000 to 2016 at country, provincial and health district (HD) levels. We found that the prevalence of gHAT in DRC was substantial decreased. However, provinces, like Mbandaka and Kivu remained most prevalent compared to others like Nord Ubangi and Sud Ubangi. HAT screening coverage, population attendance rate to active screening, the proportion of patients who received treatment, and the therapeutic efficacy rate have been identified as factors associated with disease trend. Around 16.0% of the 257 endemic HDs, that reported at least one case of HAT between 2000 to 2016, had not yet reached the target of HAT elimination as PHP according to Franco et al (2020) in 2016. Although the number of people actively screened annually remained almost stable, active screening was poorly implemented according to the recommended algorithm. Nearly two-thirds of endemic HDs were not covered by a health facility (HF) implementing the full range of HAT activities and approximately 40% of endemic health areas were not covered by a HF capable of screening for HAT. Active screening as implemented and the low coverage of passive screening may hide some cases that could be a source of resurgence in the future and do not appear to be sufficient to ensure surveillance in the post-elimination period and provide sufficient confidence in sustainability towards an EoT. And indeed, one fact that appears to support this conclusion is a relative increasing number of cases in the country between 2020 and 2021 from 306 to 425. There is a need to improve coverage and implementation of active and passive screening strategies and monitor to confirm current achievement in DRC.
Environmental surveillance, using detection of Salmonella Typhi DNA, has emerged as a potentially useful tool to identify typhoid-endemic settings; however, it is relatively costly and requires molecular diagnostic capacity. We sought to determine whether S. Typhi bacteriophages are abundant in water sources in a typhoid-endemic setting, using low-cost assays. We collected drinking and surface water samples from urban, peri-urban and rural areas in 4 regions of Nepal. We performed a double agar overlay with S. Typhi to assess the presence of bacteriophages. We isolated and tested phages against multiple strains to assess their host range. We performed whole genome sequencing of isolated phages, and generated phylogenies using conserved genes. S. Typhi-specific bacteriophages were detected in 54.9% (198/361) of river water samples and 6.3% (1/16) drinking water samples from the Kathmandu Valley and Kavrepalanchok. Water samples collected within or downstream of population-dense areas were more likely to be positive (72.6%, 193/266) than those collected upstream from population centers (5.3%, 5/95) (p=0.005). In urban Biratnagar and rural Dolakha, where typhoid incidence is low, only 6.7% (1/15, Biratnagar) and 0% (0/16, Dolakha) samples contained phages. All S. Typhi phages were unable to infect other Salmonella and non-Salmonella strains, nor a Vi knockout S. Typhi strain. Representative strains from S. Typhi lineages were variably susceptible to the isolated phages. Phylogenetic analysis showed that S. Typhi phages belonged to two different viral families (Autographiviridae and Siphoviridae) and clustered in three distinct groups. S. Typhi bacteriophages were highly abundant in surface waters of typhoid-endemic communities but rarely detected in low typhoid burden communities. Bacteriophages recovered were specific for S. Typhi and required Vi polysaccharide for infection. Screening small volumes of water with simple, low-cost plaque assays enables detection of S. Typhi phages and should be further evaluated as a scalable tool for typhoid environmental surveillance.
Cross Protection of Heterologous Shigella Flexneri 2a and S. Sonnei Challenge in Healthy Adults in the United States

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Shigella is a major cause of diarrhea in low- and middle-income countries, as well as becoming increasingly antibiotic resistant. Efforts are underway to develop vaccines against Shigella; however, the diverse serotypes of disease-causing strains necessitate a multivalent vaccine. Understanding conserved epitopes and correlates of protection can aid in the design of broadly protective vaccines. This controlled human infection model (CHIM) study sought to assess cross-protection and markers of protection after challenge and heterologous rechallenge with different Shigella serotypes. Cohort 1 was challenged with S. sonnei, followed by a S. flexneri 2a challenge 3 to 4 months later. Cohort 2 was challenged with S. flexneri, followed by a S. sonnei challenge 4 months later. Naïve volunteers were included with each rechallenge to ensure acceptable attack rates. Stools were collected and tested for the challenge organism, Blood and stool samples were saved for immunologic assays. A total of 46 individuals were enrolled; 28 received S. sonnei, of which 19 were rechallenged with S. flexneri. Eighty subjects received S. flexneri 2a; 10 were rechallenged with S. sonnei. In preliminary analyses, of the naïve volunteers who were challenged with S. sonnei, 18/28 (64%) met the consensus criteria for shigellosis or required early treatment. Of the volunteers challenged initially with S. flexneri 2a, 13/17 (77%) met shigellosis or early treatment criteria. Among volunteers initially challenged with S. sonnei, 4/19 (21%) met shigellosis or early treatment criteria following subsequent challenge with S. flexneri. In contrast, among volunteers initially challenged with S. flexneri, 8/10 (80%) met shigellosis or early treatment criteria following S. sonnei challenge. Stool culture data support the clinical findings. Immunologic analyses are ongoing. In conclusion, prior S. sonnei exposure provides significant protection against S. flexneri 2a. Prior S. flexneri 2a infection provides no clear protection against subsequent S. sonnei infection. These data raise intriguing questions about conserved epitopes and immunological memory.

Development of a Shigella Multivalent Bioconjugate Vaccine: A Phase I/II Randomized, Controlled and Age Descending Study Including Doze Finding in Kenyan Infants

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Shigella is among the most common causes of severe diarrhea and dysentery worldwide, especially among young children from lower resourced countries and travelers. Although several oral Shigella vaccines have been clinically evaluated, risk of reactogenicity and potential reversion back to a pathogenic phenotype have proven challenging. Thus, a new type of vaccine to prevent shigellosis is key. The O-antigen serotype-specific immune response detected in association with convalescence from shigellosis has encouraged development of a new generation of glycoconjugates as an alternative vaccine strategy against Shigella.

A Shigella flexneri 2a bioconjugate has shown, in a controlled human infection model, protection from the most severe form of the disease after challenge with S. flexneri 2a strain 2457T. In addition, immune responses post vaccination were associated with a lower disease severity score. The potential of bioconjugation to develop a high fidelity and cost-effective multivalent Shigella vaccine targeting the most common serotypes contributing to global morbidity and mortality has been exploited. The tetravalent Shigella4V bioconjugate, including O-antigens from S. flexneri 2a, 3a, 6 and sonnei, has been evaluated for safety and immunogenicity in the target population of 9 months old infants. The trial was conducted in Kenya starting with a step 1, age descending and dose-escalating cohort, followed by a step 2 dose finding cohort where the target population received a 3 dose schedule of the bioconjugate delivered with and without alum adjuvant. The doses used were 4, 12, 24 and 48 µg which each represents the total polysaccharide across the four Shigella O-antigens. The last patient last visit occurred in Q4 2022 and immunogenicity and safety data (from the target population) from the interim analysis (IA) was made available in Q3 2022, the remaining data will be available in Q4 2023. The IA data has shown good immunogenicity 1 month post 2nd vaccination across all treatment groups and serotypes along with well distributed and mostly mild safety events. Following the positive IA results, we have progressed towards the next steps.

Human Milk Oligosaccharides and Campylobacter Jejuni Infection Risk in Nicaraguan Children

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Campylobacter jejuni infection causes acute gastroenteritis and is associated with malnutrition, stunting, and cognitive delays. C. jejuni uses α-1,2 fucosylated and sialylated oligosaccharides on the intestinal epithelia as binding factors to infect the gut. Human milk oligosaccharides (HMOs) in breastmilk may protect against C. jejuni infection by acting as decoy receptors for C. jejuni and by shaping the gut microbiome. We examined HMOs and C. jejuni infection risk in an observational cohort of non-exclusively breastfed infants in Nicaragua. HMO composition at 2 months (range 1.1-4.8) was measured with fluorescent high-and-ultra-high-pressure liquid chromatography. Children were surveilled weekly for diarrhea, and C. jejuni in stool was detected using qPCR of the 16S rRNA and cadF genes followed by Sanger sequencing. We mapped cumulative risk of C. jejuni infection over 36-months by tertiles of concentrations of the α-1,2 fucosylated HMOs 2’-fucosyllactose (2’FL) and lacto-N-fucopentaose-I (LNFP-I), and the sialylated HMOs 3’-sialyllactose (3’SL) and 6’SL. We also assessed the 12 and 36-mo relative hazards (HR) of C. jejuni infection for each HMO stratum, adjusting for total weeks of any breastfeeding at the time of HMO collection. Analyses were stratified by child secretor and Lewis B phenotypes which determine oligosaccharide expression in the gut epithelium. Of 419 mother-child pairs with breastmilk analyzed, 49 (12%) had at least one C. jejuni infection. Surprisingly, in the first 12 months of life, Lewis B and secretor children consuming the top tertile of 3’SL (HR=2.09, 95% CI 1.02, 4.30), had twice the risk of C. jejuni infection compared to children consuming the bottom tertile of both HMOs. This difference attenuated slightly between 12-36 months. As expected, Lewis B children consuming milk in the top tertile for 2’FL (HR=0.5, 95% CI 0.24, 1.03) and LNFP-I (HR=0.49, 95% CI 0.25, 0.99) had half the risk of infection of children consuming milk in the lowest tertile for both HMOs. Our findings support evidence that the α-1,2 fucosylated HMOs 2’FL and LNFP-I may protect against C. jejuni infection in breastfeeding children.
POLIO VACCINATION IN CHILDREN IN LOW- AND MIDDLE-INCOME COUNTRIES

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While there has been tremendous progress in the eradication of poliovirus, close to 50 countries are either experiencing or are at high risk of re-emergence of polo. The majority of polo infections are in low and middle income countries (LMICs), where oral vaccine efficacy is lower than in higher-income countries, associated with poorer vaccine immunogenicity. Our goal was to develop a clinical prediction rule to identify children likely to fail to seroconvert after polo vaccination. We used clinical and demographic data from the Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) study to build predictive models to identify children who fail to seroconvert following oral polo vaccination (OPV) in eight countries. Failure to seroconvert was defined as a log2 titer <3 for polo-virus serum neutralizing antibody titers measured using WHO-standardized microneutralization assays collected at 7 and 15 months of age. We screened variables using random forests, and assessed predictive performance with random forest regression and logistic regression using cross-validation. Of the 1294 children analyzed who received at least three doses of OPV, 15.1% and 11.5% had failed to seroconvert at 7 and 15 months, respectively. Top predictors were well-aligned with known risk factors of seroconversion failure, including breastfeeding practices, indicators of household crowding, maternal education, and acute diarrhea and respiratory symptoms. Our ability to predict seroconversion failure was poor for serotype 1, serotype 3, and polio of any serotype (AUC)=0.58 (95% CI: 0.55, 0.62) for a model including the top five predictive variables. For serotype 2, we achieved an AUC=0.77 (95% CI: 0.73, 0.81) with five predictive variables. Our findings indicate that use of prediction rules may help identify children at risk of failure to seroconvert after vaccination, which could help target polo eradication efforts. We also demonstrate that predictive ability differs by serotype, and how risk factors may not be the best predictors of vaccine immunogenicity.

ASSESSING THE CONTRIBUTION OF NUCLEOTIDE VARIATIONS IN THE MAYARO VIRUS GENOME TO ITS ADAPTIVE LANDSCAPE IN AEDES AEGYPTI AND ANOPELEAS ALBOPICUS MOSQUITOES

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Mayaro virus (MAYV) is an arbovirus of the Alphavirus genus and the etiologic agent of Mayaro fever (MAYF). MAYV is maintained in forests by an enzootic cycle, and no urban outbreaks have been confirmed to date. With the expansion of the Aedes (Stegomyia) mosquito distribution, and MAYV continuous circulation, we hypothesized that infection of these mosquitoes by MAYV could result in adaptive mutations that favor transmission. To investigate mutations that could arise in MAYV infection of A. aegypti and A. albopictus from Salvador, Brazil, we fed these mosquitoes a blood meal containing 7 log10 PFU/ml of clonally derived MAYV strain CH (genotype D) which resulted in an 85% infection rate at 14 days post feeding. The salivary glands were dissected and organized into 3 pools of 15 glands each. The RNA was deep sequenced with Illumina (NextSeq 550), and analyzed for single nucleotide polymorphisms using LoFreq software. For A. aegypti and A. albopictus, respectively, we identified 128 and 30 minority variants that were not present in the parental virus. We selected mutations occurring in the genome of at least one MAYV strain on the Pubmed database for study, to reflect those that could be occurring in nature, and applied a Z-score to rank them. We have cloned and rescued four of these naturally occurring mutations in the Mayaro CH background and tested their fitness for infection and transmission using competition assays in mosquitoes and dissected the salivary gland for analysis. Only one of these viruses, with a mutation in nsp3, had increased fitness as assessed using salivary gland samples. For future studies of nucleotide variations in the context of MAYV genotype L, we also described and characterized a novel MAYV clone, strain Be1505465. These results suggest that minority variants that provide a modest fitness advantage for MAYV transmission by Aedes mosquitoes could arise after a single infection round and may contribute to the risk of urban outbreaks. This work underscores the critical importance of surveillance efforts to quickly identify urban emergence and the urgent need to develop effective vaccines and treatments against MAYF.

USING BARCODED WEST NILE VIRUS TO QUANTIFY THE IMPACT OF TISSUE-ASSOCIATED BOTTLENECKS ON VIRUS POPULATIONS IN ENZOOTIC AND BRIDGE VECTORS OF WEST NILE VIRUS

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Each step during arthropod infection constitutes a physiological barrier to virus transmission. These barriers impose stochastic reductions on arbovirus populations, frequently termed bottlenecks. In vectors of West Nile virus (WNV), the main bottlenecks associated with infection, dissemination, and transmission occur during infection of and escape from the midgut and salivary glands. The severity of these bottlenecks varies by tissue and potentially mosquito species. Arboviruses such as WNV are maintained in nature by multiple mosquito species with varying levels of vector competence (VC, efficiency of pathogen transmission). Importantly, the extent to which population bottlenecks and VC are linked is poorly understood. Additionally, quantitative analyses of mosquito bottlenecks on virus population dynamics are limited. To address these knowledge gaps, we used molecularly barcoded WNV (bcWNV) to quantitatively measure tissue-associated population bottlenecks in Culex tarsalis, Culex quinquefasciatus, and Aedes aegypti - three variably competent WNV vectors. In all species we observed reductions in bcWNV population richness and complexity upon escape from the midgut and entry of the salivary glands. In Aedes mosquitoes, barcode diversity in the midgut was significantly lower compared to Culex species. Importantly, population richness and complexity did not differ significantly between salivary glands and saliva from any species, indicating that bottleneck severity post-midgut infection does not differ between vectors of varying competence. Barcode frequency in the input population was positively correlated with successful transmission in Culex, however, high frequency in the bloodmeal did not guarantee transmission in any species. Cx. tarsalis, had the highest probability of transmitting rare barcodes when compared to lower competence vectors. This work provides insight into stochastic influences on virus population dynamics during mosquito infection in vectors of varying competence and suggests that vector competence may influence the successful transmission of rare virus variants in a population.
INTRA-HOST DIVERSITY IN VACCINATED COVID-19 PATIENTS INFECTED WITH DIFFERENT SARS-COV-2 VARIANTS
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Virus Intra-Host Genetic Diversity (IHGD) can influence transmission and virulence by evading host immune response and disease severity, especially for SARS-CoV-2 variants. First, to evaluate the IHGD in unvaccinated (Uv, individuals infected with SARS-CoV-2, without any dose of vaccine) and vaccinated (V, individuals vaccinated with two doses of CoronaVac - Butantan/Sinovac) patients, we analyzed 120 COVID-19 samples from São José do Rio Preto and surrounding cities, obtained from April to July 2021. Total RNA was extracted, and the whole-genome sequencing was performed using Illumina CovidSeq. Using Pangolin COVID-19 Lineage Assigner Tool, these genomes were classified into Gamma lineage. The intra-host single nucleotide diversity analysis was carried out using LoFreq, and annotation and prediction of genetic effects was annotated using the SnpEff. The inference of selective pressures was performed using HyPhy to detect codons evolving on diversifying (DS) or purifying selection (PS). Our results evidenced that vaccination with CoronaVac favors negative selection at the intra-host level, in different genome regions, especially in nonstructural protein-coding genes, preventing further SARS-CoV-2 genetic diversity and reinforcing the importance of vaccination to reduce virus transmission. After this, we aim to analyze the influence of booster doses on IHGD of SARS-CoV-2 in COVID-19 patients infected with other variants. So far, total RNA and whole-genome sequencing have been carried out on 762 samples from patients with two or three doses of vaccine, obtained from October 2021 to December 2022. Genomes were classified in the VOCs Delta and Omicron, of which 475 correspond to patients who received two doses of vaccine, and 287 correspond to patients who received three doses. Our next steps are subdividing the samples based on vaccination status, vaccine type (inactivated virus, viral vector, and RNA technologies), and SARS-CoV-2 variant to compare intra-host diversity in different conditions and investigate possible immune-escape mutations among vaccinated individuals.

VIRAL SEQUENCE DATA FOR EPIDEMIOLOGICAL CHARACTERIZATION OF GLOBAL DENGUE VIRUS OUTBREAKS
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Dengue is a re-emerging arbovirus that poses a significant public health risk. Dengue virus exhibits complex disease dynamics driven by a range of factors including those acting at the evolutionary scale such as clade replacement, ecological scale such as climate change, and demographic scale such as urbanization. These factors influence the emergence and expansion of the virus, resulting in outbreaks in previously naïve areas. Despite the increase in frequency of dengue virus outbreaks in recent years, a detailed characterization of these outbreaks and identification of potential evolutionary, ecological and demographic drivers of dengue introduction and subsequent spread has not been conducted. In this analysis we use publicly available viral sequence data downloaded from online databases to characterize detected outbreaks of dengue virus, and determine the influence of evolutionary, ecological and demographic drivers on dengue outbreaks. Viral sequence data annotated with date and location information corresponding to the envelope gene portion of dengue 2 virus was downloaded from publicly available online databases. Maximum likelihood phylogenetic trees were inferred in RaxML from aligned sequences and used to define sequence clusters corresponding to dengue outbreaks. Outbreak sequences were defined as genetically similar clusters of sequences collected within a date range from the same location. Birth-death skyline models in BEAST2 were used to infer key epidemiological parameters of each identified outbreak cluster, including time varying effective reproductive number and sampling proportion. Epidemiological parameters derived from phylogenetic models were used to compare global outbreaks of dengue virus and show the potential influence of evolutionary, ecological and demographic drivers on dengue outbreaks. Our analysis utilizes a global sequence dataset to determine the factors influencing dengue virus outbreaks.

THE IMPACT OF TEMPERATURE ON WEST NILE VIRUS MOSQUITO BOTTLENECKS AND ANTIVIRAL IMMUNITY
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West Nile virus is an arbovirus that is mainly transmitted by Culex mosquitoes. Within a mosquito, viruses encounter four physical barriers during systemic infection: midgut infection barrier, midgut escape barrier, salivary gland infection barrier, and salivary gland escape barrier. The strength of each of these barriers varies by virus, mosquito species, and other factors (e.g., microbiome, coinfection, etc.). Temperature is frequently the strongest abiotic factor impacting the efficiency of WNV transmission by Culex. However, the relationship between temperature and the strength of each bottleneck within the mosquito is poorly understood. Arboviruses exist in nature as genetically complex mutant swarms, with bottlenecks stochastically reducing the size and complexity of these virus populations. To mimic this genetic diversity, we utilized a barcoded West Nile virus (bcWNV) containing ~106 unique genetic markers that are approximately fitness neutral. We infected Cx. tarsalis with bcWNV and held them at 22°C, 26°C and 30°C to determine the role of temperature on the strength of population bottlenecks during arbovirus infection of mosquito vectors. We observed similar infection rates and viral loads in all mosquito tissues regardless of temperature. Using deep sequencing, we will determine the extent to which temperature impacts WNV population diversity and complexity. Additionally, increased temperature is known to alter many aspects of mosquito biology, but its impacts on mosquito antiviral immunity are currently unclear. We will thus characterize how temperature impacts mosquito antiviral responses to determine if altered temperatures change expression of innate immunity (focusing on the JAK-STAT, IMD, Toll, and RNAi pathways). Together, these results will shed light on the relationships between temperature, virus evolutionary dynamics and antiviral immunity.

GENOMIC SURVEILLANCE DURING THE FIRST-EVER HYPERENDEMIC TRANSMISSION OF ALL FOUR DENGUE VIRUS SEROTYPES IN NICARAGUA IN 2022 REVEALS NEW VIRAL INTRODUCTIONS POST-PANDEMIC
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Nicaragua, as with most Central and South American countries, has been severely impacted over the last decades by major epidemics of dengue. Since dengue virus (DENV) detection in 1985, co-circulation of serotypes has been limited to two or three, with one always predominating. However, for the first time in the country’s epidemiological history, we detected the co-circulation of all four serotypes of DENV in Nicaragua in 2022. Here, we describe the molecular and epidemiological characteristics of the 2022 epidemic to better understand the evolutionary histories of DENV currently circulating. Our findings show an introduction of a new clade of DENV-1 into the country more closely related to viruses circulating in Ecuador in...
2014 than to prior DENV-1 circulating in Nicaragua in 2016, although both fall within the American/African genotype V. DENV-2 sequences were all members of the same lineage that recently dominated in Nicaragua, in association with the 2016 and 2019 epidemics, falling within the American/Asian genotype I. DENV-3 viruses, last circulating in Nicaragua in 2014, fell within the same genotype III (Indian subcontinent) as before but formed a distinct group more closely related to Southeast Asian strains from as recent as 2019 as well as strains circulating in Florida and Cuba in 2022 than to prior Nicaraguan strains. The sequences of DENV-4, which very rarely causes clinical cases in Nicaragua, all fell within genotype IIB and descended from a widespread South American lineage with strong similarity to recent records from Mexico (2021) and Florida (2022). Our study reveals a shift in the epidemic dynamics in Nicaragua with a rise to co-dominance of DENV-1 and DENV-4 taking over where DENV-2 was formerly most common, and a strong influence of introduction and global exchange shaping the pattern of DENV circulation for three of the four serotypes. With a resurgence of travel following pandemic-related border closure and re-openings, DENV co-circulation of multiple serotypes across much broader regional scales could impact herd immunity and case severity rates and have other public health impacts yet to be determined.

**METAGENOMICS IDENTIFIES EMERGING AND RE-EMERGING VIRUSES IN NIGERIAN COHORTS WITH ACUTE FEBRILE ILLNESSES, INCLUDING PATHOGENS OF GLOBAL CONCERN**

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Malaria and schistosomiasis are two major parasitic vector-borne diseases that are a particular threat to young children in rural areas of Sub-Saharan Africa. In the present study, we investigated factors that are associated with malaria, schistosomiasis, and co-infection among school-aged children, using an explanatory sequential mixed-methods approach. A cross-sectional study was conducted in January 2022 in Misungwi, Lake Victoria zone, Tanzania, that sampled 1,300 children aged 5 to 14 years old for malaria and schistosomiasis. Mixed-effect logistic regression models were used to assess the association between infection prevalence and seroprevalence, and environmental determinants that create favorable conditions for vectors and parasites and social determinants that relate to disease exposure. Community mapping combined with direct field observations were conducted in August 2022 in two selected villages from the cross-sectional study to understand specific water use behaviours and to identify potential malaria mosquito larval breeding sites and freshwater snail habitats. The prevalence of malaria, seroprevalence of schistosomiasis, and co-infection in this study were 40.4%, 94.3%, and 38.1%, respectively. Community maps identified many unimproved water sources in both villages that were used by humans, cattle, or both. We found that children primarily fetched water, and that unprotected wells were dedicated for drinking water whereas ponds were dedicated for other domestic uses and cattle. Although not identified in the community maps, we found hand pumps in both villages that were not in use because of unpleasant taste and cost. This study improves our understanding of social and environmental factors that are associated with malaria, schistosomiasis, and co-infection which can inform potential entry points for integrated disease prevention and control.
ONE HEALTH APPROACH TO NIPAH VIRUS OUTBREAK INVESTIGATION AMIDST OF COVID-19 PANDEMIC IN BANGLADESH, 2021-2022

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Nipah virus (NV) is an emerging bat-borne virus causing fatal encephalitis outbreaks. Until 2020, Bangladesh reported 319 NV infections with 70% mortality. Amidst the COVID-19 pandemic, a multidisciplinary team investigated five suspected NV outbreaks in three districts of Bangladesh between 2021 and 2022 to identify the source, behavioral risk practices, and environmental exposures. We collected epidemiological, behavioral, and ecological data from outbreak communities and biological specimens of bats. We captured 60 Pteropus medius bats per outbreak event and environmental pool urine samples (N=314) from seven bat roosts within 10km radius of the outbreak’s epicenter. The bat samples were tested for NV by rRT-PCR and consensus PCR assay targeting rdp genes of paramyxovirus (PMV) to detect known and novel PMVs and to screen serum samples using multiplex Luminex assay for henipavirus panel. We detected two novel henipa-like paramyxoviruses in bats. The seroprevalence of NV was 15.2% (34/224, CI: 10.7%-20.56%), and Hendra virus was 1.3% (3/224), Cedar 1.3% (3/224), Mojiang 1.3% (3/224), Mojiang 0.9% (2/224), and Kumasi virus 1.3% (3/224). The behavioral investigation showed that all cases had a history of drinking contaminated raw date palm sap (RDPS) within 14 days prior to the onset of symptoms. In the outbreak communities, the participants have the habit of drinking RDPS 73.1% (CI: 67.04%-78.51%), eating bat bitten fruits 17.96% (CI: 13.36%-23.35%). Moreover, around 47% of the respondents had date palm trees in their household, among 74.78% were nursing. Serological and virological data showed diverse henipavirus circulating in bats. The high density of date palm trees and habits of drinking raw sap and eating half-eaten fruits in households acts as a source of baseline infections throughout the year, particularly in higher elevation areas where flood-driven pathogen dispersion is limited. Periods of intense rainfall then drive excess infections through large flooding-associated outbreaks. In addition to informing targeted intervention for urban leptospirosis, this study provides a methodological basis to model interactions between reservoirs, environment and human risk behaviours which may be generalisable to other One Health threats with complex epidemiology.

BREAKING TRANSMISSION: A TRANSDISCIPLINARY ONE HEALTH APPROACH TO IMPROVE HOOKWORM CONTROL

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Hookworm disease is a major global public health concern, despite targeted control programs of at-risk populations. The success of these programs has been hindered by the rapid re-infection rates linked to persistent reservoirs and the low sensitivity of conventional diagnostic techniques employed. In study 1, we used standard faecal flotation (SFF) and a multiplex qPCR assay to calculate and compare species-specific cure and egg reduction rates of single dose albendazole (400 mg) against hookworm infections at community level. In study 2, we combined microscopic and molecular typing to unveil infections by hookworm-like parasites in people living in rural communities. In study 3, we developed a novel multiplex PCR transmission model of Ancylostoma ceylanicum, the main zoonotic hookworm in Asia, and compare the effectiveness of human-only and “One Health” (human plus dog) MDA strategies under a range of eco-epidemiological assumptions. We revealed a substantial difference in cure rate of hookworm infection(s) following albendazole treatment using the SFF (81.5%) and mqPCR (46.4%) assays, and provide the first data on the efficacy of this drug against the zoonotic hookworm A. ceylanicum. In study 2, we identified infections with Trichostrongylus spp. of livestock origin and spurious passage of Melodogynine eggs in people living in remote communities. Finally, we show that One Health interventions — targeting both dogs and humans — could suppress prevalence in humans to ≤ 1% by the end of 2030, even with only modest coverage (25-50%) of the animal reservoir. With increasing coverage, One Health interventions may even interrupt transmission. These findings show that the adoption of refined a transdisciplinary One Health approach to improve hookworm control.
approach is central to monitoring hookworm infections and the success of control strategies, which can ultimately aid in reducing associated morbidity in human populations. We provide evidence that One Health MDA strategy could be highly effective against A. ceylanicum hookworm in endemic regions of Southeast Asia and beyond and will be essential for sustained elimination and reaching the WHO 2030 goals.

5080

BARRIERS AND ENABLERS TO THE IMPLEMENTATION OF THE ANTIMICROBIAL RESISTANCE NATIONAL ACTION PLAN IN MALAWI

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Antimicrobial resistance (AMR) is a major threat to global health resulting in over 1 million deaths annually. The WHO Global Action Plan on AMR (2015) was adapted in Malawi, as the National Action Plan (NAP) on AMR, retaining similar objectives. Adaptation of global policies to local contexts often besets by challenges. We therefore conducted a qualitative study to explore barriers and enablers to the implementation of AMR NAP in Malawi. National data collection involved 22 in-depth interviews (IDIs) with policymakers and stakeholders, and 2 series of semi structured interviews with AMR core group members. District data was collected through 15 IDIs with district policymakers within one health sectors in Blantyre and Chikwawa districts. Data was analyzed thematically. Results showed that AMR in Malawi is socially constructed as an issue of one health, development, healthcare policy, health security threat, and innovation. Enablers for the NAP in Malawi include the use of One Health approach; strong Ministry of Health leadership; use of AMR policy champions; political will; and media engagements. Barriers within sectors included lack of priority setting, coordination, relationships, reporting and use of data, capacity, resources, and evidence. Outside MoH, AMR was viewed as a common health issue, there was incoherence, sectors working and being resource in silos, lesser engagement in environmental sector, water splits between ministries, duplicated functions among agencies. For improved AMR fight in Malawi, our study recommends updating legislation and enforcement; increasing awareness of communities and politicians; improving data for AMR in humans and animals; linkage and use of “strong” existing programmes e.g., HIV, decentralisation; and increased and improving data for AMR in humans and animals; linkage and use of “strong” existing programmes e.g., HIV, decentralisation; and increased and reallocation of resources.

5081

A PLANETARY HEALTH INNOVATION FOR DISEASE, SUSTAINABILITY, FOOD, WATER, AND POVERTY CHALLENGES IN WEST AFRICA

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Blastocystis sp. is a globally distributed enteric protozoan parasite that infects humans and a wide range of non-human animals. Its global distribution and wide host range contribute to its high prevalence and strain diversity in humans and other animals, particularly in low-income countries, where it is estimated to infect 30-60% of people. By applying the One Health Disparities framework, our research aims to study exposure disparities by identifying characteristics that expose some community members more than others to Blastocystis sp. We used a mixed-methods approach to investigate the predictors of Blastocystis sp. infection in a population of rural small stakeholder farmers in northeast Madagascar undergoing rapid change in their natural environments. We hypothesized that low socio-economic status, low educational attainment, low health literacy, hygiene practices, and high animal contact would increase the risk of Blastocystis sp. infection. We surveyed and collected fecal samples from 184 adults in one rural village. Fecal samples were screened using DNA metabarcoding, and predictions were tested using generalized linear models (GLM). We found that 64% of participants were infected with Blastocystis sp. representing 114 genetic variants and 4 subtypes. In feeding trials, the removed vegetation was as effective as traditional livestock feed but 41-179 times cheaper and converting the vegetation to compost provided private crop production and total (public health plus crop production benefits) benefit-to-cost ratios as high as 4.0 and 8.8, respectively. Thus, the approach yielded an economic incentive—with important public health co-benefits—to maintain cleared waterways and return nutrients captured in aquatic plants back to agriculture with promise of breaking poverty-disease traps. To facilitate targeting and scaling of the intervention, we lay the foundation for using remote sensing technology to detect snail habitat. By offering a rare, profitable, win-win approach to addressing food and water access, poverty alleviation, infectious disease control, and environmental sustainability, we hope to inspire the interdisciplinary search for planetary health solutions to the numerous and formidable, co-dependent global grand challenges of the 21st century.

5082

APPLYING A ONE HEALTH DISPARITIES FRAMEWORK TO ADDRESS THE SOCIAL GRADIENT AND HEALTH DISPARITIES OF BLASTOCYSTIS SP. INFECTION IN NORTHEAST MADAGASCAR

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Many communities in low- and middle-income countries globally lack adequate resources to address disease, food, water, and poverty challenges, despite their inherent sustainability, cost-effective, mutually beneficial solutions for infectious disease control, and environmental sustainability, we hope to inspire the interdisciplinary search for planetary health solutions to the numerous and formidable, co-dependent global grand challenges of the 21st century.

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EMERGENCE AND SPREAD OF HEARTLAND AND BOURBON VIRUSES IN NEW YORK STATE

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Heartland virus (HRTV, Phenuviridae: Bandavirus) and Bourbon virus (BRBV, Orthomyxoviridae: Thogotovirus) are emerging tickborne viruses in the United States (US). HRTV and BRBV were both identified from human cases in the Midwestern US in 2009 and 2014. Since the discovery of HRTV, more than 60 cases have been recorded in 14 states and at least 4 cases of BRBV in 3 states. Vector surveillance conducted during epidemiological investigations for HRTV and BRBV and results of experimental infection studies led to the incrimination of Amblyomma americanum as the vector for both viruses. While A. americanum are typically found in midwestern and southern regions of the US, this species has experienced a recolonization of former territory and an expanded geographic range now extends as far north as southern coastal Maine. Passive surveillance through commercial laboratory testing first identified HRTV and BRBV in New York State (NYS) in 2018 and 2019, respectively. Enhanced surveillance efforts conducted in Suffolk County since have consistently detected the viruses in A. americanum with high seropositivity rates observed in white-tailed deer. To further our understanding of the emergence and spread of these viruses in NYS and the northeastern US, we conducted full genome sequencing and phenotypic characterization on HRTV and BRBV isolates from 2018-2022. Preliminary sequencing results revealed the existence of distinct clades of both HRTV and BRBV when compared to midwestern isolates. Characterization in cell culture and experimentally infected A. americanum revealed little phenotypic distinction among and between the midwestern and NYS HRTV clades. NYS BRBV isolates, however, display high levels of both genetic and phenotypic variability compared to other eastern and representative midwestern BRBV isolates. We also found BRBV but not HRTV NY isolates infect and replicate in Ix. scapularis following virus immersion. Genotypic divergence of both HRTV and BRBV suggest multiple, separate introductions of each virus into NYS with emergence of varying phenotypes potentially due to adaptation to geographically distinct transmission cycles.

BORRELIA BURGDORFERI ENZOOTIC CYCLE IN CONSTANT FLUX

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The enzootic cycle of Borrelia burgdorferi, the agent of Lyme disease, relies on larval deer ticks feeding upon mammalian hosts to acquire infection. White-footed mice are thought to be the most important reservoir for immature deer ticks, but other hosts can also serve as a source of infection. The relative importance of a host is determined by local habitat conditions that often favor certain species. Identification of the dominant reservoir host at individual sites would greatly facilitate risk reduction measures by allowing targeted efforts. However, very little work has been done to understand the stability of host species’ contribution to the enzootic cycle over time. Using our established methods for bloodmeal analysis on questing nymphal ticks, we followed two field sites, Martha’s Vineyard and Nantucket Island, for 4 years to determine how bloodmeal hosts of nymphal ticks change over time. In addition, we examined the stability of the enzootic cycle at each site by identifying Borrelia-infected ticks by PCR and determining the host from which they had acquired infection. In 2018, mice were the predominant host on Nantucket having fed 58% overall and 63% of the infected nymphs. Over time, this steadily declined to 13% overall and none of the infected ticks. Borrelia rates fluctuated between 12% and 26%, and the hosts responsible for infected nymphs differed each year. On Martha’s Vineyard, shrews were the dominant host for ticks in 2019, feeding 57% overall. However, they fed only 18% of infected ticks. Instead, mice were the predominant reservoir host, having fed 41% of the infected nymphs, despite only having fed 15% overall. Shrews remained the dominant host throughout the study, but the prevalence of infection steadily dropped from 17% to 5%. We conclude that the enzootic cycle of B. burgdorferi is in constant flux; the species contributing Borrelia-infected ticks at a single site can vary greatly from year to year. Furthermore, the species that feeds the most ticks may not be the most important source of infection.

INTERSPECIES CO-FEEDING TRANSMISSION OF HEARTLAND VIRUS BETWEEN A NATIVE TICK SPECIES, AMBLYOMMA AMERICANUM, AND THE INVASIVE EAST ASIAN TICK, HAEMAPHYSALIS LONGICORNIS

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The Asian Longhorned Tick, Haemaphysalis longicornis, is an invasive species from East Asia that has recently established populations in 18 states in the U.S. and continues to expand its geographic range. The predominant reservoir host, having fed 41% of the infected nymphs, is dependent on localized skin infection. Using an in vivo tick transmission model, we tested our hypothesis by co-feeding HRTV-infected A. americanum nymphs with uninfected H. longicornis larvae or nymphs and screened the fed H. longicornis ticks for the presence of HRTV at different co-feeding proximities on the host. Using q-RT-PCR, HRTV RNA was detected in fed H. longicornis larvae and nymphs collected from multiple mice, providing evidence of interspecies co-feeding transmission of HRTV. Interestingly, this interspecies co-feeding transmission of HRTV occurs in the absence of host viremia; therefore, it is possible that a localized skin infection facilitates HRTV transmission between co-feeding ticks in the absence of host viremia. Experiments are underway to further examine the role of host skin in co-feeding transmission of HRTV.

SPATIOTEMPORAL EVOLUTION OF LYME DISEASE IN NORTH CAROLINA FROM 2010 TO 2020

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Lyme disease is the most common vector-borne disease in the United States with a majority of cases occurring in the Northeast and upper Midwest. While historically a low incidence state, North Carolina (NC) has seen substantial increases in reported Lyme disease cases over the past decade. The aim of this study was to characterize the spatiotemporal evolution of Lyme disease in NC from 2010 to 2020. Confirmed and
probable cases reported to the NC Division of Public Health were included in the analysis. Cases with a documented travel history to high or medium-transmission states were excluded. The study period was divided into four subperiods: 2010-2012, 2013-2015, 2016-2018, and 2019-2020. Data were aggregated by zip code of residence of the identified case. Absolute change in incidence per 100,000 from 2010-2012 to 2019-2020 was mapped to identify zip codes with the largest changes.

Global and local spatial autocorrelation analyses were performed to evaluate the overall distribution of cases and identify high incidence zip codes with high incidence neighboring zip codes (i.e., hotspot areas) for each period. Overall, we found the largest absolute changes in incidence, up to 464.7 cases per 100,000 people in zip codes located in Ashe County, in the northwestern part of the state and extending towards Buncombe County.

The distribution of cases became increasingly and significantly clustered over the study period, from a Moran’s I of 0.012 (p = 0.126) in 2010-2012 to a Moran’s I of 0.403 (p < 0.05) in 2019-2020. Hotspots included 22 high incidence zip codes in the 2019-2020 subperiod in the northwestern part of the state, largely co-located with areas experiencing the largest absolute changes in incidence. The results demonstrate the rapid emergence of Lyme disease in northwestern NC over the past decade. Incidence rates in many of the highest transmission zip codes now approach those reported in high-transmission states such as Maine and Rhode Island. Efforts targeted to these geographic areas are urgently needed to educate local residents, medical providers, and public health officials in order to prevent and ultimately reduce the burden of Lyme disease.
coupled with existing serosurveys, or leverage banked samples to rapidly characterize scrub typhus burden and generate scrub typhus seroconversion estimates that are comparable across populations, regions, and time.

5091

A CONTENT REVIEW OF COVID-19 RELATED APPS USED IN VIETNAM

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Various digital applications (apps) have been developed to combat the spread of COVID-19. In Vietnam, many COVID-19 apps have been launched by private developers or the Ministry of Health with overlapping features and purposes, which greatly confused Vietnamese residents. This problem has raised a concern about the usefulness and effectiveness of these apps during the pandemic in Vietnam. We aim to evaluate all current Vietnamese COVID-19 apps including features, purposes and functionality, advantages, disadvantages, and ethical issues of each app, to eventually provide reliable resources for the Vietnamese government to develop a unique national COVID-19 app. We conducted a systematic and manual search on 1st October 2022, on PubMed, Scopus, Google, and BBC News’s official website to identify all available COVID-19 apps in Vietnam. The relevant apps were discussed to reach a consensus and comply with a list of included apps for further assessment. Thirty Vietnam-based COVID-19 mobile apps were identified in the Apple and Google Play Store, which were evaluated through opinions on these stores, along with an analysis carried out by the research team members. These are duplicated features and functions among apps detected and some apps have lacked important functionality such as vaccination-related features. It is crucial to develop one complete app that fulfills the most useful features, addresses the common errors, and replaces all current apps to facilitate user experience.

5092

COMPREHENSIVE COST-EFFECTIVENESS ANALYSIS OF A NEW COMPARTMENTAL MODEL FOR BACTERIAL MENINGITIS CONSIDERING THE INFLUENCE OF THE MEDIA

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Mathematical epidemiology has paid some attention to the study of bacterial meningitis because of the severity of the disease and the way it spreads through a population. In this paper, we formulated a new compartmental model for meningitis to study the impact of media on reducing the severity of the disease in Ghana. We obtain the control reproduction number and the herd immunity rate in the presence of vaccination. We noticed that the meningitis death rate could be controlled with an increase in publicity of the dynamics of the spread of the disease and the ability to seek immediate medical treatment. We conduct a sensitivity analysis using Latin hypercube sampling. It is noticed that the transmission rate, vaccination, and the media have a negative nonlinear correlation. The effects of media, vaccination and treatment as a function of time are also investigated using an optimal control model. We display the efficiencies of the controls and demonstrate the mean and incremental cost-effectiveness ratios. The cost of controlling meningitis in the presence of media, vaccination, and treatment is discussed, and we hope this study will further educate the public on this topic.

5094

COST-EFFECTIVENESS ANALYSIS OF FOURTH GENERATION RAPID DIAGNOSTIC TESTING FOR HIV AMONG MEN WHO HAVE SEX WITH MEN IN NIGERIA

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Fourth-generation HIV rapid diagnostic tests for HIV include both HIV antibodies and p24 antigen, increasing the ability to detect acute HIV infections. The study compared the costs and health outcomes of using fourth-generation rapid diagnostic tests to third-generation (antibody-only) tests when screening men who have sex with men (MSM) from a health system perspective in Nigeria. A health economic cost-effectiveness model was developed to compare outcomes for different screening options. A decision-tree structure was used for evaluating the screening outcomes based upon the diagnostic accuracy and timing of the test. Health and costs associated with the different screening outcomes were modelled using a Markov-based framework to reflect clinical progression of HIV infection with early and late treatment. The population was modelled throughout a lifetime horizon, and captures costs, quality-adjusted life years (QALYs), disability-adjusted life years (DALYs), and transmission of new cases. Input parameters were derived from local sources where available and adapted from regional estimates where Nigeria-specific estimates were lacking. Given the absence of HTA guidelines in Nigeria, an international reference case for economic evaluation in low-middle income countries was used. Given an assumption that 0.7% of patients are pre-seroconversion at the point of testing, screening MSM who are unaware of HIV status yields an additional cost of US $2,260 per DALY averted, and US $802 per QALY gained, both of which are below Nigeria’s GDP per capita. The relative value of fourth-generation tests increases with a higher proportion of cases with asymptomatic and acute infections. The analysis shows that switching to fourth-generation tests may be an effective strategy for improving detection of new cases - especially those who are pre-seroconversion - and prevent further transmission of HIV at a feasible cost.

5096

DIGITIZATION OF THE NATIONAL LONG ACTING INSECTICIDE TREATED MOSQUITO NET MASS DISTRIBUTION CAMPAIGN IN GUINEA: PROCESS, CHALLENGES AND LESSONS LEARNED

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In Guinea, mass distribution of Long-Acting Insecticidal Nets (LLINs) is a key component of malaria prevention strategies and has contributed to a significant decrease in malaria prevalence since 2013. In 2022, the country implemented the first ever digitization of the fourth, tri-annual national LLIN distribution campaign to improve data quality and optimize the different stages of the campaign. The purpose of this study was to analyze the implementation process and document the challenges and lessons learned from this digitization. We conducted a descriptive study to document the planning and implementation of the campaign stages. A mobile application called “MILDA2” integrated with the District Health Information Software 2 (DHIS2) was used to enumerate households, scan QR codes for distribution and manage the flow stock of LLINs in real time. Campaign results, challenges encountered, and lessons learned are described in this work. A total of 17,160,359 individuals were enumerated (< 5.8% of the projection) among 2,746,044 households nationwide, which was 4% more than the
United States

use this poster session to present the tool, highlight the functionality, and countries with an active NTD programme in the WHO African region. We will data. Since launching 2021, CHIP as been scaled up to be available to all national programmes and partners to make informed decisions using these year, and elimination of PC-NTDs as a public health problem requires delivered to eliminate these NTDs, and the impact surveys conducted visualization tool, utilizing cloud hosting, web-APIs, and business Sightsavers, Covington, GA, United States

Alexandre Laurent Pavluck WHO ESPEN COUNTRY HEALTH INFORMATION PLATFORM

The Country Health Information Platform (CHIP) is a web-based data visualization tool, utilizing cloud hosting, web-APIs, and business intelligence software, that allows anyone to review key NTD data from official annual reporting forms submitted to the World Health Organization (WHO) and the International Trachoma Initiative (ITI). These annual forms are single year forms that report on endemicity for the five PC-NTDs, treatments delivered to eliminate these NTDs, and the impact surveys conducted to determine the future treatment strategy. Because the forms are single year, and elimination of PC-NTDs as a public health problem requires multiple years of intervention, CHIP presents these data overtime to assist national programmes and partners to make informed decisions using these data. Since launching 2021, CHIP as been scaled up to be available to all countries with an active NTD programme in the WHO African region. We will use this poster session to present the tool, highlight the functionality, and present the results from pre-test and post-test training surveys.

systematic review and meta-analysis on prevalence of oral submucous fibrosis

Savitha Satish

Johns Hopkins Bloomberg School of Public Health, South Windsor, CT, United States

Systematic Review and Meta analysis on prevalence of Oral Submucous Fibrosis. ABSTRACT Oral submucous fibrosis (OSF) is a potentially malignant condition, linked to the causative habit of chewing areca nut. Since the discovery of this condition in the 1960s, numerous epidemiological studies have been conducted to assess its prevalence. With the proliferation of commercially available areca nut products and its increasing popularity amongst youth, the prevalence has been on the rise. The present meta-analysis was initiated to collate and analyse the data on the prevalence of the condition in India and different parts of the world. Literature search from databases like Pubmed, PubGet, NLM, Ovid, Medline, Cochrane and Elsevier was conducted using specific keywords. The search period ranged from 1960 to 2022. After screening the studies based on inclusion and exclusion criteria, data on study designs, associated habits, prevalence rates, country of study were extracted. Meta-analysis, using random effects model was performed to obtain pooled prevalence rate. Additionally, subgroup analysis, funnel plot and sensitivity analysis were also performed. The pooled prevalence of OSF among the included 61 studies was 9% (95% CI:5% to 35%). Majority of the studies were conducted in Asia (95.1%) and 52 studies belonged to India. The prevalence of OSF varied from least being 0.03% to highest rate of 85.5% in the present study. The funnel plot and Egger's test indicated presence of publication bias. The present meta-analysis/review shows that reported prevalence rates of OSF in worldwide vary in wide degree dependent upon the region and sample size. The variation in a large part is also due to skewed-based biased study designs and sample selections. Thus, the need for a comprehensive epidemiological survey spread across many countries to assess the prevalence rate of OSF becomes even more necessary.

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among healthy populations ranging from 0% to 30% (median 11.5%). Reported incidence was found to be increasing in countries with established surveillance systems, with China reporting the highest incidence (527.7/100,000). The case fatality rate varies widely by region and time, with India reporting the highest case fatality rate (32.8%). Meteorology, landscape, and human activities were identified as key factors of disease acquisition. Boosted regression trees (BRT), ecological niche modelling (ENM) approach and Bayesian hierarchical models were employed to estimate risk. A substantial global burden of scrub typhus exists, with significant variations in occurrence and distribution across different regions and time periods. The meta-analysis will be continued, and we believe that this comprehensive systematic review will result in more attention for this neglected disease and help establish the research agenda for producing new generalizable information to bridge gaps in comprehension of the burden of scrub typhus.

5101

IS SUB-SAHARAN AFRICA READY FOR DIGITAL CLINICAL TRIALS?

Dawit Asmamaw Ejigu1, Eyasu Makonnen1, Thy Pham2, Brenda Okech3, Kristin Kristin Croucher4, Abebaw Fekadu1


Digital Clinical trials, also known as Decentralized Clinical Trials (DCTs), leverage digital technologies to facilitate recruitment/retention, data collection, and analyses and hence, increase the efficiency of clinical trials in developing interventions against various health problems. DCTs are gaining traction after their iterative implementations during the COVID-19 pandemic. Guidelines and recommendations for implementing DCTs in developed countries are in their formative stage, while applicable recommendations for developing countries are yet to be described. DCTs have several utility features, which make them very attractive for implementation in Sub-Saharan Africa (SSA). Opportunities that enable the implementation of DCTs in the continent, such as an increasing trend in innovative health technologies, are also flourishing in the continent. However, several potential challenges still require a workaround before implementing such trials in SSA. This paper highlights the potential opportunities and challenges surrounding the implementation of DCTs in SSA and puts forward suggestions on how to exploit the opportunities and address the challenges. The paper also highlights the survey’s findings on the perceptions of experts with experience conducting DCTs in SSA on the opportunities and challenges of implementing DCTs in the continent.

5102

SPATIO-TEMPORAL OCCURRENCE, BURDEN, RISK FACTORS AND MODELLING METHODS FOR ESTIMATING SCRUB TYPHUS BURDEN FROM GLOBAL TO SUBNATIONAL RESOLUTIONS: A SYSTEMATIC REVIEW

Qian Wang1, Tian Ma2, Fangyu Ding2, Kartika Saraswati3, Benn Sartorius1, Nicholas Philip John Day1, Richard James Maude1

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Scrub typhus, caused by Orientia tsutsugamushi, poses a significant public health threat, especially in the Asia-Pacific region. This systematic review aims to synthesize the spatio-temporal occurrence, burden, risk factors, and modelling methods for estimating scrub typhus burden across various geographical resolutions (PROSPERO #CRD42022315209). We searched PubMed, Scopus, Web of Science, China National Knowledge Infrastructure and other databases for articles published up to May 2022, with no language restrictions, for studies that quantified the occurrence, burden, risk factors, and modelling methods of scrub typhus. Study quality was assessed using a modified version of the Newcastle-Ottawa Scale. Our database search returned 13,272 articles, of which 2,109 were deemed eligible for full-text review. Ultimately, 640 articles were incorporated into our systematic review and meta-analysis, spanning 29 countries from 1957 to 2020. Overall, the global prevalence of scrub typhus varied significantly across different regions and time periods with seroprevalence

5103

MATHEMATICAL MODELS OF PLASMODIUM VIVAX MALARIA: A SYSTEMATIC REVIEW

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Plasmodium vivax causes significant morbidity and mortality worldwide. With an extensive geographic distribution and specific considerations for its diagnosis and treatment, P. vivax poses unique challenges for malaria control and elimination. Mathematical modelling is a useful tool to aid decision making. As the scientific community’s understanding of P. vivax and mixed infections continues to evolve and expand, there is an opportunity to review and learn from existing models to improve future model development and application. We conducted a systematic literature review of mathematical models of P. vivax malaria transmission. Our aim was to investigate the characteristics, key features, parameter values and evolution of these models; to explore common themes and identify knowledge gaps. We searched MEDLINE, Scopus, Web of Science, and Embase, for articles published in English until 8 August 2022. We included population- and individual-based mathematical models of P. vivax or mixed species models incorporating P. vivax. We excluded pharmacokinetic/pharmacodynamic models, models that had no human component, and models that were within-host only. Two reviewers independently conducted a title-abstract screen, followed by a full text screen. Disagreements between reviewers were resolved through the authors’ consensus. The systematic review yielded 1,923 results. After removing duplicates, we screened 842 abstracts. We screened 105 full text publications, of which 58 were included in the final analysis. We present summary characteristics of the included publications (e.g., geographic focus, species included, type of model, interventions). We identify and discuss key model features, exploring the similarities and differences between model structures and approaches. We show a map of the evolution and relationship between the models. We synthesise model parameter values, presenting the distribution and source of values. Finally we critically discuss gaps and challenges for modeling P. vivax and mixed species infections.

5104

STRATIFICATION OF MALARIA BURDEN AND SUBNATIONAL TAILORING OF INTERVENTIONS TOWARDS TO INFORM THE DEVELOPMENT OF THENATIONAL MALARIA ELIMINATION STRATEGIC PLAN IN GHANA

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As part of the pillars of HBHI - use of strategic information for decision making, stratification of malaria burden at subnational level is imperative.
to inform appropriate interventions to be deployed. In 2022, the name of the Ghana National Malaria Program was changed from Control to an Elimination Programme. This called for a new strategic plan to be developed to reflect the scope and strategies to be implemented under the elimination agenda. This abstract outlines the processes and methods used in developing risk stratification of malaria at district level and tailoring of interventions towards malaria elimination in Ghana. A subnational level of malaria burden was combined with prevalence estimates from Malaria Atlas Project (MAP) and All-cause mortality estimates from the Institute for Health Metrics and Evaluation to categorize the districts into very low, low, moderate, and high epidemiological strata. Appropriate interventions and strategies were developed for each strata and discussed with multi-sectoral stakeholders for buy-in and ownership. The plan is to whip up political commitment and ensure all sectors are involved in the further reduction of malaria in the country.

Molecular Biomarker Identification in Seasonal Cardiovascular Comorbid Diseases (SCCD) Using Network Metaanalysis

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The start of many illnesses is known to be significantly influenced by seasonal variations in the human cardiovascular system. Confounding variables include behavioral and environmental factors; failure to account for such factors makes determining the real temporal effect of certain disorders challenging. Numerous clinical investigations, on the other hand, suggest that only some groups of people are more seasonal sensitive, and that the maladaptation may contribute to a variety of disorders. As a result, evaluating the etiological and seasonal sensitive patterns of cardiovascular diseases (CVDs), which affect the majority of the human population, is crucial. The study’s premise was that cardiovascular and related disorders have significant links with seasonal and etiological fluctuations. Thus, in the current study, data mining was used to find 852 disease association connections between cardiovascular and related illnesses from a systematic review of 4519 papers. To focus on only the most prevalent CVDs, a disease ontology-based semantic similarity network (DSN) study was carried out. Furthermore, topological analysis was employed that predicted the seven CVDs in three clusters. The seasonal sensitivity and temporal association of these seven CVDs were then investigated using Mann-Kendall and Cox-Stuart models and their temporal connections were validated using LOESS and TBATS. The study provides indirect evidence of a significant etiological relationship between three cardiovascular diseases, including MI, atrial fibrillation, and atherosclerosis, all of which are seasonal in the majority of the world’s population. As a result, these three conditions qualify as seasonal cardiovascular comorbidities (SCCD). Following that, secondary network meta analysis using GEO data from GSE2244 (atrial fibrillation) and GSE132651 (atherosclerosis) reveals a triad of NRF1-hsa-miR-124-3p-NRF2 which is a physiologically and statically significant module, and both NRF-1 and NRF-2 might trigger a cascade that inhibits GSK-3 phosphorylation. This may minimize the risk of myocardial infarction while also improving heart pathology.

The Role of Beliefs in Malaria Prevention and Treatment Behavior: Analysis of the 2021 Nigeria Malaria Indicator Survey

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Malaria remains a major public health problem globally and especially in Sub-Saharan Africa. In 2021, Nigeria accounted for 26.6% of global cases and 38.4% of global deaths among children under the age of five. Underuse of effective tools for prevention and treatment undermines progress against the disease. For example, recent household surveys from Sub-Saharan Africa suggest that only 47% of the population slept under a net, and 33% of children under the age of five did not receive treatment for a fever. We analyzed the 2021 Nigeria Malaria Indicator Survey to examine how people’s knowledge and beliefs about malaria were associated with their prevention and treatment behaviors. The survey included 14,476 women respondents as well as data on 39,476 children who had a fever in the two weeks prior to the survey. Preliminary analyses suggest that when controlling for age, wealth quintile, education level, region and residence type (urban/rural), women who had heard a message about malaria in the previous six months had significantly higher odds of sleeping under a bed net (OR=1.21, 95% CI [1.07 1.35]), as did women who believed their families were susceptible to malaria (OR=1.30, 95% CI [1.05 1.60]), who believed malaria was a serious disease (OR=1.16, 95%CI [1.02 1.32]) and who perceived widespread community norms around malaria prevention and treatment (OR=1.42, 95% CI [1.23 1.63]). Hearing a message about malaria, perceived susceptibility to malaria, and beliefs about community norms were also significantly positively associated with children under the age of five getting treatment for a fever outside the home. Our results indicate that people’s beliefs play an important role in malaria prevention and treatment behavior. Moreover, the findings suggest that sharing information about malaria, particularly regarding the risk of infection and severity of disease, and highlighting the degree to which the community engages in malaria prevention and treatment behaviors, could be effective strategies to increase both use of bed nets and treatment of fevers.

Systematic Review: Mathematical Modelling Parameters of the Nine World Health Organization Priority Pathogens

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Mathematical modelling of infectious pathogens has been extensively used to understand endemic diseases and respond to outbreaks such as Ebola, measles and COVID-19. Modelling is a useful tool to predict infectious disease dynamics, project the likely future epidemic trajectory and estimate the potential impact of interventions to guide the public health response. In 2019, the World Health Organization updated its list of blueprint priority infectious pathogens that pose the greatest public health risk, due to their epidemic potential and/or lack of suitable interventions or therapeutics. We systematically reviewed parameter values, mathematical models and historical outbreaks for all listed pathogens, excluding disease X: Crimean-Congo haemorrhagic fever, Ebola virus disease, Lassa fever, Marburg virus disease, Middle East respiratory syndrome coronavirus, Nipah and henipavirus diseases, Rift Valley fever, Severe Acute Respiratory Syndrome and Zika virus. We searched OVID Medline and EMBASE and Web of Science until 8th March 2019 and retrieved 95,144 results. After title and abstract screening and full-text review, we found 1,684 papers for data extraction. We collate information required to enable rapid mathematical modelling of these nine pathogens including, but not limited to, fatality ratios, mutation rates, reproduction numbers, risk factors, model structure, sero prevalence and historical outbreaks. Our work highlights areas of uncertainty, especially for pathogens having not yet caused large human epidemics, where it is hard to quantify transmission or severity. By looking
at multiple pathogens simultaneously, we can archetype pathogens into profiles based on their characteristics. This will be useful in future epidemics of these pathogens, to rapidly identify existing parameters, models and outbreak data, enable mapping to these typologies for similar novel pathogens and identify potentially effective interventions. This work will serve as the basis for a dynamic database that could be further enriched by other contributors to ensure this review provides a "live" picture of the current knowledge landscape.

GEOSPATIAL MODELLING OF FEBRILE ILLNESS PREVALENCE AMONG CHILDREN AGED UNDER FIVE YEARS IN UGANDA

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Febrile illness is still one of the major public health problems in Africa. Although it has been recognised as crucial to better understand the spatial distribution, the evidence is still limited. We aim to predict the prevalence of fever cases among children aged under 5 years in Uganda, whilst exploring the association with potential risk factors. We analyse household records from the 2016 Demographic and Health Surveys (DHS) conducted in Uganda. We develop a geostatistical Binomial model to predict the prevalence of fever cases among children aged under 5 years, accounting for environmental, nutritional, and socio-demographic risk factors obtained from publicly available sources. The median crude (empirical) febrile illness prevalence for each cluster was 30.4% (interquartile range:13.6-50.0). There is extensive within-country spatial variation in children’s febrile illness prevalence in Uganda and the model indicates the presence of strong spatial correlation, predicting a higher prevalence in the eastern and north-eastern regions. The model indicates the association with some potential risk factors, and the inclusion of them improves the predictive performance of the model. The findings could assist in targeted public health policy-making for fever case management and in hypothesis generation for aetiology.

THE ROLE OF COMMUNITY HEALTH WORKERS IN TREATMENT MONITORING OF RADICAL CURE FOR PLASMODIUM VIVAX MALARIA IN PAPUA, INDONESIA: A MIXED METHODS STUDY

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Adherence to 14-day regimen of radical cure for Plasmodium vivax malaria is improved by supervision. In a highly malaria endemic area in Papua, Indonesia, clinic staff cannot supervise treatment through home visits due to the high number of cases. As part of strengthening malaria case management, a system was developed for clinic staff to refer patients to community health workers (CHWs) for treatment monitoring. The mechanisms and contexts that underlie the effectiveness of this intervention were assessed in a mixed methods study using realist evaluation. Between May 2019 and December 2022, patient referral to CHWs for treatment monitoring was introduced in five public health clinics. Through meetings with malaria program managers, the need to establish communication channels between clinic staff and CHWs was identified as essential for patient referral. CHW treatment monitoring practices were identified by calculating the proportion of referred patients who were monitored, participant observations at the clinic and patients’ homes during visits, and interviews with clinic staff and CHWs. CHWs can succeed in finding and visiting patients when they received complete patient information upon referral. Monthly meetings with clinic staff facilitated CHWs acceptance of treatment monitoring as their task. If the CHWs perceived good communication from clinic staff, there is greater incentive to find patients even without complete information. Identifying non-compliance, such as patients who reduced the number of tablets taken per day or stopped taking drugs, increased CHW confidence in treatment monitoring. When an ACT shortage led to prescription of second line drug (quinine), CHWs became more motivated to monitor treatment out of concern over patients not completing antimalarial treatment. The refined referral system was tested with new CHWs recruited later, confirming the importance of regular communication from clinic staff and complete patient information upon referral. In addition, perception of getting direct positive results (e.g. non-compliant patient becomes compliant) directly influenced CHW treatment monitoring practices.

COVID-19 VACCINATION IN GHANA: THE DISCOURSE OF RELIGION, GENDER, PERCEIVED SAFETY OF VACCINE AND GHANAIANS’ READINESS TO BE VACCINATED

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Covid-19 was declared a global pandemic in 2020 by the world health organization due to its global damage it caused to human life and the negative effect on the global economy. To deal with this pandemic was the global plan to vaccinate globally to achieve a herd immunity. The vaccine exercise was faced with some conspiracy theories ranging from vaccine safety and religious believes. This study examine vaccination in Ghana vis-a-vis the religious and safety paradigm. The cross-sectional survey randomly sampled 2000 participants but 1409 properly completed the questionnaire representing 70% response rate. The study reveals that participants willingness to be vaccinated and be part of the first group of people to be vaccinated was dependent on the religious affiliation of the participants (X2=23.9, p-value =0.02). The study further shows that the Ghanaian perception about the safety of the vaccine was also dependent on the religious belief of the participants (X2=25.9, p-value =0.001).The study however revealed that participants perception about the safety of covid-19 vaccine and the willingness to participates in the vaccination was independent on the gender of the respondent. The study finally revealed that participants willingness to be vaccinated was independent on the gender of the participant. The study concludes that religious belief significantly influenced participants willingness to be vaccinated. The study recommends to the government of Ghana and the Ministry of Health to be sensitive to the religious leaders in their drive to get the Ghanaian people vaccinated to obtain herd immunity.

PREVALENCE, RISK FACTORS AND CONSEQUENCES OF MICROCEPHALY IN LOW- AND MIDDLE-INCOME COUNTRIES: A CALL TO ACTION FOR THE GLOBAL MATERNAL AND CHILD HEALTH COMMUNITY

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The recent Zika Virus epidemic heightened awareness of the high prevalence of microcephaly in infants in low- and middle-income countries (LMICs). Despite being considered a very rare condition in high-income countries (HICs), the prevalence of microcephaly has been found to be up to 5% in LMICs, irrespective of the presence of Zika Virus circulation, indicating a long-running, unrecognized public health concern. However, microcephaly prevalence estimates in LMICs are still sparse and calculation of microcephaly is not standardized. Furthermore, while risk factors for microcephaly have been identified in HICs, the risk factors for microcephaly may be dramatically different in both type and prevalence in LMICs. The
limited available data suggest that generational malnutrition and related intrauterine growth restriction and low birth weight, frequent infections and malnutrition in infancy, and other poverty-related stressors that are much more common in LMICs may be associated with the high prevalence of microcephaly. Finally, emerging evidence demonstrating the association between microcephaly and poor subsequent neurodevelopment in LMICs highlights the serious consequence of high microcephaly prevalence. Therefore, more in-depth understanding of the prevalence, risk factors and consequences of microcephaly in LMICs is urgently needed. We review existing evidence to identify an appropriate definition of microcephaly for research implementation, and recommend that measurement of head circumference be routinely incorporated in clinical and public health practice in LMIC settings. We then describe the factors that have been associated with microcephaly in LMICs and review the evidence on the association between microcephaly and poor neurodevelopment in LMICs. To encourage and guide advancement of this field, we provide a set of recommendations to improve collection, analysis, and interpretation of the risk factors and consequences of microcephaly in LMICs.

5112

ASSESSMENT OF DIETARY HABITS AND IODINE STATUS AMONG PREGNANT WOMEN IN SOUTHERN GHANA

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Iodine is a micronutrient essential in the production of thyroid hormones for normal neurodevelopment. Based on iodine nutrition global data, nearly two billion (28%) of the world’s population, including more than 321 million Africans (39%), are in danger of inadequate iodine intake. The WHO estimates that 60% of pregnant women worldwide do not meet the required intake. Current studies have associated iodine deficiency during pregnancy with a wide range of disorders; stillbirth, spontaneous abortions, hearing defects in infants, and cretinism. The aim of the study was to determine the iodine level and dietary habits in pregnant women. This cross-sectional study was performed among women attending antenatal clinics at Pentecost Hospital, Madina (PMH) in the Greater Accra Region. Dietary information related to iodine was obtained by using a food frequency questionnaire (FFQ). Urine iodine concentration (UIC) was performed on freshly collected urine samples, using the Sandell-Kolthoff reaction method with ammonium persulfate as the digesting agent. Results obtained showed that 50.6% (80/158) of participants had urine iodine levels below the WHO optimum range. Week of gestation had a positive association with the iodine levels in pregnant women. Regarding dietary habits, oats, yogurt, salted fish, and meat intake were significantly associated with urine iodine levels (r ranging from 0.1 - 0.2, p<0.05 in all cases). The study highlights the need for greater advocacy for pregnant women to take in iodine-rich sources of food in order to avoid possible iodine deficiency disorders in themselves and their unborn children.

5113

EFFECT OF PARTICIPANTS AGE AND OCCUPATION ON PERCEIVED SAFETY OF COVID-19 VACCINE AND PARTICIPANTS WILLINGNESS TO BE VACCINATED WITH COVID-19 VACCINE IN GHANA

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The COVID-19 pandemic, declared a global health emergency by the World Health Organization in 2020, had a severe impact on the Ghanaian economy and caused harm to human life globally. This study aimed to provide evidence on the role of age and occupation in determining perceived safety and safety-related information regarding COVID-19 vaccines. The study examined the effect of age on participants’ perceived safety of the COVID-19 vaccine, as well as the effect of occupation on their perceived safety and safety-related information. A cross-sectional survey was conducted, randomly sampling 2,000 participants, representing a 70% response rate. The study’s results showed that the perceived safety of the COVID-19 vaccine was independent of the participants’ occupation (X2=18.14, p-value =0.059). Additionally, the participants’ willingness to be vaccinated was independent of their occupation (X2=1.86, p-value =0.86). Finally, the study found that participants willingness to be vaccinated was independent on the age categorization of the participants who participated in the survey. The study concluded that participants perception of the covid-19 vaccine safety was not influenced by the type of occupation and the age categorization of the participants.

5114

EVALUATION OF THE CLINICAL TRIAL OPERATION TRAINING CONDUCTED BY CENTER FOR INNOVATIVE DRUG DEVELOPMENT AND CLINICAL TRIALS FOR AFRICA

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The number of clinical trials conducted in low and middle income countries (LMICs) is not satisfactory. Their quality is also questionable. This might be attributed to shortage of qualified personnel. Competency-based training for trial staff is required to build their capability. To this effect, CDT-Africa has conducted clinical trial operation (ClinOps) training in collaboration with course partners. As alignment of course contents to the local priorities of LMICs is critical for the success of clinical trial training, local tutors who were cognizant of the most common problems faced while conducting clinical trials in LMICs were involved. The training program activities were jointly planned with product development partners. A competency framework with andragogic innovations, jointly set by the Tropical Diseases Research program and The Global Health Network, was used in developing the curriculum. The training used Moodle Learning Management System as its platform for uploading VoiceThread links, learning materials, tasks, forum discussions and other assignments for access to trainees. This paper addresses impact of the training on trainees’ confidence in conducting GCP compliant trials. We carried out a survey and used four core competencies: trainees’ confidence in i) conducting, ii) managing, iii) designing & maintaining quality system and iv) effectively closing out & reporting results of clinical trials to evaluate the training outcome. Data taken from 69 trainees who took part in both the pre and post training survey were analyzed. Most of the respondents were trial site coordinators. Repeated measure t-test was carried out on four pair of scores in the four core competencies to see the presence of statistically significant difference in the scores of the respondents in confidence measured at the two different times. The test indicated that their confidence in all the four core competencies significantly increased after training compared to what they had before. In conclusion, the ClinOps training helped trainees acquire relevant knowledge & develop confidence in conducting GCP compliant clinical trials.

5115

THE RELATIONSHIP BETWEEN DISTANCE TO PRIMARY HEALTH CENTER, CHILD MORTALITY, AND AZITHROMYCIN MASS DISTRIBUTION IN NIGER: A SUBGROUP ANALYSIS OF THE MORDOR I CLUSTER-RANDOMIZED TRIAL

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Increased distance to health centers has been associated with increased child mortality in several West African settings. Azithromycin mass drug delivery outside health centers is an important element of the intervention in the MORDOR trial. In this paper, we aim to assess the relationship between distance to primary health centers, child mortality and azithromycin mass distribution in Niger using an analysis of a subgroup from the MORDOR I cluster-randomized trial.
administration (MDA) has been found to reduce child mortality. Identifying subgroups where mortality reductions are the largest could help target future programs. Our objective was to assess whether distance to primary health center modified the effectiveness of azithromycin MDA to reduce mortality in a subgroup analysis of the MORDOR I-Niger trial. This cluster-randomized trial enrolled 594 rural and peri-urban communities in Dosso and randomized them to biannual oral azithromycin or placebo to children 1-59 months old for 2 years. A population-based census was conducted every 6 months to collect location data, administer treatment, and collect data on mortality and person-time at risk in the target group. Distance from each community center to the nearest primary health center was calculated. Negative binomial regression was used to determine whether the community-level effect of azithromycin on mortality differed by distance to primary health center, controlling for average community age. Median distance from community center to the nearest primary health center was 5 kilometers (km; interquartile range 3.2 to 7.1). For each kilometer increase in distance in the placebo arm, mortality increased by 5% (adjusted incidence rate ratio [aIRR] 1.05, 95% CI 1.03 to 1.07, P-value < 0.001). The effect of azithromycin MDA on mortality was found to vary significantly by distance (P-value for interaction term = 0.02). Overall, we found no difference in mortality by arm in communities closest to primary health centers and an increasing reduction in mortality in the azithromycin arm compared to placebo as distance increased. For example, the reduction in mortality with azithromycin vs placebo was 0% at 0 km from the health center (95% CI -19% to 17%), 4% at 1 km (95% CI -12% to 17%), 16% at 5 km (95 CI 7% to 23%), 28% at 10 km (95% CI 17% to 38%), and 39% at 15 km (20% to 54%). Children who live farthest from existing healthcare facilities may benefit the most from azithromycin MDA.

**5116**

**SPLILLOVER EFFECT OF AZITHROMYCIN MASS DRUG ADMINISTRATION ON ANTIMICROBIAL RESISTANCE IN NIGER**

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MORDOR was a cluster-randomized mass drug administration (MDA) trial that compared child mortality in communities receiving two years of biannual azithromycin versus placebo. Azithromycin MDA has been shown to increase antimicrobial resistance (AMR) in the target 1-59-month age group. Given the community-based nature of this intervention, AMR may spill over into non-target populations as well. To better characterize the full population-level impact of azithromycin MDA on AMR, this study aimed to evaluate the prevalence of AMR spillsovers in children 7-12 years of age not targeted for MDA. Thirty communities in the MORDOR morbidity trial were randomized to biannual azithromycin or placebo to children 1-59 months old. After 2 years of distributions, nasopharyngeal swabs were collected from a random sample of up to 40 children 7-12 years of age from all communities. Genetic determinants of resistance to macrolides, beta-lactams, tetracyclines, and fluoroquinolones were assessed at the individual level using TaqMan Array card. The presence of resistance determinants for each class was compared by arm using generalized estimating equations to account for clustering by community. Nasopharyngeal swabs were collected from a total of 1,103 children 7-12 years old. Compared to placebo communities, those in azithromycin communities had 1.34 times the odds of macrolide resistance (95% CI: 0.82 - 2.20), 1.06 times the odds of beta-lactam resistance (95% CI: 0.71 - 1.57), 0.99 times the odds of tetracycline resistance (95% CI: 0.45 - 2.20), and 0.45 times the odds of fluoroquinolone resistance (95% CI: 0.20 - 1.01). These findings suggest that there may be an increase in macrolide resistance in untreated children in communities receiving azithromycin versus placebo, although the MORDOR trial was not powered to detect this difference. More research is warranted to further our understanding of such spillover effects as a result of azithromycin distribution in MDA programs.

**5117**

**TAKING BLOOD FROM CHILDREN FOR RESEARCH PURPOSES - WHAT DO PEOPLE THINK ABOUT IT? A QUALITATIVE STUDY TO EXPLORE THE FACILITATORS AND BARRIERS FROM A CLINICAL TRIAL CONDUCTED IN LALITPUR, NEPAL**

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Clinical trials are a critical part of evidence-based medicine and blood sample collection is its important component. However, refusal of consent for blood draw is a common challenge associated with it. Literature exploring the perceptions of people regarding blood draw is scarce. We are conducting a qualitative research as a part of a large randomised controlled trial for a typhoid conjugate vaccine in children from Nepal. For the qualitative aspect of the trial, we are seeking to identify the motivators and barriers for blood draw from children for clinical research purposes. We are conducting in-depth interviews (IDIs) with the parents of children less than 18 years and the participants of the vaccine study who are now 18 years or older. There are around 17 study clinics in the community and a tertiary hospital where people with fever can come for check-up and are asked for blood draw if they meet the study fever criteria. So far, 16 in-depth interviews (IDIs) have been conducted with the participants who have consented and denied for blood draw for research purposes. IDI will be conducted until data saturation. We found that most of the participants did not understand the purpose of drawing blood for research purposes and their major motivation to provide consent was the trust towards the health professionals. The perception that it will be painful to draw blood from a sick child, making them weaker, is the major factor for blood draw denial. We also found that people had no problem in drawing out blood from sick adults but they felt the volume of blood drawn from a sick child was excessive, hence their hesitation. We have not heard any social belief related to blood draw so far that could lead towards denial of blood draw. To facilitate research that entails blood draw, health professionals and especially medical officers or nurses should continue to explain in detail about the purpose of blood draw for research purposes. Pain relief ointment can also be considered prior to blood draw. Additionally, engagement activities can be conducted to address the concern of volume and health consequences of blood draw thus improving consent procedures in future studies.

**5118**

**EPIDEMIOLOGY OF LEPROSY IDENTIFIED THROUGH ACTIVE CASE DETECTION IN SIX DISTRICTS OF NEPAL**

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Pediatric cases and grade-2 disabilities (G2D) indicate recent transmission and late diagnosis respectively, which necessitate active and early case detection. This operational research was performed to identify approaches best suited for early case detection, determine community-based leprosy epidemiology, and identify hidden leprosy cases early and respond with prompt treatment. Active case detection was performed by: door-to-door visits among vulnerable populations (n=26,469), contact examination and tracing (n=7,608) and screening prison populations (n=4,428) in Siraha, Bardiya, Rautahat, Banke, Lalitpur and Kathmandu districts of Nepal. New case detection rates were highest for contact tracing (250), followed by house-to-house visits (102) and prison screening (45) per 100,000 population screened. However, cost per case identified was cheapest for house-to-house visits (Nepalese rupee (NPR) 76,500/case), then contact tracing (NPR90,286/case) and prison screening (NPR298,300/case).
(PB:MB) ratios were 59:41 and 68:32; female/male ratios 63:37 and 57:43; pediatric cases 11% in both approaches; and G2D 11% and 5% respectively. Developing leprosy was similar among household and neighbor contacts (Odds ratios (OR)=1.4, 95% confidence interval (CI), 0.24-5.85) and for contacts of MB versus PB cases (OR=0.7, 0.26-2.0). Attack rates were similar among household contacts of MB cases (0.32%, 0.07-0.94%) and PB cases (0.13%, 0.03-0.73) and neighbor contacts of MB cases (0.23%, 0.1-0.46) and PB cases (0.48%, 0.19-0.98). BCG vaccination with scar presence had a significant protective effect against leprosy (OR=0.42, 0.22-0.81). In conclusion, the most effective case identification approach here is contact tracing, followed by house-to-house visits in vulnerable populations and screening in prisons, though house-to-house visits were cheaper. The findings suggest hidden cases, recent transmission, and late diagnosis in the community exist and highlight the importance of early case detection.

5119
ADDRESSING PROVIDERS’ DISTRUST OF MALARIA RAPID DIAGNOSTIC TESTING THROUGH PEER-TO-PEER ENGAGEMENT
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In Nigeria, 40% of public sector providers prescribe antimalarials without parasitological confirmation. Studies show that provider norms and misconceptions influence adherence to fever case management (FCM) guidelines. From January 2019 to December 2022, Breakthrough ACTION-Nigeria convened quarterly small group discussions with Officers-in-Charge (OICs) from 417 primary health facilities in 5 states to discuss diagnostic norms and misconceptions, exchange experiences, review data, and develop action plans to improve FCM. Selected facilities are designated by the government to provide a minimum package of services within each ward. Before each session, 417 OICs completed self-administered surveys about their own knowledge and attitudes toward FCM. After sessions, OICs conducted similar “step-down” meetings with 712 facility staff. District Health Information System (DHIS) data was analyzed to examine trends in testing and adherence. The testing rate rose from 85% to 94% between Dec 2018 and Dec 2022. The proportion of test-positive cases who received artemisinin-combination therapy (ACT) increased from 82% to over 98%. While the DHIS does not capture test-negative results, clinical diagnosis (proportion of fever cases receiving ACTs without a malaria test) decreased from 20% to 0.8%. Though the number of fever cases seen decreased during the pandemic, testing and adherence rates did not. Average OIC knowledge and attitude scores rose from 44% in 2019 to 80% in 2022, and during meetings, OICs reported improved norms and attitudes toward malaria rapid diagnostic tests (RDTs) among staff. Scores improved after one session, but meeting discussions reflected lingering doubts such as concerns about RDT storage conditions prior to arrival at the facility that diminished with time. Results suggest that a sustained, peer learning approach may address entrenched concerns and contribute to improved FCM adherence. Future research should track attitudinal shifts among facility staff in addition to OICs, use control sites, and compare the peer-to-peer approach to other quality improvement initiatives.

5120
LIVED EXPERIENCES AND COPING STRATEGIES ADOPTED BY ADOLESCENTS IN THE MANAGEMENT OF ONCHOCERCIASIS IN A RESOURCE LIMITED SETTING OF GHANA
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Onchocerciasis is a neglected tropical disease that continue to be a major contributor to disabling skin and eye conditions. Sub-Saharan Africa accounts for 99% of the total global prevalence of the disease, with about 37 million people infected and about 300,000 permanently blind. This is a cross-sectional study adopting a phenomenological qualitative approach. We conducted in-depth interviews among 16 onchocerciasis adolescent patients between June 2022 – July 2022 in order to explore the lived experiences and coping approaches adopted by adolescents in the management of onchocerciasis. Data was analysed using Interpretative Phenomenological Analysis (IPA) with ATLAS.ti version 7.5.7, and results presented in themes and supported with quotes. Half (50.0%) of the adolescents interviewed were aged 15-17 years old; majority (62%) of them were still in school. Also, 25.0% of them had lived with the condition for over 10 years. The major initial symptoms experienced by adolescents were blurred vision, ball-like moving nodules in the body, and frequent fatigue. Majority of the participants reported that their experience with hospital-based management of their condition in terms of quality of treatment and care they received from the health professionals. Majority of participants preferred hospital management as compared to home-based treatment. The main coping strategies adopted by participants in dealing with their conditions were religious in nature expressed through faith and belief in God by prayer; and recreation by playing football and watching movies. The findings revealed that most participants delayed in seeking healthcare, and only did so after complications had developed. The delay we believe could be due to a lack of access to appropriate information and education about onchocerciasis. Religion and recreation were the main strategies adopted by participants in coping with their conditions. This points to the need for health authorities and policymakers to intensify education and communication campaigns on NTDs in general and onchocerciasis in particular in the last miles settings of developing countries like Ghana.

5121
SPATIAL INEQUALITY IN CHILDHOOD IMMUNIZATION COVERAGE IN NIGERIA: A GEOSTATISTICAL APPROACH
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Routine vaccination for children and mass immunization campaigns are key global public health strategies for reducing morbidity and mortality from infectious diseases. The Expanded Program on Immunization (EPI) aimed at providing routine immunization to all children less than two years of age in Nigeria. Though initial progress towards attaining universal coverage was documented, structural and organizational challenges soon constitute barriers to the ambitious targets of the EPI strategy. Estimates from the 2018 Nigeria Demographic and Health Survey (NDHS) indicate that only 37% of children aged 12-23 months received all basic vaccinations while 21% received all age-appropriate vaccinations. Combining data from 2008, 2013, and 2018 NDHS with ancillary data sources, we adopt a model-based geostatistics approach to characterize and quantify spatial variations in full immunization coverage (defined as children aged 12-23 months who have received one dose of BCG vaccine, three doses of DPT-containing vaccine, three doses of oral polio vaccine, and one dose of measles vaccine) as explained by factors such as women disempowerment, intimate partner violence (IPV), and other child’s and mother’s characteristics. We also considered the influence of travel time to nearest health facility, fear over personal safety, and local population size on the spatial patterns of immunization coverage. Women disempowerment, IPV and travel time...
are among the barriers to uptake of childhood vaccines, exacting varying influence at different locations in Nigeria. Locally tailored intervention would be effective in enhancing coverage in the country.

5122

UNDERSTANDING COVID-19 VACCINE HESITANCY IN THE JOHNSONVILLE, PEPPER WULU TOWN COMMUNITY: A QUALITATIVE STUDY

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The COVID-19 pandemic has had widespread morbidity and mortality, with nearly 6.2 million deaths globally. In Liberia, fewer cases and deaths have been reported than in other countries, but the limited healthcare resources in this low-income country makes the population vulnerable, in the event of more virulent variants. The COVID-19 is no exception. To protect against the COVID-19 virus, vaccines are being distributed; yet coverage targets have not been met. The objectives of this research were to understand COVID-19 vaccine hesitancy among people in the Johnsonville Pepper Wulu Town community, and to gather community feedback on what can be done to encourage the uptake of COVID-19 vaccines. A qualitative study was conducted with purposive sampling identifying 12 community members who were expected to have knowledge and understanding about the COVID-19 vaccine. All participants were aged 18 years or older and had resided in the community for not less than six (6) months. Audio recordings of in-depth interviews were transcribed and manually coded; thematic analysis was undertaken. From the study conducted, three themes were generated to group participants’ statements into categories which better explained what they know about the COVID-19 vaccine. Out of the 12 participants interviewed, 10 said they have not gotten any dose of the COVID-19 vaccine and were not willing to take the vaccine due to reasons like fear to die in two years after taking the vaccine and lack of trust in Government. Eight indicated that they are not prepared to recommend the vaccine to their children, friends or relatives. The results of the study showed that COVID-19 vaccine hesitancy exists in the community and it comes from issues such as misinformation, lack of information, and/ or mistrust. Mobilization through prominent individuals and Community based-Organizations along with building emotional support, and increased knowledge to overcome misinformation would help to alleviate the myths of death, and liaising with stakeholders to address concerns around trust could help improve uptake.

5123

AN ETHNOBOTANICAL STUDY ON MEDICINAL PLANTS USED AS ANTIDOTES FOR SNAKEBITE AND AS SNAKE REPELLENTS IN THE HAUTS BASINS AND SOUTHWEST REGIONS OF BURKINA FASO

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Ophidian envenomation is a public health problem in the tropics and subtropics. Expensive cost of antivenoms forces most of the population to resort to medicinal plants as a first-line treatment. The use of plant extracts for therapeutic purposes is a common practice in traditional African medicine. The present study aimed to contribute to a better knowledge of medicinal plants used in the treatment of snakebite envenomations and as a snake repellant in the Hauts-Bassins and Southwest regions of Burkina Faso. In the province of Houet and the South West region, ethnobotanical information was collected during six months of the year 2022 by 117 people (traditional health practitioners and herbalists) using a questionnaire. Knowledge was assessed quantitatively using relative citation frequency. A total of 31 plant families divided into 58 species have been identified by both traditional practitioners and herbalists. The distribution of these species by family showed that Polygalaceae (28.2%), Annonaceae (14.52%), Fabaceae (13.67%) followed by Ebenaceae (3.41%) and Apocynaceae (3.41%) were the most mentioned. Roots were mostly used, 68.96% (40/58) in the preparation of remedies. The majority of traditional healers were male (84.61%, 99/117). More than 80% (94/117) of respondents were not literate. Almost all of therapeuticians, 90% (105/117), had knowledge from their ancestry. The surveys made it possible to inventory a diversity of medicinal species and to collect as much information as possible concerning local therapeutic or repellent uses against snakes. Extensive pharmacological and toxicological studies need to be conducted for the reported medicinal plants to contribute to the well-being of local communities in tropical and subtropical regions.

5124

MIXED EFFECTS MODELS IN THE ANALYSIS OF EPSTEIN BARR VIRUS SEROLOGICAL RESPONSES IN CHILDREN FROM CHULAIMBO WESTERN KENYA

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In malaria-endemic regions, infants acquire Epstein Barr virus (EBV) as early as 6-months of age, resulting in poor viral control, which may contribute to endemic Burkitt’s lymphoma development. Maternal malaria infection during pregnancy impairs vertical transfer of EBV specific antibodies. The impact of this impairment on the subsequent decay rate of maternally derived anti-EBV specific antibodies in infants remains to be determined. There exist two preferred classes of mixed effects models for analysis of longitudinally collected data; linear (LMMs) and nonlinear NLMMs mixed effects models. Whether LMMs perform sufficiently in scenarios suitable for NLMMs remains unclear. A total of 66 infants from Western Kenya, born to HIV-negative women, with or without malaria during pregnancy were enrolled and followed up from birth through 24 weeks of age. We assessed the performance of mixed effects models in the analysis of EBV serological responses comparing infants born to women with and without malaria infection during pregnancy (MEU and MUU respectively). The levels of anti-VCA and anti-EBNA1 IgG against EBV infection in cord (neonatal) and infant blood samples were quantified using multiplex bead-based assay and analyzed using LMM and NLMM. To evaluate model performance, Pearson correlation test was used to analyze observed versus predicted antibody levels. Decay rates of anti-VCA IgG between MEU and MUU did not differ significantly (P = 0.45). However, the decay rates of anti-EBNA1 IgG were significantly faster in the MUU group compared to MEU (P = 0.03) with a decrease rate of 0.26 and 0.14 per month, respectively. Correlations between observed versus predicted VCA and EBNA1 IgG levels demonstrated the competent performance of LMM in fitting both VCA and EBNA1 IgG responses (r: 0.89 and 0.71, respectively) compared to NLMM (r: 0.95 and 0.89). These findings suggest that in-uterine malaria exposure does not impact decay rates of maternally derived anti-VCA IgG and EBNA1 IgG. Moreover, both LMM and NLMM performed well with NLMM being slightly more robust in the analysis of EBV serological responses in infants over time.
MOLECULAR CHARACTERIZATION OF THE RHESUS D (RHD) GENE IN BLOOD DONORS WITH THE DEL PHENOTYPE AT THE NATIONAL BLOOD TRANSFUSION CENTER (CNTS) OF BAMAKO, MALI

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The Rh Del phenotype, expressed very weakly on the surface of red blood cells, can only be detected by adsorption/elution. The RhD1227 allele is the most common Rh Del with a grossly intact RhD gene. In Mali, there is very little information on the prevalence of the Del phenotype. The objective of this study was to perform a molecular characterization of the Del phenotype in blood donors. After confirmation of Rh Del by adsorption/elution on donor blood bags at the CNTS, DNA from the samples was isolated with the QiAamp blood DNA mini kit and amplified by PCR using primers specific to the Rhd gene and the RhD1227A allele. Amplicons were visualized on a 1% agarose gel under UV light. 365 serologically negative RhD donors were included in this study. The majority was male with 90.4%. The age range [26-39] represented 52.9% and the mean age was 32.5±33.53 years. The Cee phenotype accounted for 72.72%. Blood group O was the most represented with 38.6%. The Rh Del phenotype was positive in 7.1% (26/265). All ten exons of the RhD gene were amplified in 69.23% of the Rh Del positives and the RhD1227A allele was present in the samples. Our study showed the presence of the intact Rh D gene in all samples with the presence of the Rh Del mutation. However, we observed other non-specific amplifiers that would be interesting to characterize by sequencing.

ACADEMIC ACHIEVEMENT AMONG CHILDREN WITH SICKLE CELL ANAEMIA IN UGANDA

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Academic achievement among children of school age is crucial in attainment of optimal learning goals. Children with Sickle Cell Anaemia (SCA) are prone to cognitive deterioration which might impact their academic achievement. Limited data exists on the academic achievement of school-going children with SCA in Uganda. We conducted a cross-sectional study among children with SCA aged 6-12 years attending the Sickle Cell Clinic at Mulago National Referral Hospital in 2019. Controls were siblings/immediate relatives without SCA. The Wide Range Achievement Test-4 (WRAT-4), was used to measure cross-sectional spelling, reading, math and sentence comprehension. Age-appropriate items were administered for each age group. The WRAT-4 raw scores were converted to z-scores based on age and gender. T-tests were used to compare the z-scores of the controls with the cases. Linear regressions adjusting for social economic status, home stimulation and environment were used to compare the association between SCA and academic achievement. A p-value <0.05 was considered significant. A total of 68 cases (30 females, 44.1%) and 69 controls (37 females, 53.6%) were enrolled. Mean age was 9.0±2 years for both cases and controls. Overall, cases performed poorer than controls (Mean difference: -0.25, 95% CI: -0.02-0.53, p-value=0.03). Results for each subtest demonstrated that cases performed poorer than controls in math, with z-scores of -0.36 vs. 0.02 (p-value= 0.002) and spelling, with z-scores -0.31 vs. 0.03 (p-value=0.02). Cases and controls did not significantly differ in reading, with both groups scoring below the test’s mean z-score. In contrast, in sentence comprehension, both cases and controls scored similarly and performed above the mean z-score. No significant differences were found between cases and controls for mother’s education, social economic status or home environment. Children with SCA in Uganda have poorer performance in math and spelling compared to their healthy siblings/peers. Additional education support in maths and spelling may be considered to reduce these differences in academic achievement.
A fundamental lesson from the COVID-19 pandemic is that dissemination of reliable, evidence-based information is essential to confront novel pathogens by enabling robust and reassuring decision-making. The sudden emergence of a vast number of diagnostic tests, without clear guidance on quality, use cases, or routes to procurement, contributed to global instability and panic. FIND established an open-access, centralized directory of COVID-19 diagnostics to track availability, regulatory status and performance of molecular and rapid tests (antibody and antigen). Today, the COVID-19 test directory contains data on 2124 tests, to support countries and other stakeholders with ongoing pandemic response and containment strategies, as well as to bring transparency to the R&D landscape. Test data are gathered via various workstreams, including proactive scouting, review of scientific publications and regulatory databases, and outreach to manufacturers to request direct submission of test data via online forms. Variables collected include product/technology features, performance characteristics, and regulatory status. Interactive dashboards and curated tables are publicly available and updated in real time on the FIND website. In response to positive feedback from stakeholders, new directories were quickly set up in response to the mpox and ebolavirus outbreaks of 2022, which currently contain 119 and 70 tests, respectively. Further, a test directory dedicated to neglected tropical diseases has been set up to bring transparency to the diagnostic pipeline and maximize the impact of scarce resources in that area. Looking to the future, the test directories are being harmonized and consolidated into one single platform, which will facilitate further expansion of test information on other diseases of outbreak potential, vaccine-preventable diseases, tuberculosis, and antimicrobial drug-resistance. By supplying a bird’s eye view of the diagnostic landscape, this suite of test directories provides actionable insights that can improve access to diagnostics and global health.

In post conflict Liberia, psycho-social problems are those that affect the mental psyche of the individual and therefore affects an individual productive capacity. Girls in secondary schools especially in developing countries endure the burden of psycho-social problems caused by parents, school and other stakeholders with ongoing pandemic response and containment strategies, as well as to bring transparency to the R&D landscape. Test data are gathered via various workstreams, including proactive scouting, review of scientific publications and regulatory databases, and outreach to manufacturers to request direct submission of test data via online forms. Variables collected include product/technology features, performance characteristics, and regulatory status. Interactive dashboards and curated tables are publicly available and updated in real time on the FIND website. In response to positive feedback from stakeholders, new directories were quickly set up in response to the mpox and ebolavirus outbreaks of 2022, which currently contain 119 and 70 tests, respectively. Further, a test directory dedicated to neglected tropical diseases has been set up to bring transparency to the diagnostic pipeline and maximize the impact of scarce resources in that area. Looking to the future, the test directories are being harmonized and consolidated into one single platform, which will facilitate further expansion of test information on other diseases of outbreak potential, vaccine-preventable diseases, tuberculosis, and antimicrobial drug-resistance. By supplying a bird’s eye view of the diagnostic landscape, this suite of test directories provides actionable insights that can improve access to diagnostics and global health.

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size of 400 and multistage sampling technique for selection of respondents. A semi-structured questionnaire was used to obtain information on socio-demographic characteristics, knowledge and attitude on vaccine and cold chain management, and practices of healthcare workers on vaccine stock management. Data was analyzed using the Statistical Package for Social Sciences software version 23. Level of significance set at p<0.05. A total of 396 respondents participated in the study with a response rate of 97%. Respondents from equipped and non-equipped health facilities had mean age of 36.8 years ± 8.7 standard deviation and 35.8 years ± 7.1 standard deviation respectively. Two hundred and sixty-five (71.6%) and 105 (28.4%) worked at primary health care centres (p<0.05). Increased knowledge (p>0.05), years of working experience (p<0.05), positive attitude (p>0.05), and good practices (p<0.05) of vaccines and cold chain management was observed among respondents from equipped health facilities as compared to those from non-equipped health facilities. Barriers to effective vaccine handling include insufficient ice packs and cost of transporting vaccines to the health facility. Drivers of effective vaccine stock management are length of years working in health facilities, good knowledge, and practices on vaccine stock management as evident among healthcare workers from equipped health facilities. The findings from this study should be used to improve effective vaccine stock management at the state, LGA, and health facility levels.

5132

THE PREVALENCE OF UNDIAGNOSED HYPERTENSION AMONG RESIDENTS OF THE DUPORT ROAD COMMUNITY

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Hypertension is an important public health and medical issue worldwide. It is the largest contributor to global burden of disease, accounting for 7% of global disability adjusted life years. Liberia is a low-income country and as such faces many challenges especially in the health sector. Most time people who visit the hospital for the first time poses serious complication of hypertension thereby placing extra burden on the already challenged healthcare services. Undiagnosed hypertension is a risk factor for mortality and morbidity among Liberians since there is no presenting symptoms, furthermore the idea is regular screening and hospital checkup is not common in Liberia. The study seeks to identify the proportion of older population living with undiagnosed hypertension. A cross sectional study conducted in the Duport Road Community in 2019 with 167 participants’ age 30 years and above who has never been diagnosed with hypertension selected for the study. Participants answered a questionnaire through direct interviews and their blood pressures were measured using automated digital blood pressure machine. Individuals who had systolic blood pressure above 139mmHg and or diastolic blood pressure above 89mmHg had two subsequent measurement of their blood pressure. The results of the study revealed that 29% (n = 49) of the participants had Hypertension (systolic blood pressure >139 mmHg and or Diastolic blood pressure of >89mmHg). Furthermore, 70% ( n=118) of the study population had not had a blood pressure check in the preceding 5 years or could not remember the last time they check their blood pressure. Out of the 49 participants who were hypertensive, 65% (n = 32) were 50 year old and above. Hypertension is significantly associated with increasing age, while the lack of regular screening regularly health care visit has attributed to the high prevalence of undiagnosed hypertension among the inhabitants of the Duport Road Community.

5133

INTEGRATED SKIN DISEASE TRAINING IN VANUATU: PEER-SUPPORTED CAPACITY BUILDING TRANSFORMING HEALTH WORKER CONFIDENCE

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Programs targeting Neglected Tropical Diseases (NTDs) are routinely implemented focused on individual diseases. The financial and opportunity costs of single disease activities are substantial, and, as a result, there has been a call to move towards integrated programs targeting multiple NTDs at once. An integral part of a successful NTD program is training health workers (HCWs) on the prevention, diagnosis, treatment, management, and reporting of NTDs in health facilities and communities. As part of a larger implementation project, the Vanuatu Ministry of Health developed and piloted an Integrated Skin Disease (ISD) training targeting yaws, scabies, and leprosy in three Vanuatu provinces between June 2021 and October 2022. The aim was to increase HCWs’ confidence and competencies in diagnosing and managing the care of patients with skin diseases, including community low-up. Unlike typical training, which is often didactic and based largely on one-way information delivery from expert to trainee, ISD used principles of adult learning and took a unique participatory, scenario-based, and peer-learning approach. The training improved the capacity and confidence of 97 HCWs to identify, diagnose, treat, and report cases of skin NTDs. ISD introduced new tools and resources to support HCW in managing cases of skin NTDs and new systems to facilitate timely and accurate reporting to the MoH. For example, for scabies, we observed increased knowledge of 30% to diagnose, 32% to treat, and 37% to refer. Participants, supported by the MoH, developed a plan and have continued rolling out the novel training approach. ISD also improved channels of communication. It catalyzed the creation of a self-sustaining network to support continuous learning and peer support. The training methodology increased dialogue between the MoH NTD programs and health facilities, which (i) enriched the learning experience and (ii) highlighted gaps and needs of health clinics. The ISD showed that participatory, peer learning that builds on the direct experience and local contexts of health practitioners and volunteers can strengthen capacity and support local transformation.

5134

“I BELIEVE BECAUSE THE VACCINE IS NOT COMING TODAY” - AN EXPLORATION OF THE SOCIO-BEHAVIORAL FACTORS INFLUENCING CHILDHOOD VACCINATION UPTAKE IN URBAN POOR SETTLEMENTS IN NAIROBI, KENYA

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Childhood vaccination uptake has been on the decline, with the World Health Organization estimating that approximately 25 million children missed out on one or more vaccination doses in 2021. Often, children residing in marginalized populations are at a higher risk of being unvaccinated. In Kenya, timely immunization coverage in urban poor settlements remains below 50%. Timely vaccination is crucial in maintaining population immunity against vaccine-preventable diseases (VPDs) and in preventing VPD outbreaks. Exploring context specific reasons for missed vaccinations facilitates the development of tailored interventions. This study aimed at exploring the behavioral and social factors that influence timely childhood vaccination uptake in the urban poor settlements of Nairobi, Kenya. Five focus group discussions (FGDs) were conducted with purposively sampled caregivers of children under five years of age residing in two urban slums in Nairobi. Each FGD involved face-to-face discussions with groups of seven to nine caregivers using an open-ended FGD guide. The development of the FGD guide was guided by the Theory of Planned Behavior. The FGDs were audio-recorded, translated and transcribed. Thematic framework
analysis was used to identify emerging themes and patterns. A total of 39 respondents participated in the FGDs. The median age for the participants was 29 years (range 20-52 years). Although vaccination was perceived to be beneficial and effective in preventing disease, uncertainties about the side effects of vaccination, lay theories and cultural beliefs negatively influenced vaccination uptake. The level of spousal support and lack of the mother’s autonomy in vaccination decisions greatly influenced access to timely vaccination. There was inadequacy of vaccination information to facilitate caregiver decisions, with male participants expressing marginalization in vaccination messaging and processes. Community derived and context specific approaches such as tailored messaging need to be tested and applied to enhance timely childhood vaccination uptake in these marginalized populations.

5135

AVAILABILITY AND ACCESSIBILITY OF SUICIDE PREVENTION SERVICES: A GLOBAL INVESTIGATION

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Suicide is a major global public health issue, worldwide. Suicide prevention services, especially helplines, are crucial resources for individuals at risk of suicide, providing immediate crisis counselling, emotional support and information. However, the availability and accessibility of these services vary greatly across countries and regions, and the effectiveness of these services in reducing suicide rates is not well established. We conducted a global, cross-sectional survey of suicide helpline services. A comprehensive directory of helpline services was created by a team of local collaborators to estimate the number of suicide prevention services available in each country. “Find A Helpline,” the largest suicide helpline resource, helped disseminate the survey to their network in over 100 countries. In areas with limited outreach, the questionnaire was directly distributed by local collaborators to increase participation. As of writing, we have collected 460 responses from suicide helplines in 104 countries. The results showed that most services (75.7%) were available nationwide, while 15.7% of services were functional at the state/province level, and 4.3% of services were available at the county/district level. Telephone-based services were the most common mode of service delivery (87.8%), with most services (77.6%) operational 24*7. However, 48.5% of the services reported a decrease in funding levels after the onset of the COVID-19 pandemic. Most helplines (82.4%) provided free services, while only 11.5% provided language translation services. Additionally, only 22.2% of the helplines provided annual booster/refresher training for their staff, and lack of funding and low pay for staff were reported as major challenges by many helplines. Youths were regarded as the most difficult groups to consult with. The findings can inform policymakers and stakeholders in developing strategies to improve the availability, quality, and sustainability of suicide helpline services worldwide. The difficulties of consulting with youths also emphasize the need for specialized training and services for this vulnerable group.

5136

COMPREHENSIBILITY OF THE EBL2007 CLINICAL TRIAL BY PARTICIPANTS FROM THEIR ENROLLMENT UP TO TWO YEARS OF FOLLOW UP

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In Boende, Democratic Republic of Congo (DRC), an Ebola vaccine trial included health care providers and frontliners. A sub-study developed a true/false questionnaire to assess the understanding of trial consent among participants (Test of Understanding, TOU). One of the eligibility criteria for participation in the trial was the ability to successfully answer at least 9/10 questions of the TOU. This sub-study assessed whether participants understanding of the consent/EBL2007 vaccine protocol evolved over years after signing consent in a trial that was at least one year in duration. In total, 699 health care providers and frontliners were enrolled (from December to February 2019). Data included all participant scores at day one at baseline, at year one, and at year 2 for their planned follow up visit. We performed a beta regression analysis using the R packages ‘betareg’ and ‘emmeans’.

5137

IMPROVED FACILITY BASED INTEGRATED SUPPORTIVE SUPERVISION- GAINS ON HEALTH SYSTEM STRENGTHENING IN OYO STATE

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Nigeria is a high-burden country, which accounts for approximately one in four of the estimated 232 million cases worldwide (World Malaria Report 2022). The US President’s Malaria Initiative for States supports Integrated Supportive Supervisory (ISS) visits to health facilities to support health system strengthening in Oyo state. The objectives of the ISS were to measure the quality-of-service delivery at the facilities, assess the functionality of various systems, update and improve the capacity of health workers. Quarterly visits to health facilities were made using an electronic checklist that covers: infrastructure, basic equipment, human resources, essential drugs, and service delivery for the malaria program. A total of 1625 health facilities were visited between September 2020 and December 2022.

astmh.org
and 69% were revisited. Stock availability of artemisinin-based combination therapy (ACT)1, ACT4, Sulphadoxine-Pyrimethamine (SP) and rapid diagnostic test (RDT) showed an incremental improvement from 28%, 34%, 59%, 60% in September 2020 to 62%, 68%, 69% and 84% by December of 2021 and 87%, 94 %, 97 % and 96% by December of 2022. Reduction from 25% at baseline to 17% in December 2021 and 8% in December 2022 was noted in the number of patients who were RDT negative and incorrectly treated with ACT. State performance scores for infrastructure, basic equipment, human resource (job presence), essential medicines were noted to improve from 79%, 71%, 60% and 31% in September 2020 to 83%, 78%, 77% and 54% in December 2021 and 81%, 85%, 72% and 64% in December of 2022. Slight decline was noted in performance scores for infrastructure and human resource in 2022. A Pearson correlation coefficient test between number of visits and performance shows a strong positive correlation for infrastructure(r=−.94) (p<0.01), basic equipment(r=.91) (p<0.01), essential medicine(r=.81) (p<0.01) and human resource (r=.93) (p<0.01), thus revealing that the more visits to the facilities the better the improvement in performance. Following up with issues identified to ensure resolutions made during these visits is key to strengthening health systems.

5138

IMPROVED DATA QUALITY FROM AUTOMATED DHIS2 DATA EXCHANGE BETWEEN THE MALARIA RAPID REPORTING SYSTEM AND HEALTH MANAGEMENT INFORMATION SYSTEMS IN ZAMBIA

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The Zambia National Malaria Elimination Programme relies heavily on robust surveillance systems not only for malaria programming but also to track progress towards elimination and identification of transmission hotspots for targeted responses. However, the Ministry of Health (MOH) collects malaria data through three separate data systems, which makes it difficult to bring these platforms into one main data source for all malaria related indicators. The three platforms are Malaria Rapid Report System (MRRS) managed by National Malaria Elimination Centre (NMECT), Integrated Disease Surveillance and Response (IDSR) managed by the Zambia National Public Health Institute (ZNPHI); and Health Management Information System (HMIS) managed by the MOH.To identify data gaps and harmonize indicators across the three systems, USAID Evidence for Health (E4H) project in collaboration with NMECT conducted a comparative analysis to assess the performance of malaria surveillance systems in Zambia. Data collection was conducted through a review of existing data collection and reporting systems, datasets, data elements, indicators, and organizational units. This was followed by interviews with key stakeholders. The main issues identified across the three systems were: 1) Inconsistencies in the naming conventions of facilities, even where the same unique identifiers were used; 2) Data element and indicator naming conventions were not consistent across all three systems, and; 3) While all the three systems largely adhered to reporting timeliness, about 96% of the data elements and indicators were either named differently or collected with different disaggregation, thus affecting data quality as well as increasing the workload on data collectors. Based on these findings, MOH collaborating with E4H and partners has prioritized the harmonization of all malaria reporting systems through standardization of indicators, data elements and automation of data exchange across platforms to ensure that the overall reporting system functions as one reliable and effective source of information that improves data quality and reduces workload at data sources.

5139

EXPLORING HEALTHCARE WORKERS’ PERCEPTION AND CHALLENGES TO PRACTICING EFFECTIVE INFECTION PREVENTION AND CONTROL IN TERTIARY CARE HOSPITALS: A MULTI-CENTERED STUDY IN BANGLADESH

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Hospital-acquired infections (HAI) are a major public health burden globally. Deficits in infection prevention and control (IPC) knowledge and perceived barriers among healthcare workers (HCWs) put hospital staff, patients, and visitors at greater risk of acquiring infections. This study identified the perceptions and key challenges on IPC including barriers to compliance with IPC measures among HCWs in Bangladesh. Between August 2020 and March 2021, a qualitative exploratory study was conducted at eight tertiary care hospitals across Bangladesh. We conducted 32 in-depth interviews (IDIs) with hospital administrators, senior physicians, nursing matrons, and heads of cleaning staff from each hospital. We also facilitated 24 focus group discussions (FGD) with physicians, nurses, and cleaning staff. IDIs and FGD findings were compared and themes were triangulated across data collection strategies. Responses were analyzed using a thematic framework. The key barriers to effective IPC practices were a lack of dedicated personnel, irregular monitoring and audit, lack of equitable distribution of personal protective equipment, and insufficient supplies of soap and alcohol-based hand rubs. HCWs identified hospitals’ physical layout and infrastructure were unsupportive of following IPC best practices. The barriers included the inaccessibility of hand hygiene stations; inadequate space to isolate patients; deficient HCW-to-patient ratios; and a lack of screening for patients with infectious diseases at admission. However, the implementation of basic IPC activities, such as environmental cleaning, was mostly hindered by the overcrowding of patients and visitors. The findings highlighted the critical IPC-related challenges faced by HCWs during their day-to-day service. The IPC practices were mostly hampered due to the insufficient supplies of IPC materials, unsupportive infrastructure, overcrowding of patients and visitors. The findings from this study could aid hospital leadership and policymakers in developing and implementing a tailored intervention to improve the IPC practices among HCWs to reduce the burden of HAI.s.

5140

A PHASE I STUDY TO ASSESS THE SAFETY AND IMMUNOGENICITY OF A RECOMBINANT ADENOVIRUS-BASED VACCINE AGAINST PLAGUE

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Plague, an ancient and lethal zoonosis caused by the bacterium Yersinia pestis, continues to present a threat globally; both to populations in endemic regions, and due to it’s potential as a bioweapon. Despite this, there is no current licenced and available vaccine in the Western sphere. The University of Oxford has developed a novel plague vaccine using the replication-deficient simian adenovirus-vector platform (ChAdOx1) and expressing known immunogenic Y.pestis LcrV and F1 antigens (ChAdOx1 Plague). The PlaVac Phase I trial (ISRCTN: 41077863) recruited healthy adult volunteers (18-55years) who received one or two doses of 5 x 1010 VP at intervals of 0 (Group 1), 0 & 56 (Group 2), or 0 & 182 (Group 3) days and were followed-up to day 365. The primary endpoint was safety and tolerability to day 28 post each dose. Secondary endpoints include antibody responses by ELISA at day 28 post each vaccination, and serious
Acute febrile illness (AFI) is a frequent syndrome among persons seeking medical care in West Africa. Determining AFI etiology remains challenging in this region, where laboratory diagnostic capacity is limited. We conducted a scoping literature review to understand the coverage of AFI investigations in West Africa and to identify knowledge gaps regarding AFI etiology in the region. We conducted title and abstract searches of Medline, Embase, Global Health, and OVID databases using the search terms, in both English and French: "undifferentiated fever," "febrile," "non-specific fever," "suspected malaria," "presumed malaria," "presumptive malaria"; and either "West Africa" or the name of any country in ECOWAS (Economic Community of West African States). Using a standardized data collection form, we extracted data from studies that met the following inclusion criteria: published between January 1, 2010, and December 31, 2020; human studies; and AFI etiology determined by laboratory diagnostics. After deduplication, we screened 743 titles and abstracts. We performed full-text screening on 145 publications, and 76 studies were included for data abstraction. Nineteen of the fifty (60%) ECOWAS countries were represented in the final publications, of which 57% of studies were in Nigeria. No consistent definition of AFI was utilized. Participant enrollment was most common in health facilities and included urban and rural populations. Studies reported a range in the number of pathogens tested (1-39), with 75% of studies testing for <3 pathogens. The most common laboratory diagnostic methods were microscopy (43%) and polymerase chain reaction (41%); 53% of studies utilized more than one laboratory diagnostic method. Malaria and dengue were the top two pathogens tested for and detected. Knowledge gaps remain about AFI etiologies in West Africa. Published data are helpful but insufficient to fully inform pathogen prioritization and guide the enhancement of surveillance systems in the region.

**SCOPING REVIEW OF ACUTE FEBRILE ILLNESS IN WEST AFRICAN REGION, 2010-2020**

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Acute febrile illness (AFI) is a frequent syndrome among persons seeking medical care in West Africa. Determining AFI etiology remains challenging in this region, where laboratory diagnostic capacity is limited. We conducted a scoping literature review to understand the coverage of AFI investigations in West Africa and to identify knowledge gaps regarding AFI etiology in the region. We conducted title and abstract searches of Medline, Embase, Global Health, and OVID databases using the search terms, in both English and French: "undifferentiated fever," "febrile," "non-specific fever," "suspected malaria," "presumed malaria," "presumptive malaria"; and either "West Africa" or the name of any country in ECOWAS (Economic Community of West African States). Using a standardized data collection form, we extracted data from studies that met the following inclusion criteria: published between January 1, 2010, and December 31, 2020; human studies; and AFI etiology determined by laboratory diagnostics. After deduplication, we screened 743 titles and abstracts. We performed full-text screening on 145 publications, and 76 studies were included for data abstraction. Nineteen of the fifty (60%) ECOWAS countries were represented in the final publications, of which 57% of studies were in Nigeria. No consistent definition of AFI was utilized. Participant enrollment was most common in health facilities and included urban and rural populations. Studies reported a range in the number of pathogens tested (1-39), with 75% of studies testing for <3 pathogens. The most common laboratory diagnostic methods were microscopy (43%) and polymerase chain reaction (41%); 53% of studies utilized more than one laboratory diagnostic method. Malaria and dengue were the top two pathogens tested for and detected. Knowledge gaps remain about AFI etiologies in West Africa. Published data are helpful but insufficient to fully inform pathogen prioritization and guide the enhancement of surveillance systems in the region.

**ROOT CAUSE ANALYSIS OF HEALTH SECTOR VIOLENCE IN NEPAL: A QUALITATIVE EXPLORATION OF STAKEHOLDERS’ VIEWS**

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The task force conducted in-depth interviews with key informants, such as healthcare service providers, patient representatives, and other stakeholders, to map out the causes of violence against healthcare professionals and suggest coping mechanisms to create a violence-free environment. The interviews revealed that poor medical services delivery and increased patient awareness of their rights and access to justice are the main reasons for these acts of violence. The trust in medical services has been eroded by unqualified medical professionals, negative media influence, catastrophic out-of-pocket expenses, poor quality of care, commercialization of health services, poor monitoring of medical services, and a lack of professional ethics among some unscrupulous medical professionals. Despite socio-political development, violence against healthcare professionals is still on the rise due to increasing patient awareness and their willingness to seek justice. The healthcare system, healthcare professionals, government, and media all contribute to these disputes, but the poor quality of care, out-of-pocket payments, and negative media influence are the main factors driving the vicious cycle of complexity. To address these issues, the government, healthcare professionals, social actors, and healthcare facilities need to adopt strong policies and mechanisms to manage the problems that arise from these disputes. Trustworthiness and cost-effective treatment approaches by healthcare professionals and facilities are critical to preventing healthcare sector violence in resource-constrained settings. In conclusion, addressing the root causes of healthcare sector violence requires a multi-faceted approach that involves all stakeholders. Improving the quality of care, reducing out-of-pocket expenses, and promoting professional ethics among healthcare professionals are vital to rebuilding patient trust in the healthcare system and reducing violence against healthcare professionals.

**ANTIBIOTIC USAGE IN LAYER FARMS: POTENTIAL ROLE IN EMERGENCE OF ANTIBIOTIC RESISTANCE**

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Tetracyclines are widely used for preventing diseases in poultry flocks since decades. It has been shown that low and sub-therapeutic doses of tetracyclines can enhance growth and production. However, their non-prudent use is leading to the occurrence of residues contaminated eggs and emergence of antibiotic resistant strains of bacteria. The present study aimed at detection of tetracycline residues in egg samples collected from

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100 layer farms located in five different districts of Haryana, India. A total of 100 pooled egg samples were analyzed using High Performance Liquid Chromatography method for the simultaneous detection of residues of four tetracyclines viz., oxytetracycline, tetracycline, chlortetracycline and doxycycline. Out of 100 samples, 13 (13%) were found to be contaminated with residues of tetracycline at concentration above limit of quantification. Out of these 13 samples, 1 (1%) was positive for tetracycline and 12 (12%) for chlortetracycline. None of the analyzed sample showed residues of oxytetracycline and doxycycline. Out of 13 samples, 5 (38.46%) (1 for tetracycline residues and 4 samples for chlortetracycline residues) exceeded the maximum residue limits established by Food Safety and Standards Authority of India. The presence of chlortetracycline and tetracycline residues above maximum residues limit in eggs is a matter of concern as it indicates non-prudent use of antibiotics in layer birds and possibility of unacceptable health risks to the consumers. The occurrence of these residues is also indicative of poor farm practices such as non-adherence to withdrawal periods. Thus, there is a need to generate awareness among layer farmers regarding the judicious antibiotics usage and sensitization for adherence to withdrawal protocols. Also, there is a need for formulation and implementation of strict legislations for the regulation of non-prudent antibiotic usage in poultry production.

5145

GLOBAL PUBLIC HEALTH INTELLIGENCE: WORLD HEALTH ORGANIZATION OPERATIONAL PRACTICES

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Two episodes of death cases occurred in the village of Kpo-Kahankro, in a health area in the Bouaké South health district, in Côte d’Ivoire. The first episode occurred on the night of Friday December 2, 2022, during which 20 children, 6 of whom died, presented with febrile gastroenteritis syndrome, adynamia and convulsions. This situation occurred in the context of funerals, but also of a Vitamin A supplementation campaign in the village. The second episode would have started on January 19, 2023, causing 58 cases including 16 deaths, where a notion of manipulation of an ecological niche was found; a traditional fetish. Epidemiological investigations were carried out respectively for the two episodes, the objectives of which were to: - Describe the phenomenon - Identify the cause - Propose control measures. The investigations were carried out by a multidisciplinary team made up of epidemiologists, environmentalists, biologists, clinicians, hygienists and socio-anthropologists. Investigations consisted of questioning parents of patients and villagers, reviewing the medical records of patients and taking biological samples from patients and suspected sources of contamination. A total of 78 cases have been identified, including 22 deaths, i.e. a lethality of 28.2%. Among the cases, 76 children from 0 to 10 years old. The subjects who died were children (20 children) and subjects aged 50 and over (54 subjects). The signs presented were vomiting (71.1%), fever (52.6%), adynamia (39.5%), diarrhea (31.6%), coma (13.2%). Both episodes occurred after manipulation of an ecological niche during rituals. On more than ten blocks in the village, 47.7% of cases live in the block where the ecological niche is located. Clostridium botulinum was found on microbiological examination of the liquid contained in the niche. The destruction of the ecological niche has allowed the phenomenon to stop.

5147


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Since the first reported outbreak in Wuhan, China, the Coronavirus disease 2019 (COVID-19) has raised serious concerns globally. The COVID-19 pandemic has caused a severe psychological impact on healthcare workers (HCWs), and especially nurses, who are the most numerous and exposed front-line group. This systematic review and meta-analysis aim to summarize extant literature on the effects of the COVID-19 pandemic on the psychological health of nurses, particularly concerning the prevalence and risk factors for post-traumatic stress disorder (PTSD). A systematic search was conducted on Pubmed, Embase, and PsychInfo from March 2020 to September 2021. Articles were included/excluded.
on predetermined eligibility criteria. A random-effects meta-analysis model was performed using proportions to determine the pooled prevalence of PTSD in nurses and evaluate sources of heterogeneity. Relatively high prevalence rates of PTSD were reported in the nurse population during the COVID-19 pandemic in eighteen different countries, globally. Risk factors associated with PTSD include having prior mental health co-morbidities, being a female, having insufficient protective conditions, working in an Intensive Care Unit, and having a young age. The overall pooled prevalence was 32.2% (95% CI = 0.239, 0.417) using a random-effects model in synthesizing 30 studies. The regression test of funnel plot asymmetry indicated a significant level of publication bias among studies. The COVID-19 pandemic is associated with significant levels of PTSD among frontline nurses globally. A high level of heterogeneity was observed across studies. Psychological, social, and administrative interventions should be implemented to mitigate heavy psychological distress in nurses.

5148

PERCEPTIONS OF YELLOW FEVER EMERGENCY MASS VACCINATIONS IN UGANDA: A QUALITATIVE STUDY

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Yellow fever (YF), a mosquito-borne viral hemorrhagic fever, is endemic in South America and Africa. Numerous outbreaks have been reported in Uganda. During emergency mass vaccinations 1.6 million people were immunized against YF in 2011 and 2016. The aim of this study was to explore local perceptions of YF emergency mass immunization to strengthen future vaccination campaigns. In this qualitative study we conducted 43 semi-structured interviews, 10 expert interviews and 4 focus group discussions. All 76 participants were from six affected districts with emergency mass vaccinations. We interviewed pregnant women and elderly people ≥ 65 years, who are excluded from YF vaccination except during mass immunization. For information on YF mass immunization participants relied on community sources. Information was spread door-to-door, during religious gatherings, in communal places, and via radio. Despite awareness campaigns most respondents had no knowledge of the vaccine, and it was unclear to them whether a booster dose was required. A concurrent presidential election during mass immunization led to distrust and rejection of the vaccine by the opposition. Moreover, distrust in YF vaccines was unclear to them whether a booster dose was required. A concurrent campaign most respondents had no knowledge of the vaccine, and it relied on community sources. Information was spread door-to-door, during case investigation forms and PPE, but some took long to investigate cases. Others lacked internet access and electricity to produce reports effectively and efficiently such that there could be delayed reporting or underreporting of actual effort. Therefore, capacity building should support development of both content and physical resource needs for DSOs to ensure more timely reporting and case investigation.

5150

ANTIBODY RESPONSE TO DIFFERENT COVID-19 VACCINES AMONG THE MIGRANT WORKERS OF BANGLADESH

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Due to the ongoing COVID-19 pandemic, various host countries such as Singapore imposed entry requirements for migrant workers including pre-departure COVID-19 seroconversion proof. To combat COVID-19 worldwide, several vaccines have acquired conditional approval. This study sought to assess antibody levels after immunization with different COVID-19 vaccines among the migrant workers of Bangladesh. Venous blood samples were collected from migrant workers who were vaccinated with different COVID-19 vaccines (n=675). Antibodies to SARS-CoV-2 spike protein (S) and nucleocapsid protein (N) were determined using Roche Elecsys® Anti-SARS-CoV-2 S and N immunoassay, respectively. All participants receiving COVID-19 vaccines showed antibodies to S-protein, while 91.36% were positive for N-specific antibodies. The highest anti-S antibody titers were found among the workers who completed booster doses (13327 U/mL), received mRNA vaccines Moderna/Spikevax (9459 U/mL) or Pfizer-BioNTech/Comirnaty (9181 U/mL), and reported SARS-CoV-2 infection in the last six months (8849 U/mL). The median anti-S antibody titers in the first month since the last vaccination was 8184 U/mL, which declined to 5094 U/mL at the end of six months. A strong correlation of anti-S antibodies was found with past SARS-CoV-2 infection (p < 0.001) and the type of vaccines received (p < 0.001) in the workers. Bangladeshi migrant workers receiving booster doses of vaccine, vaccinated with mRNA vaccines, and having past SARS-CoV-2 infection, mounted higher antibody responses. However, antibody levels waned with time. These findings suggest a need for further booster doses, preferably with mRNA vaccines for migrant workers before reaching host countries.
combined four essential components to perform the sequence of RT-LAMP steps: a heating block for thermal lysis followed by an isothermal incubator for amplification, a transilluminator module for visual detection, and a controller to provide user interface, control device components and wireless monitoring through a smartphone app. The White Lotus demonstrated excellent positive and negative agreement percentages (PVA 90.91% (90.33); NPA 96.55% (90.90)) compared to the reference RT-PCR, and significantly reduced instrument capital costs. This low-cost POCT will next be coupled with multiplexed diagnosis panels to differentiate between pathogens, species, and variants and could play a critical role in controlling the spread of infectious diseases, particularly in LMIC and resource-limited regions.

**IMPACT OF ARTHROPODS ON THE TRANSMISSION OF DISEASES AND EPIDEMICS IN POPULATIONS WITH POOR HYGIENIC CONDITIONS AND INAPPROPRIATE BEHAVIORAL HABITS IN THEIR HOMES: CASE STUDIES ON POPULATIONS IN THE HEALTH DISTRICTS OF KINYINYA AND GISURU, NOVEMBER 2022 TO FEBRUARY 2023**

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Burundi is an East Africa Country with 27,834 km square and a population of 12,574,569 (2022). The country is endemic for malaria, onchocerciasis, schistosomiasis and STH. Some districts have low trachoma rates, but none reach the MDA threshold for azithromycin. Cholera is sporadic every year. The districts of Kinyinya and Gisuru are two out of 4 districts in the health province of RUYIGI, they are located in the eastern part of the country and share the common border with Tanzania. The total population of the two districts in 2021 is 176,487. In 2021 Kinyinya district had 18 health centers and 2 district hospitals while GISURU had 14 health centers and 1 hospital. The two districts recorded 2,101 patients placed under observation for malaria with intestinal disorders or severe malaria, severe acute malnutrition. They received new hygienic facilities fitted out (2,473 standpipes, 3,400 hand washing devices, 4,765 garbage pits, 3,004 latrines. Despite all these efforts, the two districts periodically record unusual public health events manifested by skin manifestations with pruritus, followed by lesions and allergies. The investigation of the unusual public health event in these health districts led to the conclusion that the health situation is the consequence of poor hygiene conditions among the affected populations living with the hens. The inappropriate management of these scratch lesions can also be the gateway for pathogenic germs and cause infections of all kinds. The precarious hygiene in the population of these districts increases the risk of persistence of malaria and neglected tropical diseases in this endemic area. In addition, these insects could also be carriers of other transmissible diseases if they are not eliminated effectively. The cohabitation between humans and animals increases the risk of emergence of zoonosis such as arboviruses which could cause new serious epidemics. Collaboration between the Administration, the Ministry of Public Health and the Ministry of Agriculture, Livestock and the Environment is essential in order to promote the hygiene of the population and healthy breeding without danger to human health.

**RAPID, LOW-COST, AND PORTABLE LAB-IN-A-BOX ‘WHITE LOTUS’ FOR POINT OF CARE TESTING OF SARS-COV-2 IN LOW MIDDLE INCOME COUNTRIES**

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Rapid identification of infectious diseases is critical in controlling their spread. Reverse Transcription Polymerase Chain Reaction (RT-PCR) is the primary approach for detecting large-scale infectious diseases but it is a complex and expensive process that involves several sample preparation and incubation steps at varying temperatures, making it impractical for low-and middle-income countries (LMIC). Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) offers a simpler alternative to RT-PCR that maintains high sensitivity and the ability to detect variants, however, large-scale implementation of such tests is challenging as it requires sophisticated and expensive equipment, complex sample preparation and specialized storage conditions for assay reagents. Point-of-care tests (POCT) can overcome this challenge by providing low-cost means to perform a rapid diagnosis at the bedside without intricate steps. This study focused on developing a customized portable lab-in-a-box platform for rapid, sensitive, and patient-centered diagnostic approach based on LAMP. The POCT dubbed “White Lotus” was validated for COVID-19 diagnosis by using saliva samples and involved a quicker detection time with a heat inactivation step instead of RNA extraction. The White Lotus

**ASSESSMENT OF POINTS OF ENTRY, ISOLATION SITES AND COUNTIES PREPAREDNESS AND RESPONSE TO EBOLA VIRAL DISEASE, OCTOBER 2022, KENYA**

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Ebola Virus Disease (EVD) is a rare and deadly disease most commonly affecting people & non-human primates (monkeys, gorillas, & chimpanzees). The Health Department in Uganda confirmed the outbreak of Ebola Virus Disease (EVD) on September 20th,2022 with 130 cases & 43 deaths as of October 31, 2022. There were possibilities of the outbreak spreading to the neighboring 20 high-risk counties in Kenya whose level of preparedness & response was unknown. Therefore, we conducted an assessment to determine the level of preparedness & response in the counties in Kenya. The methodology used was the administration of a standardized questionnaire to determine the level of preparedness & response at the points of entry, ground crossings, & the County Health Management Team between October, 20th 2022 to November, 2nd 2022. The surveillance pillars under assessment were; Coordination, Rapid Response Team, Case Management, Laboratory investigation, Infection Prevention & Control, Isolation & Referral system, Training, Risk Communication, Surveillance, & Community Engagement. The eligibility of the counties for assessment was based on the risk of exposure & proximity to the Uganda border which was experiencing an active Ebola virus disease outbreak. Data were analyzed using excel version 2016 to show the level of preparedness & response in percentages for each surveillance pillar. The results after the assessment were as follows; the average score for all nine surveillance pillars was 21%. The performance on individual pillars was as follows; Risk Communication 58%, Infection Prevention & Control 36%, Rapid Response Team 23%, Coordination 22%, Case Management 14%, Referral services 14%, Training 11%, Isolation & Surveillance 6.6% & Laboratory capacity 4% respectively. In conclusion, there was generally very poor performance on Ebola Virus disease preparedness & Response in all the counties under assessment. Therefore, we recommended all the high-risk counties to develop contingency/emergency plans specifically for Ebola Virus Disease and Disease outbreaks in the future.

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Priorities and gaps. The strategies and policies prioritize the strengthening of genomic surveillance, and a stakeholder engagement process to identify review of existing policies, a situational analysis of the current state of Africa CDC, Member States, academia, non-governmental organizations and policies are being developed through a collaborative effort involving disease surveillance, preparedness, and response in Africa. The strategies for sustainable integration of genomic into existing systems for outbreak detection and disease surveillance. Here discuss the priorities, progress, and challenges of developing national strategies and policies for sustainable integration of genomic into existing systems for outbreak detection and disease surveillance. Here discuss the priorities, progress, and challenges of developing national strategies and policies for sustainable integration of genomic into existing systems for outbreak detection and disease surveillance. Here discuss the priorities, progress, and challenges of developing national strategies and policies for sustainable integration of genomic into existing systems for outbreak detection and disease surveillance. Here discuss the priorities, progress, and challenges of developing national strategies and policies for sustainable integration of genomic into existing systems for outbreak detection and disease surveillance. Here discuss the priorities, progress, and challenges of developing national strategies and policies for sustainable integration of genomic into existing systems for outbreak detection and disease surveillance.

Brucella spp. are highly infectious pathogens causing brucellosis, a significant zoonotic disease affecting livestock, wildlife, and humans. In livestock, brucellosis could cause abortion and reduced milk production. Brucellosis is endemic in Tanzania; however, the prevalence of brucellosis amongst livestock species still needs to be fully established. This study aimed to determine the seroprevalence of brucellosis in cattle, goats, and sheep at Kitengule ranch in Kagera region of Tanzania. A cross-sectional sampling was conducted in early 2023. Blood samples were collected from cattle, goats, and sheep for serological examinations. Collection was used to evaluate the risk factors associated with brucellosis. The presence of serum immunoglobulin with Brucella was tested using Rose Bengal Test and confirmed by cELISA. Descriptive statistics were used to determine the disease's prevalence among livestock species. Serum from 541 cattle and 185 small ruminants (goats/sheep) were tested for Brucella spp.

The overall seroprevalence in livestock was 34.2%, comprising 68.1% in small ruminants (n=126, 95% CI= [60.9-74.8]) and 22.6% in cattle (n=122, 95% CI= [19.1-26.3]). Seroprevalence was significantly different between herds (Chi-square p< 0.0001). Multiple logistic regression analysis was run on variables, including animal species, herds, sex, and age. Results indicated that small ruminants are more likely to test positive than cattle (OR=14.0, CI= [6.7-31.4], p< 0.0001). Seropositivity was considerably higher in females than males (OR=3.1, CI= [1.2-4.2], p=0.001). Our results also showed that seropositivity was higher in older animals (> 2 years) compared with younger animals (OR=1.8, CI= [1.2-3.6], p=0.024). Our study suggests a high rate of seroprevalence of brucellosis (84.2%) at the Kitengule ranch compared to the national pooled average (8%). This calls for an urgent unmet need for further investigation of risk factors associated with brucellosis in livestock in other areas of Tanzania to help inform the development of evidence-based control plans benefiting both animal and public sectors.

A STUDY ON KNOWLEDGE, ATTITUDE AND PRACTICE (KAP) ON YELLOW FEVER AMONG COMMUNITY MEMBERS IN FOUR DISTRICTS AFTER AN OUTBREAK IN THE SAHARA REGION, GHANA

In 2021, Yellow Fever (YF) outbreak occurred in, rural and mainly nomadic communities in the Savannah Region of Ghana with over 40 deaths, including children. We determined the knowledge, attitude and practice (KAP) of community members in four districts in the Savannah Region using a questionnaire survey on 869 participants from June to July, 2022. Out of the total inhabitants interviewed, majority 702 (80.5%) indicated they heard about YF through healthcare personnel. 191 (22.0%) had knowledge that transmission is through mosquito bites. Two districts; Central Gonja, 171 (86.5%) and Sawla-Tuna-Kalba 172 (86.0%) showed high knowledge on YF, the difference in participant’s high-level knowledge on YF between the two districts is statistically not significant (Mann-Whitney test =2570.000 P = 0.262), while North Gonja 58 (21.6%) and West Gonja 52 (26.0%) showed little knowledge on YF. The difference in participant’s low-level knowledge on YF between the two districts is statistically not significant (Mann-Whitney test=19900.000 P = 0.886). Among the participants 611 (70.3%) adopted the use of mosquito net, 605 (69.6%) clearing bushes and 636 (73.2%) clearing of their surroundings as a mosquito preventive measure against the spread of YF. The vaccination of respondents or any household member was likely not influenced by time taken to access health service as explained by the lower correlation coefficient (Spearman Rho correlation coefficient=0.264). The emergency mass YF vaccination carried out by the Ghana Health Service in response to the outbreak in the region could have contributed to the high knowledge of community members on the disease. Regular education on YF in these YF hotspot communities in the Savannah Region and Northern Ghana as a whole will help increase awareness and consequently reduce the risk of transmission of the disease.

STRATEGIES AND POLICIES FOR SUSTAINABLE PATHOGEN GENOMIC SURVEILLANCE IN AFRICA: PRIORITIES, PROGRESS, AND CHALLENGES

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Pathogen genomic surveillance is one of the crucial components of regional and global health security. Africa, with its diverse population and high burden of infectious diseases, has a critical need to integrate genomics into existing systems for outbreak detection and disease surveillance. Here discuss the priorities, progress, and challenges of developing national strategies and policies for sustainable integration of genomic into existing disease surveillance, preparedness, and response in Africa. The strategies and policies are being developed through a collaborative effort involving Africa CDC, Member States, academia, non-governmental organizations (NGOs), and other stakeholders. The process involves mapping and review of existing policies, a situational analysis of the current state of genomic surveillance, and a stakeholder engagement process to identify priorities and gaps. The strategies and policies prioritize the strengthening of laboratory and bioinformatics infrastructure, the development of human capacity, and the establishment of sustainable funding mechanisms. The strategies also emphasize the importance of collaboration and sharing of data, resources, and expertise among member states and with other global partners. Furthermore, the policies highlight the need for ethical considerations in genomic surveillance, including data privacy, data governance and sharing. Implementation of the strategies and policies will require a multi-sectoral approach and sustained commitment from governments, donors, and other stakeholders. Overall, we highlight the need for sustained investment in genomic surveillance by African Union Member States and underscore the role of international collaborations and partnerships in achieving sustainable disease surveillance and response.

COMMUNITY ENGAGEMENT IN A NEW TRIAL SITE OF THE PARTNERSHIP FOR RESEARCH ON EBOLA VACCINATION IN MALI

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Since the 2014 Ebola virus disease outbreak in West Africa, several clinical trial initiatives have been implemented to accelerate the search for an effective vaccine against the disease. We report on a community engagement process to identify issues related to the understanding and
acceptability of participation in a community-based vaccine trials of the Partnership for Research on Ebola Vaccination (PREVAC) in Mali. To identify a community engagement strategy for a new vaccine against Ebola in Mali. A questionnaire and focus groups (8) involving heads of households, women and community leaders were conducted. 22 community mobilizers were trained on Ebola vaccination and on responsible conduct of research and ethics. “Ice-breakers” meetings with traditional authorities were conducted to establish a participatory framework between the research team and the communities. Study participants expressed a very good knowledge of Ebola, including mode of transmission (32%), prevention (88%) and 97.9% recognized the importance of vaccination. The baseline survey showed that 75.2% were inclined to participate in the vaccine trial. The community engagement strategy, through the series of “Icebreakers” meetings and the interface between the communities and the research team made it possible to dispel the concerns raised for non-participation in clinical trials (lack of confidence in a new vaccine). They made it possible to achieve a retention rate of 94% of the volunteers who made the scheduled 9 visits in the research protocol and more than 4000 home visits. The participatory approach through inclusion of traditional community legitimacy, awareness and information on the vaccine process helped to build trust and acceptability of the vaccine trial. This experience gathered during PREVAC with the NIH can be used to guide future vaccine trials in developing countries.

COVID-19 ATTITUDES AND VACCINE HESITANCY AMONG AN AGRICULTURAL COMMUNITY IN SOUTHWEST GUATEMALA: A CROSS-SECTIONAL SURVEY

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Despite offering free-of-charge COVID-19 vaccines starting July 2021, Guatemala has one of the lowest vaccination rates in Latin America. During September 28, 2021 to April 11, 2022, we conducted a cross-sectional survey of community members adapting a Center for Disease Control and Prevention (CDC) questionnaire to evaluate COVID-19 vaccine access and hesitancy. Of 233 participants ≥12 years, 127 (55%) received ≥1 dose of COVID-19 and 4 (2%) reported prior COVID-19 illness. Persons ≥12 years old (eligible for vaccine at the time) who were unvaccinated (n=106) were more likely to be female (73% vs 41%, p<0.001) and homemakers (69% vs 24%, p<0.01) compared with vaccinated participants (n=127). Among those ≥18 years old, vaccinated individuals were more likely to be moderately or very worried about COVID-19 (n=36, 31%) compared to unvaccinated individuals (n=13, 18%; p=0.04); unvaccinated individuals were more likely to have little no confidence in public health institutions (55% vs 38%, p=0.02). Participants’ primary reported motivations for COVID-19 vaccination were to protect family/friends (88% vaccinated vs 73% unvaccinated, p=0.54), to protect their own health (24% vs 11%, p<0.01), and to protect the health of the community (2.5% vs 3%, p=0.94). Community- and/or home-based vaccination programs, including vaccination of families through the workplace, may better reach female homemakers and reduce inequities and hesitancy, and a such a program is ongoing. In follow-up, beginning March 14, 2023, we began a repeat survey in the same household cohort to assess changing attitudes and access around COVID-19 and COVID-19 vaccines as well as drivers (risk associations) of those changes.

DESCRIPTION OF AN ACTIVE SURVEILLANCE SYSTEM CONDUCTED IN OUTPATIENT CLINICS FOR PRIORITY ACUTE INFECTION SYNDROMES IN GUATEMALA

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Due to the high burden of infectious disease and limited diagnostic capabilities in Guatemala, the Vigilancia integrada colaborativa de enfermedades infecciosas agudas prioritarias (VICO-ip) was established to estimate the burden and characterize etiologies for key infectious disease syndromes. VICO-ip operates in Quetzaltenango, a department with a majority urban-dwelling population of nearly 800,000. We examined data from two ambulatory clinics in the San Juan Ostuncalco (SJQ) and El Palmar municipalities, which differ in climate, altitude, and ethnic makeup. Patients of all ages who provide consent or assent are screened for signs of acute respiratory (ARI), diarrheal (ADI), or febrile (AFI) syndromes, and epidemiological data and samples (nasopharyngeal and/or oropharyngeal swabs, stool, blood, and urine) are systematically collected. Etiologies are identified by LIAT, FilmArray, and PCR testing. Analysis of surveillance data from a period of continuous enrollment (1 March 2022 to 25 January 2023) reveal differences in disease frequency, notably respiratory and diarrheal, between the two clinics. Overall, of the 644 enrolled participants, mean age was 27.6, and 57.8% were female. 92.2% of patients presented with symptoms of ARI, 16.3% ADI and 28.9% AFI. 97.2% provided samples for etiological testing. Few cross-syndrome etiologies were seen. SARSCoV2 (198/409, 48.4%) was the most common respiratory disease etiology found, whereas E. coli strains (12/37, 32.4%) and Leptospirosis bacteria (7/13, 53.8%) were the most common diarrheal and febrile etiologies identified, respectively. Significantly more laboratory-confirmed diarrheal disease was seen in El Palmar (OR: 14.1, 95% CI: 2.18-283) than SJQ. SJQ demonstrated significantly greater laboratory-confirmed burden of respiratory disease (OR: 1.80, 95% CI: 1.19-2.75) than El Palmar, indicating local site characteristics should be used to decide on control and mitigation interventions. Examining syndromic data from similar-level facilities can assist public health officials in better understanding population needs and tailoring appropriate interventions.


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The Global Health Security Index (GHSI) is a composite index developed in 2019 to assess countries’ preparedness for epidemics and pandemics under the International Health Regulations framework. However, recent research showed that the GHSI was not predictive of countries’ performance during the COVID-19 pandemic, raising questions about its validity as an assessment tool. We empirically explored if and how subcomponents of the GHSI were associated with their ability to safeguard essential health service delivery during the pandemic. Using a difference-in-difference methodology, we assessed the relationship between countries’ ratings pertaining to their overall preparedness and six subcategories and childhood immunization coverage rates during the pandemic. We operationalized the GHSI 2019 and its 6 subcomponents and 34 indicators to assign countries to the treatment (above 80th percentile) and control groups and defined 2020 and 2021 as post-pandemic years. The results were cross-checked using the World Bank’s governance indicator to ensure the reliability. All analyses were adjusted for potential confounders. While the overall effect of the GHSI on childhood immunization coverage
rates during 2020-2021 was statistically non-significant, countries’ commitments to sharing data (coef: 6.0; p = 0.017), infrastructure (coef: 3.2; p < 0.001), and overall environmental risks (coef: 2.94; p < 0.001) were the subcategories most positively associated with preventing declines in childhood immunization coverage. These results were confirmed with the World Bank’s governance indicators, which demonstrated that countries’ rule of law (coef: 3.58; p < 0.000), effective governance (coef: 3.27; p < 0.000) and control of corruption (coef: 3.16; p < 0.000), were most strongly associated with preventing the declines. Our findings underscore the importance of core health system capacities that shape countries’ overall risk landscapes in mitigating the public health consequences of the COVID-19 pandemic and provide prioritization guidance to policymakers to improve countries’ preparedness for future epidemics and pandemics.

5161
THE SYNERGISTIC IMPACT OF UNIVERSAL HEALTH COVERAGE AND GLOBAL HEALTH SECURITY ON HEALTH SERVICE DELIVERY DURING THE COVID-19 PANDEMIC: A DIFFERENCE-IN-DIFFERENCE-IN-DIFFERENCE STUDY OF CHILDHOOD IMMUNIZATION COVERAGE FROM 192 COUNTRIES
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Universal health coverage (UHC) and global health security (GHS) are two high-priority global health agendas that seek to foster health system resilience against health emergencies. Many countries, however, have had to prioritize one agenda over the other due to scarce resources and political pressures. To aid policymakers’ decision-making, this study investigated the individual and synergistic effects of countries’ UHC and GHS capacity in safeguarding essential health service delivery during the COVID-19 pandemic. We used a quasi-experimental difference-in-difference and difference-in-difference-in-difference methodology to quantify the relationship between 192 countries’ progress towards UHC and GHS and those countries’ ability to provision 12 essential childhood immunization services between 2010 and 2021. We used the 2019 UHC Service Coverage index to divide countries into a “high UHC group” (above 80th percentile) and the rest, and similarly used the 2019 GHS Index to divide countries into a “high GHS group” and the rest. All analyses were adjusted for potential confounders. Countries with high UHC scores prevented a 3.34% (95% CI: 2.17%; 4.52%; p-value < 0.001) reduction in immunization coverage across 2020 and 2021. Countries with high GHS scores prevented a 2.02% (95% CI: 0.44%, 3.59%; p-value = 0.012) reduction in immunization coverage in 2021 but no statistically significant effect in 2020. The DIDID model showed that high GHS capacity needed to be augmented with high UHC to prevent a decline in immunization coverage while high UHC alone was able to safeguard immunization coverage during the pandemic. This study found that greater progress towards both UHC and GHS safeguarded essential health service delivery during the pandemic but only progress towards UHC was both a necessary and likely sufficient element for yielding this protective effect. Our results call for strategic investments into both health agendas and future research into possible synergistic effects between the two health frameworks.

5162
WASTEWATER GENOMIC SURVEILLANCE AS AN APPROACH TO TRACK INFECTIOUS DISEASES PATHOGENS IN THE AGADIR REGION OF MOROCCO
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Wastewater genomic surveillance is a promising approach to monitor pathogens that may constitute a health threat for humans and animals. This approach can be applied as an early warning tool and to enable acting before pathogens spread to the general population. In collaboration with Moroccan health and environmental authorities, we established a platform of experimental and analytical resources to monitor antibiotic resistance genes (ARGs) and SARS-CoV-2 dynamics. The workflow developed comprises sample collection and processing, nucleic acid purification, and downstream genomic pipelines using a real-time quantitative PCR and NGS platforms. Using these resources, first we demonstrate the feasibility of ARGs monitoring by successful profiling of the resistome in 6 wastewater treatment plants (WWTPs) in the Agadir region for the first time in Morocco, providing a better understanding of the status of ARGs and highlights their dissemination potential. Second, we performed genomic surveillance of SARS-CoV-2 during the initial Omicron wave in January 2022. Following automated RNA extraction, SARS-CoV-2 N gene was qPCR amplified and quantified. The results showed that viral load in wastewater influents from these WWTPs ranged from 52.7E+03 to over 75.8E+04 viral N gene copies/L. The evolution of the number of confirmed cases identified by the Ministry of Health, during the same period and in the same geographical area correlates with our viral load findings. NGS sequencing of SARS-CoV-2 genomes recovered from wastewater clearly indicated the predominance of the BA1 variant (99%) with less than 1% for the BA2 variant. Furthermore, the number of infected individuals was predicted by using a SEIR-model and the mass rate of SARS-CoV-2 RNA in wastewater. The results estimated the number of infected persons to be ~25 fold higher than the reported number of cases. This may be due to asymptomatic and undiagnosed cases, and individuals who are positive but do not undergo testing or report to clinics. These results highlight the power of wastewater genomic surveillance as a valuable tool to monitor, better understand and control pathogens.

5163
THE VIRTUAL BIORESPOSITORY SYSTEM FOR OPEN ACCESS TO SAMPLES: THE ONLINE DELPHI PRIORITIZATION OUTCOME
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An equitable, federated, virtual biorepository system (VBS) with stewardship focused at local sites broadens access to specimens and associated data for outbreak-prone infectious diseases. This would accelerate the development of diagnostics, assessment of vaccine efficacy, and support surveillance and research that is reflective of the populations being served. The VBS is aligned to complement other specimen-sharing efforts as a force multiplier to strengthen global tools for countering epidemics. Our work reveals significant gaps in diversity, equity, and inclusion in accessing specimens/data especially for LMICs. We used an online Delphi process (oDp) as an inclusive approach to engage stakeholders to re-imagine and empower low-resource partners to drive the VBS concept at scale, where they will prioritize the benefits and define sustainable operations in their context. Participants included principal investigators (23), public health/clinical laboratories (9), research institutions (22), commercial diagnostics (3), and biorepository managers (5) working from Africa (15), N. America (10), Europe (9), Latin America (9) and Asia-Pacific (5). We posed 34 questions for participants to rank preferences presented as % in agreement. There was complete consensus (100%) about the need for collecting clinically well characterized specimens and associated data and implementing a cost recovery scheme; followed by serially collected blood and blood-derived samples (95%), access to qualified reference standards (93%), and willingness to share specimens (92%). The VBS is envisaged to combine centralized and federated components (82.9%) with networking and collaboration as the highest ranked benefits. Regulations prohibiting sharing samples out of country (56%) were seen as the greatest challenges. By using the oDp, we were able to successfully use a grassroots effort to
THE VIROME OF PHLEBOTOMINE SAND FLIES FROM SELECT REGIONS OF KENYA

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Sand fly-associated viruses are inadequately researched in Sub-Saharan Africa. Whereas recent surveillance studies in parts of Kenya have revealed a number of arboviruses circulating among sand fly populations, our overall knowledge of the identity of viruses in these vectors remains limited. The present study used metagenomics, viral isolation and Next Generation Sequencing to characterize RNA viruses in sand flies. Sand flies were collected between September 2020 and July 2022 in Baringo, West Pokot, Nakuru, Kisumu, Kiif, Kwale and Isiolo counties in Kenya using CDC light traps. The collections were sorted into pools of 10 or less flies and homogenized. Virus isolation was performed in vero cells with CPE positive samples being subjected to NGS. For metagenomic analyses, super-pools were generated based on site of origin. Libraries were prepared and sequenced on the Iseq 100 platform. The unassembled short reads were classified using Kraken 2 metagenomic sequence classifier and mapped to the genomes of isolated viruses. Contigs were generated in MegaPhat and further taxonomic classification done. Two CPE positive samples resulted in isolation of the Koutango lineage of West Nile Virus (WNV) and Borogia virus, a phlebovirus. Read classification led to identification of several viral sequences including Pamir flavivirus, Marnava virus, Marabu virus, Corajas virus, Pyi virus, Koutango virus (WNV) and Borogia virus. From assembled metagenomic sequences, 14 phylogenetically distinct insect specific viruses belonging to Dicistroviridae, Chuviridae, Flaviviridae, Nodaviridae, Tombusviridae, Polycipoviridae families and Neguvirus were identified. These results have shown that sandflies from Kenya harbour diverse RNA viruses, some of which are of public health importance.

SYSTEMATIC REVIEW ON TICKS AND TICK-BORNE DISEASES IN ASIA AND AUSTRALIA

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Globally, tick-borne pathogens of human concern pose a threat to deployed war fighters (DWFs). DWFs pyrethroid treated uniforms are their first line of defense against tick vectors. As climate change shifts vector habitats and behaviors adapt to climate change, continued surveillance will be vital to understanding the risk towards human health. Gaps for future surveillance were noted, especially in Buthan, Guam, and Palaau where few records were found. This valuable knowledge can ensure well informed decisions are made to protect DWFs in these areas.

MITE-TRANSMITTED INFECTIOUS DISEASES: WIDELY DISTRIBUTED AND NEGLECTED

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Mites are among the smallest arthropod vectors of infectious diseases (IDs) with most species barely visible at less than one µ in length. Bites by house mouse mites can transmit Rickettsia akari, the causative bacterial pathogen of rickettsialpox. Bites by larval trombiculid mites can transmit Orientia tsutsugamushi, the causative bacterial pathogen of scrub typhus. Rickettsialpox occurs worldwide, and scrub typhus is considered regionally confined to the Tsutsugamuchi Triangle in Asia and Northern Australia. The ecology, epidemiology, clinical manifestations, differential diagnosis, laboratory diagnosis, management, and prevention of mite-transmitted IDs are presented. Internet search engines are queried in order to provide evidence that mite-transmitted IDs are both widely distributed and neglected. Rickettsialpox is widely distributed for several reasons including its animal reservoir, the common house mouse, and its mite vector are ubiquitous; and subclinical and doxycycline cured cases are often underreported worldwide. Scrub typhus is also widely distributed for several reasons including a global distribution of potential larval mite vectors with new cases of scrub typhus described in the Middle East and South America, far away from the Tsutsugamuchi Triangle. Rickettsialpox and scrub typhus are super-neglected and not included among the WHO listing of neglected diseases. Neglect of these mite-transmitted IDs is evidenced by a lack of research and new drug development as compared to mosquito-borne and tick-borne IDs. In addition, there are no vaccines to prevent rickettsialpox and scrub typhus. Targeted research into mite-transmitted IDs, new drug development to counter increasing doxycycline resistance and replace chloramphenicol, and vaccines, especially for scrub typhus, are needed now.

POPULATIONS STRUCTURE ANALYSIS OF PHLEBOTOMUS PAPATASI POPULATIONS USING TRANSCRIPTOME MICROSEUALLITES: POSSIBLE IMPLICATIONS ON GENE EXPRESSION

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Phlebotomus papatasi considered the primary vector of Leishmanina major parasites which causes zoonotic cutaneous leishmaniasis (ZCL) in the Middle and far East and in North Africa. P. papatasi populations have been extensively studied and revealed the existence of different genetic populations and subpopulations over its large distribution range. Genetic diversity and population structure analysis using transcriptome microsatellite markers is important to uncover the vector distribution dynamics, an essential for controlling ZCL in endemic areas. In this study, we investigated for the first time the level of genetic variation using expressed sequence
tags EST-SSRs among field and colony P. papatasi samples collected from 25 different locations in 11 countries. The genetic variation reveals the existence of high-level population structures expressed in five distinct populations. These great genetic differences in expressed genes may enable P. papatasi to adapt in different environmental conditions along its distribution range and affects dispersal and control of this species probably by upregulating insecticide resistant genes which is common among vectors of infectious diseases. Moreover, anthropogenic changes can also lead to an increase in the dispersal of sand flies and hence the disease transmission. The investigation of population structuring of P. papatasi may have a great contribution to the L. major containment efforts in these countries. Moreover, the level of genetic variation among these populations may have a great benefit for understanding parasite-vector interaction and contribute to the efforts of vaccine development based on P. papatasi salivary proteins.

5168

RELATIONSHIP BETWEEN ENVIRONMENTAL FACTORS AND PHYSICO-CHEMICAL PARAMETERS IN THE DISTRIBUTION AND DENSITY OF MOLLUSC INTERMEDIATE HOSTS OF SCHISTOSOMIASIS IN SENEGAL

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A change in the epidemiology of bilharzia has been noted with the appearance of hybrid strains in northern Senegal. The objective of this work is to study the relationship between physico-chemical parameters (water temperature, conductivity, salinity, pH, total dissolved solids), environmental factors (plastic waste, vegetation) and the distribution of molluscs in 14 sites in the Ferlo valley, Lac de Giers and Taouey canal. At each site, prior to any survey, information concerning the habitat was collected, namely the geographical coordinates, pollution, type of vegetation and the presence of animals. To measure the physico-chemical parameters such as water temperature, conductivity, salinity, pH and total dissolved solids we used multiparameteric tester with digital probes PC60 (APERA instruments). The technique consisted of immersing the probes in the water to collect these data. The surveys identified 8 species of molluscs, four of which are involved in the transmission of human schistosomes ( Biomphalaria pfeifferi, B. globosa, B. truncatus, B. senegalensis) and four other species that are not involved in the transmission of schistosomiasis (B. forskali, Lymnaea natalensis, Bellamyia unicolor and Mellanoides tuberculata). The density of molluscs collected is a function of the nature of the site. This study also shows that environmental parameters (vegetation, pollution) and physicochemical parameters (total dissolved solids, salinity, pH) have effects on the distribution and density of mollusc populations intermediate host of human schistosomes.

5169

FIRST RECORD OF MOSQUITO BORNE SINDBIS VIRUS <GENOTYPE I> IN BURKINA FASO, WEST AFRICA

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Several mosquito-borne viruses represent a major threat to human health worldwide. Beyond those well-known pathogens, a large diversity of arboviruses transmitted by mosquitoes remains largely unstudied despite an established potential for emergence in some cases. Beyond a high diversity, mosquito arboviruses have also shown an impressive capacity for spread into new regions. Thus, health services require updated characterisations of the arbovirus diversity in a given region to optimize diagnostics. Nevertheless, such updates are rarely carried out, especially in low-income countries. Thus, diagnostics of potential arboviral infections is often limited to high-profile viruses, like dengue virus. This situation probably leads to a large fraction of undiagnosed cases due to arboviruses neglected or recently established in a region. Here, we have characterized the diversity of mosquito-borne viruses in two regions of Burkina Faso. To this end, we have screened a recent and large mosquito collection using untargeted metagenomics. The analysis focused on two mosquito species, Aedes aegypti and Culex quinquefasciatus, considered among the most important vectors of arboviruses worldwide. The screening detected Sindbis virus (SINV, Togaviridae) for the first time in Burkina Faso. This zoonotic arbovirus has spread into Europe from Africa and is the cause of disease outbreaks mainly in Europe. SINV was detected at low prevalence and only in C. quinquefasciatus from one of the regions and at a single year. A phylogenetic analysis placed the nearly-full SINV genome within the cluster of Central African sequences at the origin of the strains that have spread into Europe. Thus, this result extends the region as potential SINV source to Western Africa. Finally, a virus isolate was obtained for future experimental studies. Overall, our results provide insights into the current arbovirus diversity in Burkina Faso and can help to improve diagnosis. Moreover, the approach used here could be applied to other regions in need of a better characterization of the arbovirus diversity in mosquitoes.

5170

CHANGING EPIDEMIOLOGICAL PATTERN OF VISCERAL LEISHMANIASIS IN NEPAL

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On the Indian subcontinent, visceral leishmaniasis VL is targeted for elimination as a public health problem by 2017. Nepal has achieved the elimination target at district level in 2013 and has been sustained the situation since then. Recently, we conducted a field surveys in non-program hilly districts in Nepal where increasing number of VL cases have been persistently reported since 2000. A house-to-house survey in 14 villages from eight hilly districts documented retrospectively 54 cases of VL, predominantly males, mostly pediatric cases who were reported in the last five years. Anti-Leishmania antibodies were found in 22/23 past-VL cases, in 40/416 9.6% persons without VL and in 12/155 7.7% domestic animals. An age- and sex-matched case-control study showed that exposure to known VL-endemic areas was no risk factor for VL, but having a VL case in the neighborhood was. SSU-rDNA PCR for Leishmania sp. was positive in 24 (5%) of the human, in 18 (12%) of the animal samples and in 16 (14%) blooded female Phlebotomus argentipes sand flies. L. donovani was confirmed in two asymptomatic individuals and in one sand fly through hsp70-based sequencing. This study proves that there is indeed ongoing local transmission of Leishmania donovani in areas at an altitude above 600 meters, districts considered higher to non-endemic for VL. This geographical expansion of cases and ongoing local transmission could challenge the aim of the VL elimination program in Nepal. Hence, policy makers should give a high priority in expanding active surveillance and control activities to achieve the realistic goal.

5171

BEHAVIORAL INTERACTIONS OF BED BUGS WITH LONG-LASTING PYRETHROID-TREATED BED NETS: CHALLENGES FOR VECTOR CONTROL

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Long-lasting insecticide-treated nets (LLINs) have historically been, and remain, one of the most commonly used vector control tools in the campaign against malaria. The emergence of pyrethroid resistant bed bugs in malaria endemic communities and failure to control infestations have been suggested to interfere with the effective use of LLINs. Therefore, the behavioral interactions of bed bugs with commonly used bed nets should be better understood. To investigate the interactions between bed bugs (Cimex lectularius) and LLINs, insecticide-susceptible and pyrethroid-resistant bed bugs were challenged to pass through two
commonly used LLINs in two behavioral assays. We found a significant impact of deltamethrin-treated nets on blood-meal- and aggregation-seeking behaviors of susceptible bed bugs, and no impact of treated nets on resistant bed bugs. Commonly used new LLINs failed to prevent the passage of susceptible and pyrethroid-resistant bed bugs in host-seeking and aggregation-seeking bioassays, resulting in overall low mortality. For the first time, we have shown the strong potential of LLINs to select for resistant non-target pests, and so their potential role in stalling malaria control programs should be further investigated.

5172

ISOLATION OF MICROSATELLITE LOCI FROM THE GENOME OF PHLEBOTOMUS ARGENTIPES, THE MAJOR VECTOR OF LEISHMANIASIS IN SRI LANKA: A PRELIMINARY STUDY

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The microsatellite markers are promising because of their high polymorphism rate, high abundance, and wide distribution throughout the genome. However, microsatellite markers have not been reported for the sandfly species Phlebotomus argentipes, possibly due to the low abundance in the genome and/or difficulty in isolation. The present study aimed to identify d- or tri-nucleotide microsatellites in Pargentipes. Whole genomic DNA was extracted from a pool of 60 sand flies, then about 9 μg were digested with RsaI restriction enzyme, and adaptors were ligated into the digested DNA. Size selection of the adaptor-ligated DNA was done using running DNA in 1.8% low melting agarose gel, followed by exciting the 200–1000 bp range from the smear and recovering the DNA fragments. A PCR evaluated the success of the adaptor ligation to the digested DNA. The DNA fragments were hybridized with 3’-biotin-labeled (GT)12 oligonucleotide and captured using streptavidin-coated magnetic beads. The enriched DNA fragments were recovered by differential stringency washes and amplified by PCR. Cleaned PCR products were ligated into pGEM-T vector, then transformed into Escherichia coli JM109 competent cells and plated onto LB/ampicillin/IPTG/X-gal media. White colonies were used to construct the library which was further screened by PCR amplification. The colonies giving two amplified bands were sequenced to confirm the presence of the microsatellite. More than 100 transformants were obtained from the blue/white screening and few of them have been screened by PCR. A total of 12 transformants were shown double bands and one transformant was shown an imperfect (TG) 6 microsatellite loci. In conclusion, the isolation procedure used in this study was successful, but the rest of the transformants should be screened to select a few microsatellite loci. In further studies, polymorphisms have to be evaluated by amplifying the microsatellite sequences using different subsets of Pargentipes with designated primers. This study would expand the knowledge related to the microsatellite markers in Pargentipes and might be helpful in population genetics studies.

5173

SPECIES COMPOSITION, ACARICIDE RESISTANCE IN AMBLYOMMA VARIEGATUM TICK SPECIES: KNOWLEDGE, ATTITUDE, AND PRACTICES OF LIVESTOCK OWNERS IN DIFFERENT ECOLOGICAL ZONES OF GHANA

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Acaricide failure on cattle has been on the rise globally. In Ghana, acaricides are still used to control ticks although some studies have identified acaricide resistance in ticks and reports the inappropriate use of acaricides. The aim of this study was to determine the distribution of tick species infesting livestock, assess the knowledge, attitude, and practices (KAP) of livestock owners and evaluate the resistance status of larval tick populations to some acaricides used in Ghana. This study was a cross-sectional study and tick populations evaluated in this study were collected from different cattle farms in the guinea savannah, transitional zone, deciduous forest and coastal savannah ecological zones from May to September 2022. Larval packet test was used to evaluate resistance status of Rhipicephalus ticks at discriminating dose (DO), half the discriminating dose (0.5DD) and twice the discriminating dose (2×DD) of three classes of acaricides; cypermethrin, amiraz and chlorpyrifos representing the pyrethroid, amidine and organophosphate groups respectively. The WHO criteria was used for determining the acaricide resistance status of the ticks. Statistical analysis was done using Microsoft Excel 2019 and Stata version 13.0. A total of 1022 ticks of three major genera; Amblyomma (519/1022), Hyalomma sp (136/1022) and Rhipicephalus species (367/1022). Out of the total number of ticks collected, Amblyomma variegatum constituted, 51%, followed by Rhipicephalus boophilus and sanguineus, 36%, Hyalomma rufipes and truncatum, 13%. Amblyomma variegatum was the most dominant species with a representation of 51% and also the most abundant in the different ecological zones, except for the Coastal savanna where Rhipicephalus was the most abundant species with a representation of 71% out of all the ticks collected in that zone. The study showed that Amblyomma variegatum were susceptible to cypermethrin, amiraz and chlorpyrifos acaricides and most farmers employed acaricidal control for treatment of their livestock during tick infestations.

5174

PHLEBOTOMINE SANDFLY SPECIES FROM OLD AND NEW LEISHMANIASIS FOCI OF COLOMBIA

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Leishmaniasis is a neglected tropical disease endemic in rural as well as urban areas of northern Colombia, which affects mainly the poorest communities; where cutaneous and visceral leishmaniasis are the prevalent clinical forms of the disease. Despite of the above, the sandfly fauna is still poorly known in several leishmaniasis foci of the north of Colombia. Even more important is the appearance of new leishmaniasis transmission foci during the last years. The aim of this work was to identify the sandfly species from old and new leishmaniasis foci of the north of Colombia. Phlebotomines were collected in ten localities of the Departments of Córdoba, Bolívar, Sucre, Cesar, and La Guajira, from 18:00 to 06:00 hours, by using CDC light traps installed in intra, peri and extra-domestic environments. Sandflies were morphologically identified up to the level of species using standard taxonomic keys for New World’s sandflies. An epidemiological survey, including the characteristics of the dwellings and their surrounding area, was conducted through a mobile application developed for the study. Seventeen sandfly species were taxonomically identified, including Lutzomyia evansi, L. longipalpis, L. nuneztovari, L. shannoni, L. gomezi, L. panamensis, L. dubitans, L. rangeliana, L. punctigentiliculata, L. cayennensis cayennensis, L. micropyga, L. atrovagula, L. carpenteri, L. sp. (series townsendi), L. venezuelensis, Helcocyctomyia sp., and L. trinidadensis. New locality records are provided for the Colombian sandflies, including recognized vectors of Leishmania spp.
**NATION-WIDE VECTOR SURVEILLANCE OF CHAGAS DISEASE IN EL SALVADOR, 2018-2020**

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Chagas disease, caused by the infection of Trypanosoma cruzi, is one of the most serious health issues in Latin American countries. In many endemic countries, infected triatomine bugs are considered majorly responsible for the persistent T. cruzi infection. However, the up-to-date vector burden has not been evaluated thoroughly due to the cessation of active vector surveillance in the Central American region. Our study focused on updating the risk of vector-borne T. cruzi infection in one of the most Chagas-endemic countries in the region. Nation-wide vector surveillance was performed by conducting house-to-house visits in the domestic environment of El Salvador. Houses were inspected by experienced surveyors for the infestation of vector insects. Infection for T. cruzi was evaluated by microscopic examination of insects’ feces, followed by a species confirmation using PCR. As a result, we identified 1529 Triatoma dimidiata from all fourteen departments of El Salvador between the years of 2018 and 2020. No other vector species were captured in this study. Microscopic examination revealed 153 specimens out of 1529 (10.0%) positive for T. cruzi infection, which was confirmed by PCR molecular diagnosis. Geographically, higher infection rates for T. cruzi in T. dimidiata were found in departments sporadically placed across the country. These insights suggested the presence of high-risk areas of Chagas disease transmission in the region.

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**PHENOTYPIC AND MOLECULAR ASSAYS CONFIRM PUTATIVE PYRETHROID RESISTANCE IN ANOPHELES ALBIMANUS IN MALARIA ELIMINATION SETTINGS IN HONDURAS**


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Vector control continues to be the principal strategy for malaria elimination worldwide. In Honduras, control relies on pyrethroid insecticide-treated bednets (ITNs) and indoor residual spraying (IRS) with carbamates. Even with adequate vector control coverage, malaria cases have risen from 330 to 1550 cases per year, between 2019 and 2021. Phenotypic insecticide resistance has been detected previously, and given those findings, we carried out an assessment of key insecticide resistance target-site mechanisms in vector populations from the main endemic areas of the country. Wild-caught adult anophelines were collected in eight localities across six malaria-endemic departments in Honduras, during 2021. Using CDC bottle bioassays, susceptibility to diagnostic doses of deltamethrin, permethrin, and bendiocarb were carried out. PCR and sequencing were used to identify mutations at positions 995 in the VGSC gene and 280 in the Ace-1 gene. Our findings revealed that An. albimanus in three sites were resistant to at least one pyrethroid. Individuals from Gracias a Dios, the main endemic region, were susceptible to deltamethrin. All populations were susceptible to bendiocarb. Populations from Yoro, Comayagua and Olancho reported mortalities ranging from 48 to 89% (permethrin) and 62 to 88% (deltamethrin). Two genotypes (TGT and TCC) equivalent to mutation L995F (16%) and L995C (30%) were detected at the VGSC in 4 of 8 localities. Comayagua showed the highest frequency for L995C (65%), and Santa Rita for L995F (77%). No mutations were found at the Ace-1 gene. A substantial number (44/184) of potential heterozygotes (TNN) for kdr were detected. This is the first report detecting two target-site mutations on the VGSC gene associated with PY resistance in malaria vectors from Honduras, with the L995C allele as the most frequent mutation across the populations screened. Further analysis will clarify the frequency of heterozygotes. The nascent pyrethroid resistance reported here should inform the choice of vector control products from alternative insecticide classes, in order to allow for proactive resistance mitigation and management.
THE GENOMIC BEHIND INSECTICIDE RESISTANCE IN ANOPHELES MOSQUITOES FROM THE BIJAGÓS ARCHIPELAGO

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Evolving insecticide resistance threatens the efficacy of vector control interventions. In turn, this threatens the control of all vector-borne diseases, including malaria. The Bijagós Archipelago is situated off the coast of Guinea-Bissau, West Africa, and is endemic for malaria. The major vectors for malaria in the Bijagós are Anopheles gambiae in the rainy season, and An. melas in the dry season. The majority of malaria control in the Bijagós is Long Lasting Insecticide treated Nets. The WHO recommends monitoring insecticide resistance to inform evidence-based vector control interventions. This study investigated the status of insecticide resistance in mosquito vectors across the Bijagós archipelago in 2019 and 2022 using both phenotypic assays and molecular monitoring of resistance markers. WHO bottle bioassays were used to assess phenotypic resistance to deltamethrin, and molecular monitoring was used to investigate the frequency of genomic markers of insecticide resistance to a broad range of insecticides. Molecular monitoring was conducted using high-throughput custom-targeted amplicon sequencing. Collected mosquitoes included a high proportion of An. gambiae/colluizi hybrids (2%). This study revealed phenotypic resistance to discriminating concentrations of deltamethrin in 50% of the mosquitoes assayed. Whole Genome Sequencing was employed to investigate signatures of directional selection associated with deltamethrin resistance, by comparing genomic diversity in resistant and susceptible mosquitoes. Investigation of molecular markers revealed the presence of mutations associated with pyrethroid resistance in the Voltage Gated Sodium Channel (VGSC) gene (kdr mutations) L995F, N1570Y and A1746S. Mutations in the GSTE2 gene associated with resistance to organophosphates and carbamates were also identified, including F120L and L119V, as well as the RDL A296G mutation associated with resistance to dielkrid and fipronil.

EVALUATION OF THE SUSCEPTIBILITY OF ANOPHELES FUNESTUS POPULATIONS IN THE CENTRE, CENTRE-WEST AND SOUTH-WEST REGIONS OF BURKINA FASO

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In recent years, insecticide resistance in the main malaria vectors has intensified in sub-Saharan Africa. This poses a threat to the effectiveness of insecticide-based vector control interventions. In Burkina Faso the main malaria vectors are members of the gambiae complex and the funestus group. Monitoring the susceptibility level of all vectors is necessary to adapt control strategies. The aim of this study was to update on the insecticide susceptibility profile of Anopheles funestus populations. Indoor resting blood-feed female mosquitoes were collected between September and November 2021 in Naaba-Zana in the Centre, La in the Centre-West and Sibera in the South-West, three villages in Burkina Faso. Female members of the An. funestus s.s. group were oviposited to generate F1. Insecticide tests were performed on the F1 progeny to assess the susceptibility profile of this vector to three classes of insecticides (pyrethroids, carbamates and organophosphates) using the WHO cylindrical-tube test guidelines. The specific composition of the funestus group members, the sporozoite infection rate and the presence of the L119F-GSTE2 gene were investigated. An. funestus s.s. was the only species of the funestus group present in all three localities. The sporozoite infection rate was 6.47%. Anopheles funestus s.s. was resistant to deltamethrin in all the three localities with mortality rates being 21.24%, 35.43% and 65.24% respectively in Naaba-Zana, La and Sibera. However, the population at the La site was resistant to bendiocarb but susceptible to pyrimiphos-methyl. The frequency of the L119F-GSTE2 mutation conferring resistance to DDT and pyrethroids was overall 24.30%. These results suggest that resistance to pyrethroids and carbamates in An. funestus in Burkina Faso could pose a threat to the effectiveness of operational insecticide-based vector control tools.

PROFILING OF INSECTICIDE RESISTANCE, MICROBIOME AND PATHOGEN PREVALENCE IN AEDES AEGYPTI IN PUERTO RICO

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Puerto Rico suffers from the circulation of arboviruses, including dengue, Zika and chikungunya, transmitted by the Aedes aegypti mosquito. The predominant control strategies across the island are breeding habitat removal, larviciding and spatial spraying in response to outbreaks. This study aims to characterise the Ae. aegypti population in Puerto Rico in terms of phenotypic and molecular insecticide resistance, microbiome composition and pathogen prevalence, as well as assessing associations between these factors. Ae. aegypti eggs were collected using ovitraps from 2 sites (Bayamón and San Juan) and reared to adults. These adults were subjected to testing against deltamethrin and malathion at three concentrations. A target amplicon sequencing approach was used to identify genetic variants such as single nucleotide polymorphisms SNPs and insertions/deletions associated with insecticide resistance and identify Wolbachia in the microbiome. We found considerable resistance in both sites to one- and five-times diagnostic dose of deltamethrin and one- and three-times diagnostic dose of malathion. Mortality at one diagnostic dose was under 25% for deltamethrin and under 40% for malathion in mosquitoes from both sites. Molecular assays revealed mutations in insecticide resistance relevant genes including: VGSC, RDL, ACE and GSTE2, including a novel mutation not previously described in Ae. aegypti, but that has been associated with resistance in other insects. Neither Wolbachia nor arboviruses were identified in the samples we screened. Understanding insecticide resistance is vital to facilitate the best possible control strategies for arboviral disease. Here we show that this Ae. aegypti population has high levels of resistance to the pyrethroid deltamethrin and the organophosphate, malathion. Insecticide resistance data should be used to inform the future selection of compounds used in larvicides and insecticide spraying for vector control.
MONITORING INSECTICIDE RESISTANCE STATUS OF ADEOS AEGYPTI AND AE ALBOPICTUS POPULATIONS IN FIVE LOCAL GOVERNMENT AREAS IN LAGOS STATE, NIGERIA

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Aedes aegypti and Aedes albopictus, the principal vectors of dengue, Zika, and chikungunya viruses, have progressively started to develop resistance against most of the currently used insecticides. This increase in resistant populations makes management crucial to the design of effective disease control. Thus, this study was carried out in Lagos State to determine the insecticide susceptibility status of Aedes species. Bioassays were performed on Aedes species, according to their abundance, in five Local Government Areas (LGAs) between July and September 2022. Aedes larvae and pupae were collected from tires, plastic containers, and abandoned water closets. Ae. aegypti were collected from three LGAs (Ibeju Lekki, Yaba, and Alimoso), while Ae. albopictus were found in the other two LGAs (Ikorodu and Somolu). Immature stages were reared to the adult stage at the insectary of the Nigerian Institute of Medical Research (NIMR). Three to five-day-old sugar-fed females were exposed to four classes of insecticides according to the standard WHO protocol: organochlorines (4% DDT); carbamates (4% bendiocarb); pyrethroids (0.75% permethrin & 0.05% deltamethrin); and organophosphates (5% malathion). Results revealed that the collected populations of Ae. aegypti were susceptible to bendiocarb and malathion (100% mortality to both insecticides), but resistant to permethrin (mortality from Ibeju Lekki was 42%, Yaba was 52%, and Alimoso was 50%), deltamethrin (mortality from Ibeju Lekki 57%, Yaba 59%, and Alimoso 52%) and resistant to DDT (mortality from Ibeju Lekki and Yaba was 100% and Alimoso was 39%). For Aedes albopictus, the Somolu population was 100% susceptible to bendiocarb and malathion but resistant to permethrin and deltamethrin. The Ikorodu population showed 100% susceptibility to malathion and resistance to other insecticides (bendiocarb resulted in 87% mortality, permethrin resulted in 62% mortality, and deltamethrin resulted in 62% mortality). The level of detected resistance in these populations prompts the need to use alternative insecticide classes for the control of Aedes populations in this region.

INSECTICIDE RESISTANCE SPECTRUM AND PREVALENCE OF L1014F KDR TYPE MUTATION IN ANOPHELES GAMBIAE S.L. IN ABIA STATE, NIGERIA

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Anopheles gambiae s.l. is the primary vector of malaria, a debilitating disease responsible for substantial mortality and morbidity in Sub-Saharan Africa. This study was conducted to evaluate the insecticide resistance status and frequency of L1014F kdr mutation in Anopheles gambiae [Diptera: Culicidae, Giles 1902] within Abia state, Nigeria. Immature stages of An. gambiae (s.l.) were collected from Umudike, Agala, and Eborn communities and reared to adulthood. Batches of twenty five sugar fed female mosquitoes, aged 3-5 days, were exposed to four types of WHO insecticide impregnated papers i.e., 4% DDT, 0.75% Permethrin, 0.1% bendiocarb, and 5% Malathion, for one hour and the mortalities were recorded after a recovery period of 24 hours. Mosquitoes were also subjected to molecular diagnosis for speciation, and genotyped for kdr type gene mutation L1014F. Anopheles gambiae (s.l.) was highly resistant to permethrin (Umudike-18.76% mortality, Agala-17.51%, Eborn-49.01%) and DDT (0%) in the three locations. Conversely, all the locations recorded complete susceptibility to malathion (100%). Although complete susceptibility to bendiocarb was reported from Umudike (100%) and Eborn (100%), some resistance was reported from Agala (87.5%). PCR analyses showed that An. gambiae (s.l.) were predominantly An. gambiae s.s., in Umudike (90%), Agala (67.5%) and Eborn (67.5%), whereas the rest were An. coluzzii. Very high frequencies of the L1014F kdr mutation was observed in all locations [Umudike (1.00), Agala (0.98), and Eborn (0.95)]. Interestingly, mosquitoes from Agala that survived the 24 hours post-exposure were put in a freezer (-12°C) for about eight hours to die, but it was observed that the mosquitoes which appeared dead in the recovery tubes got resuscitated shortly after they were brought out of the freezer. The worrisome resistance to bendiocarb in Agala suggests existence of metabolic resistance that needs to be clarified and calls for urgent implementation of integrated vector control strategies, whereas a study on the possibility of hibernation in An. gambiae s.l. mosquito populations during cold season is suggested.

EPITHELIAL NITRATION RESPONSE TO PLASMODIUM FALCIPARUM IN INSECTICIDE RESISTANT ANOPHELES COLUZZII MOSQUITOES

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Insecticide-based vector control tools are the most valuable resources to fight malaria infections with insecticide treated bed nets (ITNs) accounting for almost 70% of the reductions in malaria cases between the years 2000 – 2015. Recently, progress in reducing malaria cases has stalled according to the World Malaria Report 2022; this corresponds strongly with the spread of insecticide resistance (IR). The threat is compounded by the use of relatively few insecticides in malaria control programs, highlighted by use of pyrethroids on all bed nets currently distributed. The mosquito harbours the longest-lived life stages of the parasite and offers an attractive target for interrupting the transmission cycle. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been shown as effective mediators of the anti-plasmodial immune response in the midgut. In particular in the epithelial nitrination reaction, a major barrier for parasite invasion, by attacking ockinetes. Unfortunately, in contrast to rodent malaria this response has been shown mostly ineffective for the human pathogenic parasite Plasmodium falciparum. Transcriptomic analysis of insecticide resistant Anopheles coluzzii mosquitoes from Burkina Faso indicated potential higher levels of metabolic activity, as reported previously. Such high levels may lead to increased RNS/ROS and thus differential vectorial capacity for P. falciparum. The potential for perturbance of the redox state offers exciting new avenues for the development of more effective or additional vector control tools, like attractive targeted sugar baits (ATSBs), for improved integrated vector management directly targeting parasite invasion. This study illustrates the relationship between oxidative stress, insecticide resistance and anti-plasmodial immunity. We demonstrate that the epithelial nitrination response and ROS state differs between insecticide resistant and insecticide susceptible mosquitoes of the An. gambiae complex, potentially leading to differential vectorial capacity.

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The challenge of insecticide resistance in malaria vector populations has led to the need for laboratory testing of new insecticide formulations using Anopheles gambiae s.l. mosquitoes. This study aimed to investigate how the insecticide resistance profile of laboratory-maintained mosquitoes changes after multiple generations of breeding without the selective pressures found in their natural breeding sites, mainly focusing on the effect of the larval water sources. Anopheles gambiae s.l. larvae from a known insecticide-resistant field population were bred in field, tap, or distilled water for 11 generations under standard laboratory conditions. The adult mosquitoes were then exposed to WHO discriminating insecticide dosage of several insecticides, and the detoxification activity of the population was assessed through a synergist assay with piperonyl butoxide (PBO) and pyrethroids. The enzymatic activity of the various colonies and physicochemical parameters of the different water types were also analyzed. The WHO susceptibility results showed high resistance to all insecticides (mortality ≤ 90%). However, mosquitoes bred in field water showed relatively high mortality upon synergist exposure. The An. gambiae colony bred in field water also showed higher enzymatic activity for α esterase, mixed function oxidases (MFO’s), and β esterase compared to the other breeding water types (P=0.05), with insensitive acetylcholinesterase (AChE) activity remaining constant across all breeding water types. Lastly, the field water was found to be polluted based on the physicochemical parameters measured with reference to WHO guidelines. This study recommends the use of field breeding water to replicate and maintain the observed field levels of phenotype, genotype, and metabolic insecticide resistance in laboratory-reared Anopheles gambiae s.l. mosquitoes, as it outperforms and maintains all three resistant mechanisms.

**BREEDING WATER EFFECT ON ANOPHELES GAMBIAE SENSU LATO INSECTICIDE SUSCEPTIBILITY DURING LABORATORY COLONIZATION**


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The challenge of insecticide resistance in malaria vector populations has led to the need for laboratory testing of new insecticide formulations using Anopheles gambiae s.l. mosquitoes. This study aimed to investigate how the insecticide resistance profile of laboratory-maintained mosquitoes changes after multiple generations of breeding without the selective pressures found in their natural breeding sites, mainly focusing on the effect of the larval water sources. Anopheles gambiae s.l. larvae from a known insecticide-resistant field population were bred in field, tap, or distilled water for 11 generations under standard laboratory conditions. The adult mosquitoes were then exposed to WHO discriminating insecticide dosage of several insecticides, and the detoxification activity of the population was assessed through a synergist assay with piperonyl butoxide (PBO) and pyrethroids. The enzymatic activity of the various colonies and physicochemical parameters of the different water types were also analyzed. The WHO susceptibility results showed high resistance to all insecticides (mortality ≤ 90%). However, mosquitoes bred in field water showed relatively high mortality upon synergist exposure. The An. gambiae colony bred in field water also showed higher enzymatic activity for α esterase, mixed function oxidases (MFO’s), and β esterase compared to the other breeding water types (P=0.05), with insensitive acetylcholinesterase (AChE) activity remaining constant across all breeding water types. Lastly, the field water was found to be polluted based on the physicochemical parameters measured with reference to WHO guidelines. This study recommends the use of field breeding water to replicate and maintain the observed field levels of phenotype, genotype, and metabolic insecticide resistance in laboratory-reared Anopheles gambiae s.l. mosquitoes, as it outperforms and maintains all three resistant mechanisms.

**NATIONWIDE ASSESSMENT OF MALARIA VECTOR SUSCEPTIBILITY TO CHLORFENAPYR, PYRIPROXYFEN, AND ALPHA-CYPERMETHRIN IN PREPARATION FOR WIDESCALE DEPLOYMENT OF NEW GENERATION NETS (INTERCEPTOR® G2 AND ROYAL GUARD®) IN BENIN**

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Pyrethroid resistance is widespread in sub-Saharan Africa, including Benin, and threatens the effectiveness of pyrethroid-treated mosquito nets. To manage this generalized resistance to pyrethroids, new-generation insecticide-treated nets (NG-ITNs) with new active ingredients (AIs) have been developed. Before the deployment of NG-ITNs, it is essential to understand the relative efficacy of Interceptor® G2 and Royal Guard® nets nationwide in Benin. The study is to assess the status of insecticide susceptibility to the new AIs on NG-ITNs across Benin, suggesting that the deployment of NG-ITNs should be effective in controlling mosquitoes. However, monitoring of these AIs should continue to detect the emergence of resistance. Although chlorfenapyr was lethal and pyriproxyfen inhibited fertility, operational evaluations will help to better understand the relative efficacy of Interceptor® G2 and Royal Guard® nets.

**ANOPHELES GAMBIAE S.L. KNOCKDOWN RESISTANT MUTANT ALLELES AND SUSCEPTIBILITY TO INSECTICIDES IN THREE SENTINEL SITES OF ZIMBABWE**

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Indoor residual spraying and long-lasting insecticidal nets are the mainstay malaria prevention measures in Zimbabwe. However, efficacy of the insecticides is affected by vector resistance mechanisms. Aim of this study is to assess the status of insecticide susceptibility to insecticides sprayed in malaria endemic areas. 1,929 mosquito larvae were collected in Mashonaland East province (Mudzi, Mutoko and Wedza district) from March 2020 to August 2022. The larvae were reared under insectary conditions. WHO tube and bottle bioassays were conducted and mortality rate was assessed after 24 hours. Anopheles arabiensis KGB strain was used as susceptible positive control. An. gambiae s.l specimens morphological identification and confirmatory PCR was determined previously. Further analysis to detect kdr and ACE1R alleles were done in previously reported studies. 5.8% were An. arabiensis, 0.73% An. gambiae ss, 0.47% An. merus and 93% An. quadriannulatus 100% mortality after 24 hrs exposure was recorded in Wedza. Kdr to clothianidin was recorded 75% in Mutoko and 86.6% mortality rate in Mudzi. PCR showed that 1.44% An. quadriannulatus was resistant to deltamethrin, DDT, pirimiphos-methyl and clothianidin in Mutoko and Mudzi. All An. gambiae ss and An. merus were susceptible whereas 2.04% An. arabiensis species were resistant to deltamethrin in Mutoko. L1014S allele was found in Mudzi 0.01% and Mutoko 0.5%, whereas L1014F allele was 0.4% Mudzi and 0.7% Mutoko. ACE1 resistance was present in Mutoko 1.04% and 0.3% in Mudzi. Deltamethrin insecticide was associated with L1014S mutant allele (p=0.01), DDT and pirimiphos-methyl were associated with L1014F mutant allele (P=0.01). ACE1R was associated with clothianidin and deltamethrin in Mutoko and Mudzi (P=0.01). An. gambiae species were susceptible to alpha-cypermethrin, chlorfenapyr and permethrin insecticides, thus using these insecticides in the study sites is recommended. Furthermore, results have identified the need for new approaches for monitoring insecticide resistance studies since larval collections can be dominated by non-vectors.
IDENTIFICATION AND INSECTICIDE RESISTANCE PROFILE OF ANOPHELES AZEVEDOI (RIEBO, 1969) IN LUANDA PROVINCE, ANGOLA: IMPLICATIONS FOR VECTOR CONTROL

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In collaboration with the National Malaria Control Program, the U.S. President’s Malaria Initiative VectorLink project re-established entomological activities in Angola in 2019 through routine assessments of species composition and susceptibility to relevant insecticides of the primary malaria vector species (Anopheles gambiae s.l. and An. funestus s.l.). In Luanda Province, the most abundant larval-reared specimens collected for insecticide susceptibility tests were initially morphologically identified as a species of the An. funestus s.l. group. A subset of specimens were analyzed at the CDC Entomology Branch, where morphological and molecular analyses determined they were An. azevedoi. This identification was further confirmed by the US National Museum of Natural History (Smithsonian Institution) where voucher specimens from these collections were indexed. Sequences for the ITS2 and CO1 barcoding genes were produced and reported to GenBank for the first time. Retroactive DNA sequence analysis of archived samples detected An. azevedoi among larval collections in the provinces of Namibe and Benguela, which had been incorrectly reported as An. gambiae s.l. Since 2021, An. azevedoi larvae have been collected in two villages in Luanda Province, WHO insecticide susceptibility assays conducted in 2021 found the species to be resistant to deltamethrin and alpha-cypermethrin (mortality of 56% and 68%, respectively), with pre-exposure to PBO fully restoring susceptibility to both insecticides. Populations were fully susceptible to chlorfenapyr.

A sub-sample of adult An. azevedoi collected through indoor CDC light traps underwent blood meal analysis and 9 of 26 had fed on humans. No Plasmodium falciparum sporozoites were detected in this set. These findings show that An. azevedoi is a prevalent species in several regions of Angola. It has been morphologically confused, is resistant to pyrethroids, is present inside houses, and feeds upon humans. Continued entomological monitoring, sporozoite assays, and research on this species is recommended to understand its role in malaria transmission in Angola.

5187

HIGH ENTOMOLOGICAL INOCULATION RATE OF ANOPHELES COUSTANI IN THE MALARIA ELIMINATION SETTINGS OF DEMBIYA DISTRICT, NORTH-WESTERN ETHIOPIA

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Despite the progress in scaling up the intervention tools in Ethiopia, malaria is still a major health problem. Therefore, a continuous monitoring of the local vector behavior & ecology is relevant to design evidence-based malaria control strategies. This study investigated the species composition & the biting & resting behaviours of Anopheles mosquitoes in selected localities of Dembiya District. Adult Anopheles mosquitoes were sampled indoors & outdoors from June 2018 to May 2019 by using CDC light traps, pyrethrum spray catches, artificial pit shelters, & mouth aspirators. Anopheles mosquitoes were identified to the species level. Their blood source & Plasmodium sporozoite infections were determined using an Enzyme-linked immunosorbent assay. PCR was used for identification of sibling species of An. gambiae s.l. Anopheles mosquitoes belonging to 11 species were identified from 2,055 collected mosquito specimens. Anopheles pharoensis & An. arabiensis were the dominant species in both Guramba Bata & Areyabi study sites. The CDC light traps caught the highest number of Anopheles mosquitoes in both study sites. The density of outdoor host-seeking & restig Anopheles mosquitoes were higher outdoors than indoors (P < 0.05). The human bite index (HBI) of indoor and outdoor host-seeking An. arabiensis were 17.4% & 15.3%, respectively. The entomological inoculation rate (EIR) of outdoor host-seeking An. arabiensis was 4.7 infective bites/person/year. Additionally, the outdoor EIR of host-seeking An. coustani was 25.7/bi/p/year. In conclusion, the indoor & outdoor density of host-seeking & resting Anopheles mosquitoes were comparably high in the Dembiya district. This contrast with the fact

5189
that the area is known for the long-term implementation of vector control strategies. Therefore, re-evaluating vector control strategies considering vector & host behaviour is mandatory to eliminate malaria in the study area. Anopheles pharoensis, An. coustari, and An. squamous were positive for Plasmodium circum-sporozoite protein, which urges further investigations to substantiate their vectorial role.

5190

NATURAL INFECTION OF NYSSORHYNCHUS DARLINGI AND NY. BENARROCHI B WITH PLASMODIUM DURING THE DRY SEASON IN THE UNDERSTUDIED LOW TRANSMISSION SETTING OF DATUM DEL MARÁNON PROVINCE, AMAZONIAN PERU

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The persistence of malaria hotspots in Datum del Marañon Province, Peru, prompted vector control units at the Ministry of Health, Loreto Department, to collaborate with the Amazon International Centers of Excellence for Malaria Research to identify the main vectors in several riverine villages that had Annual Parasite Incidences (API) >15 in 2018-2019. Anopheline females were collected indoors and outdoors for two 12-hr nights/community during the dry season in 2019 using Human Landing Catch. We identified four species: Nyssorhynchus benarrochi B, N. darlingi, N. triannulatus and Anopheles gambiae. The most abundant, Ny. benarrochi B, accounted for 96% of the total (7550/7844) of which 62% were captured outdoors (4826/7844). Nine mosquitoes, 4 Ny. benarrochi B and 5 Ny. darlingi, were collected indoors and outdoors for two 12-hr nights/community during the dry season in 2019 using Human Landing Catch. We identified four species: Nyssorhynchus benarrochi B, N. darlingi, N. triannulatus and Anopheles gambiae. The most abundant, Ny. benarrochi B, accounted for 96% of the total (7550/7844) of which 62% were captured outdoors (4826/7844). Nine mosquitoes, 4 Ny. benarrochi B and 5 Ny. darlingi, were infected by Plasmodium falciparum, P. vivax or both. Human Biting Rates ranged from 0.5-592.8 bites per person per hour for Ny. benarrochi B and 0.5-32.0 for Ny. darlingi, with EIR values as high as 0.75 infective bites per night for Ny. benarrochi B and 0.50 for Ny. darlingi. These data demonstrate the risk of malaria transmission by both species even during the dry season in villages in multiple watersheds in Datum del Marañon province.

5191

HOUSES IMPROVING AS A SUPPLEMENTAL INTERVENTION TOOLS FOR REDUCING INDOOR VECTOR DENSITIES AND MALARIA PREVALENCE IN EMANA, CENTER CAMEROON

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Improvement of typical rural houses can effectively reduce indoor vector densities and consequently malaria transmission. We assessed this supplemental control effects in a MILDA low coverage area of center Cameroon. 16 houses were firstly selected based on their indoor density of resting malaria vectors. Half of them randomly chosen for eaves screens (experimental) with fibreglass coated wire mesh and half left unscreened (control). Entomological baseline were collected monthly in both groups. Outdoors and indoors adults mosquitoes were sampling for entomological data collection in each houses using Human Landing Catch (HLC). Malaria prevalence surveys were conducted after mosquitoes sampling in both groups. A total of 300 mosquitoes were collected over six months period using HLC in 16 houses (mean mosquitoes =18.75). Among Anopheles funestus, 63.9% were unfed, 32.3% blood fed, 0.99% gravid and 1.56% half gravid females. 17.7% of An. gambiae were unfed and 82.2% blood fed. More indoor adult mosquitoes were collected in the control (n=74) than experimental houses (n=56). Parasitological surveys results to relatively low malaria parasite prevalence rates in screened houses compared to the control houses. Overall, malaria prevalence was 57.8% (95% CI: 0.32-0.74) n=90, with baseline prevalence rate of 58.5% (95% CI: 0.67-1.13), n=65 and 2nd follow-up survey prevalence of 42.0% (95% CI: 0.52-0.76) n=66. At all the two parasitological follow-up survey points, house screening significantly reduced the malaria prevalence by 43% (p< 0.001). Housing improvement has potential to reduce indoor vector densities and malaria prevalence.

5192

SPATIO-TEMPORAL DISTRIBUTION OF Aedes species (DIPTERA: CULICIDAE) IN FOUR LOCAL GOVERNMENT AREAS IN LAGOS STATE, NIGERIA

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The frequency and magnitude of arbovirus outbreaks that are transmitted by Aedes mosquitoes are increasing globally, driven by ecologic, economic, and social factors. Therefore, it is imperative that more detailed monitoring be conducted to enhance the distribution and abundance predictions of the Aedes species to update the risk assessment of arbovirus transmission and improve the planning capabilities of public health decision-makers. Here we aimed at developing a Geographic Information System-based overlay on the spatio-temporal distribution of Aedes species collected from four local government areas (LGAs) in Lagos State (urban; Yaba and Somolu, and rural; Ikorodu and Badagry). Adult trapping was conducted for three consecutive nights on a monthly basis from October 2021 through August 2022, using BioGents lure baited traps. A total of 2,204 mosquitoes were collected and identified revealing 2,148 Aedes mosquitoes belonging to two species: Aedes albopictus (94.7% of specimens) and Aedes aegypti (5.3% of specimens). Results indicated dominant occurrence of Aedes albopictus in the four LGAs in comparison to Aedes aegypti. The highest number of Aedes albopictus was collected from Somolu (accounting for 80.5% of collection records), followed by Badagry (8.1%), Ikorodu (6.2%) and Yaba (5.2%). Of the 114 collected Aedes aegypti, 72% were collected from Somolu compared to other LGAs (22.8% Ikorodu, 5.2% Yaba and 0% Badagry). Both Aedes species were most prevalent during the rainy seasons (accounting for 67% of total) compared to the dry seasons.

5193

RESIDUAL OF ANOPHELES ARABIENSIS AND A. MELAS IN CENTRAL SENEGAL

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Understanding the behavior and ecology of local malaria vectors is essential for the effectiveness of the commonly used vector-targeted malaria control tools in areas of low malaria transmission. This study was conducted to
Determine species composition, biting behavior and infectivity of the major Anopheles vectors of Plasmodium falciparum in low transmission settings in central Senegal. Adult mosquitoes were collected using human landing catches during 2 consecutive nights and Pyrethrum Spray Catches in 30-40 randomly selected rooms, from July 2017 to December 2018 in 3 villages. Anopheline mosquitoes were morphologically identified using conventional keys; their reproductive status assessed by ovary dissections, and a sub-sample of An. gambiase s.l. were identified to species level using polymerase chain reaction (PCR). Plasmodium sporozoite infections were detected using real-time quantitative PCR. During this study 3684 An. were collected of which 97% were An. gambiase s.l., 0.6% were Anopheles funestus, and 2.4% were An. pharoensis. Molecular identification of 1,877 An. gambiase s.l. revealed a predominance of An. arabiensis (68.7%), followed by An. melas (28.8%), and An. coluzzii (2.1%). The overall human-biting rate of An. gambiase s.l. was highest in the inland site of Keur Martin with 4.92 bites per person per night, while it was similar in the deltaic site, Diofor (0.51) and the coastal site, Mbine Coly (0.67). Parity rates were similar in An. arabiensis (45%) and An. melas (42%). Sporozoite infections were detected in both An. arabiensis and An. melas with the respective infection rates of 1.39% (N = 8) and 0.41% (N = 1). Results suggest that low residual malaria in central Senegal is transmitted by An. arabiensis and An. melas. Consequently, both vectors will need to be targeted as part of malaria elimination efforts in this area of Senegal.

TOWARDS ENVIRONMENTAL SURVEILLANCE OF THE INVASIVE VECTOR SPECIES ANOPHELES STEPHENSI IN SUB-SAHARAN AFRICA

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Anopheles stephensi is a highly competent malaria vector whose historical distribution encompassed the Indian subcontinent, parts of South-East Asia and the Arabian Peninsula. Recently it has become an invasive vector species in the Horn of Africa, where it was first reported in Djibouti in 2012 and since then has spread to Ethiopia, the Republic of Sudan, Somalia, Yemen and most recently Nigeria and Kenya. Unlike An. arabiensis, the main regional malaria vector species, An. stephensi breeds in man-made water containers, buckets, discarded tyres, and water storage tanks for domestic use and construction. These are sites that may not be under routine surveillance by the National Malaria Control Program; such larval habitats are often used by arbovirus-transmitting Aedes species. To inform prospective control strategies, novel surveillance methods for tracking An. stephensi dispersal dynamics and insecticide resistance mechanisms are urgently required, which are both agnostic to mosquito larval morphology and simple to implement at the sampling stage. Using new multiplex TaqMan assays, specifically targeting An. stephensi and Ae. aegypti, we validated the use of environmental DNA (eDNA) for simultaneous vector detection in shared artificial breeding sites. Study findings demonstrated that An. stephensi and Ae. aegypti eDNA deposited by as few as one second instar larva in 1L of water was detectable. Characterization of molecular insecticide resistance mechanisms, using amplicon-sequencing panels targeting genetic fragments of the voltage-gated sodium channel, acetylcholinesterase and glutathione-s-transferase epsilon 2) for both vector species, was possible from eDNA shed by as few as 16-32 s instar larvae in 50ml of water. An. stephensi edNA, derived from emerging pupae for 24h, was remarkably stable, and still detectable 2 weeks later. eDNA surveillance has the potential to be implemented in local endemic communities and at points of country entry, to monitor the spread of invasive vector species. Ongoing community studies are validating the feasibility of this technique under field conditions.
were sampled using indoor CDC light traps from February to July 2022. Morphological and molecular identification techniques were used to identify the species and Plasmodium falciparum (Pf) infection status of collected Anopheles mosquitoes. The presence of knockdown resistance mutations (kdr) in An. gambiae complex was also investigated. Of the 715 female An. sp. mosquitoes collected indoors, 60.3% were identified as An. funestus s.l., 11.7% as An. gambiae s.l, and 28% as other Anopheles species. Molecular identification revealed that An. gambiae s.l comprised of 56.5% An. gambiae ss and 31% An. arabiensis. An. funestus group consisted of 83.3% An. funestus ss and 0.5% An. vaneeni. The overall Pf infection rate was 5.3% in An. funestus ss and 2.3% in An. gambiae ss. The Human Blood Index for An. funestus ss and An. gambiae ss was 100%. Notably, the 1014S (kdr-east), an allele associated with pyrethroid insecticide resistance, was identified for the first time in An. gambiae ss but only in heterozygotes. An. gambiae ss also exhibited a high frequency of the 1014F (kdr-west) kdr mutation. An. arabiensis were found to be wild type. The study highlights An. funestus as the primary malaria vector in the Alto Catumbela during the collection period. The low number of An. gambiae s.l captured indoors may indicate a possible behavioral adaptation to previous indoor vector control methods. The presence of kdr mutations in An. gambiae ss is concerning and warrants attention regarding potential pyrethroid insecticide resistance. Overall, this study provides valuable information on the abundance, species composition, and infection status of malaria vectors in the Benguela province, which can inform effective malaria control strategies in the region.

### 5197

**COMPOSITION AND SEASONALITY OF ANOPHELES GAMBAE S.L. AND AN. FUNESTUS S.L. IN LIBERIA**

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Vector monitoring is critical in understanding the potential dynamics of malaria transmission and making informed decisions in the selection of control interventions and evaluating their impact. To better understand malaria vector composition and seasonality in Liberia, we collected mosquitoes monthly from eight sites in seven counties from September 2021 to October 2022. Three adult mosquito collection methods were used: pyrethrum spray catch (PSC), human landing catch (HLC) and CDC light traps (CDC-LTs). A total of 7748 Anopheles gambiae s.l (76%), 2417 An. funestus s.l (24%) and 21 other Anophelines species (0.2%) were collected. More than 98% of mosquitoes collected were An. gambiae s.l. at Jackson Farm, Madina, Saint John and Obedin Camp. The population started to increase in March and reached peak from May to July, coinciding with the beginning of the rainy season. After July, the vector population start to decrease gradually. Anopheles funestus s.l was dominant in Zensanne (89%), where the population started to increase in January and peaked in March-May, which is during the dry season. During the collection period, the average number of An. gambiae s.l collected by method were: 0.87/house/day by PSC, 1.6/trap/night by CDC-LT and 5.8 bites/person/night by HLC; with a similar pattern observed for An. funestus s.l. Of the three collection methods, HLC was the most efficient in mosquito trapping. Twenty one percent (2138/10165) of the combined An. gambiae s.l and An. funestus s.l were collected from Zensanne, 19% (1882/10165) were from Saint John and the lowest was from Fissabo, 2% (250/10165). Malaria vector density and composition varied by site with a combined high peak over five months per year. Sites with higher density of malaria vectors, An. gambiae s.l. and An. funestus s.l., could indicate a higher risk of exposure for malaria transmission. Further molecular analysis for of An. gambiae complex and the An. funestus group will help to identify the species composition, parasite infection rate and host blood meal analysis to better understand trends in malaria transmission risk by space and time.

### 5198

**THE IMPACT OF FOUR YEARS OF INDOOR RESIDUAL SPRAYING WITH PIRIMIPHOS METHYL AND CLOTHIANIDIN ON ENTOMOLOGICAL DRIVERS OF MALARIA TRANSMISSION IN BURKINA FASO, WEST AFRICA**

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Since 2018, indoor residual spraying (IRS) has been performed by the National Malaria Control Program in the high-burden districts of Kampti and Solenko using SumiShield® 50 WG (clothianidin: neonicotinoid), Actellic® 300CS (pirimiphos-methyl: organophosphate) and Fludora Fusion® WP-SB (clothianidin + deltamethrin: pyrethroid) in Burkina Faso. Routine entomological surveillance was conducted to measure the impact of IRS on entomological drivers of malaria transmission. Anopheles gambiae s.l. were sampled monthly from June to December by human landing and pyrethroid spray catches in two sprayed and two unsprayed sentinel sites to measure entomological inoculation rates (EIRs). The residual activity of insecticides was also assessed using the WHO cone test. After the first round of IRS in 2018, there was a significant decrease in malaria transmission in all sprayed districts compared with unsprayed districts as shown by reduced EIR (RR=19.75, CI95%= [8.71-33.45], P<0.001). From 2019 to 2021, the EIR was significantly lower in Kampti compared to Gaoua (unsprayed district) (RR=13.12, CI95%= [4.35.71-21.18] P<0.001). In Solenko, the EIR varied from one year to the next, but the reduction was not significant. SumiShield® 50 WG and Fludora Fusion® WP-SB lasted more than 7 months covering the malaria transmission period, compared to Actellic® 300CS, which lasted 5 months. These findings highlight varying entomological impact of the IRS at the two sites, in an effort to reduce malaria transmission.

### 5199

**CIRCUMSPOROZOITE POSITIVE ANOPHELES LONGIPALPIS C MOSQUITO IDENTIFIED IN ZIMBABWE**

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Surveillance of malaria vectors in Zimbabwe has historically focused on the two major vectors Anopheles gambiae s.l. and An. funestus s.l., both morphologically indistinguishable species groups that require molecular tools for species identification. During routine malaria vector surveillance in rainy and fertile Buma Valley, 174 samples that were identified morphologically as An. funestus s.l., but failed to amplify when processed using the standardized An. funestus s.l. species ID multiplex PCR assay were sequenced for follow-up and 35 were identified as Anopheles longipalpis C. The finding prompted the Africa University laboratory to investigate the distribution and behavior of An. longipalpis C in Zimbabwe.
Of 1841 An. funestus samples, 529 that had previously amplified the double band pattern matching An. longipalpis C in the An. funestus PCR assay were revisited and analyzed for distribution, biting behaviour, and sporozoite parasite detection. The findings indicate that An. longipalpis C was first collected in 2016 in Buruma Valley and through 2022 in Beitbridge, Chakwaka, Buruma Valley, Zindi, Actururs, Uumba, Mubairakanda, Kawera, Makarara, Dandera, and Chiyadzwa. Of 180 blood fed samples, 74.4% were cattle-fed, 11.6% exhibited multiple host meals with a combination of human and animal blood, and 13.8% fed on other animals. Circumsporozoite ELISA testing found one Makarara sample from 2021 to be positive for Plasmodium falciparum (0.5% infectivity). In this study revealed An. longipalpis C to be widely distributed in Zimbabwe and supports that An. longipalpis C is still predominantly zoophagic, preferring cattle blood. However, the number of samples positive for combined animal and human blood and the single positive sample suggest An. longipalpis C, its distribution, and behaviour should be closely monitored by national malaria programs. This study highlights the importance of investigating PCR species identification results that don’t amplify, to characterize Anopheles species composition and detect previously unidentified Anopheles species and their potential contributions to the spread of malaria.

5200

RESURGENCE OF MALARIA IN UGANDA COINCIDES WITH AN INCREASE IN ABundance OF ANopheles FUNESTUS WITH EVIDENCE OF VARIATION IN SUSCEPTIBILITY TO CLOTHIANIDIN

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Historically, Anopheles gambiae mosquitoes are responsible for the majority of malaria transmissions in Uganda where vector control is reliant upon indoor residual spraying (IRS) or long-lasting insecticidal nets (LLINs). After 5 years of using Bendiocarb followed by Actellic® for IRS, in 2020 Uganda switched to clothianidin-based formulations (Fudora® and Sumishiled®). Following this switch, there has been an apparent resurgence in malaria cases, prompting an investigation. From 2022-23, a 12-month longitudinal survey was conducted in Tororo, one of the IRS districts, utilizing 60 households enrolled in a prospective cohort study. Vector abundance was estimated using CDC light traps, and assessment of the effectiveness of the IRS used wall cone bioassays and HPLC analysis of samples from sprayed surfaces. Resistance phenotyping was also performed using CDC bottle assays. The data showed a significant change in the abundance and composition of malaria vectors with An. funestus proportion increasing from 10.4% (8.9 - 12.1; 95% CI) of total collections before IRS with clothianidin to 65.2% (61.8 - 68.5; 95% CI) afterwards. Wall cone assays showed that the average mortality in An. gambiae mosquitoes was high (90% - 98%) up to 12 months post spraying. When tests were performed in the same houses, An. funestus had a significantly lower knockdown (7.3% (F=12.49, df=20, P=0.00083) and 48h mortality (76.1%) (F=3.34, df=20, P=0.0048) compared to An. gambiae (knockdown = 50.9%, 48h mortality = 98.2%). In CDC bottle assays, mortality of wild-caught An. gambiae was 100% at 48h post exposure. HPLC analysis revealed that there was a marked variation in the amount of insecticide on walls ranging from <5 mg/m2 to >200 mg/m2. All houses in all rounds of sampling had less than the recommended dosage of 0.5g/m2. Overall, these investigations suggest that the malaria resurgence may be driven by An. funestus that is more tolerant to clothianidin-based formulations than An. gambiae. Further investigations including full resistance characterization and examination of behavioural response to clothianidin treated surfaces is currently ongoing.

5201

IMPACT OF ENVIRONMENTAL MODIFICATION ON THE DYNAMICS, BEHAVIOR, TRANSMISSION RISK AND INSECTICIDE RESISTANCE OF MALARIA VECTORS: THE CASE OF ARJO-DIDESSA SUGARCANE IRRIGATION SCHEME, SOUTHWESTERN ETHIOPIA

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

5202

DISTRIBUTION OF ANOPHELES MOSQUITOES AND THEIR ROLE IN MALARIA TRANSMISSION IN SOUTHWESTERN ETHIOPIA

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The species distribution of malaria mosquitoes and their role in disease transmission varies from place to place. Hence, updating the species distribution and identifying their role is essential to design appropriate interventions. This study aimed to assess the Anopheles mosquito species and their infection rate in Southwest Ethiopia. A cross-sectional multistage sampling technique of adult malaria mosquitoes was done from June 2018 to July 2019. With a purposeful selection process, four malaria-endemic zones in the region, two malaria-endemic districts in each zone, and two malarious villages in each district were chosen. Ten per cent of households in each village were visited once to collect adult mosquitoes using Center
2019 mass-campaigns, and yearly IRS implemented from 2017 to 2021. As part of the regular monitoring on the impact of interventions, monthly entomological surveys were conducted using human landing catches to assess malaria vectors feeding behavior. Mosquitoes were collected both indoors and outdoors with four collectors for two consecutive nights in four selected houses. The indoor and outdoor human biting rates (HBRs) of the An. gambiae s.l. compared using the Kruskal-Wallis test. The mean HBR (bites/person/night) of An. gambiae s.l. was similar indoors and outdoors in 2017 (p = 0.27); in 2018 (p = 0.46), and in 2019 (p = 0.16). Contrary to the previous years, the biting activity was higher outdoors than indoor in 2020 (p = 0.04) and 2021 (p = 0.001). The results indicated the shift in biting behaviors of An. gambiae s.l., from equally feeding indoor and outdoor to predominantly outdoor feeding in Kiremba might be associated with continuous indoor vector control interventions in the area.

5203

UPD A TE D AS SE SS ME N T OF ANOPHELE S STEPHEN SI PRE NCE IN SOUTHER N Y EMEN

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The invasive malaria vector Anopheles stephensi has currently been the focus of attention in the horn of Africa and adjacent countries, since its 2013 introduction to East Africa. Although malaria is endemic in Yemen, the introduction of this vector to the country could drive urban malaria transmission, adding to the current malaria burden, traditionally transmitted by the native vector An. arabiensis in rural areas. Vector surveillance was conducted in nine Governorates covering all southern areas, including Socotra Island, with sites specifically selected from Aden, Lahij and Hadramout based on malaria cases and human populations’ records. Larval collection and spray catch methods were conducted to collect mosquitoes An. stephensi larvae were found either alone or coexisting with other species in artificial and natural containers in urban settings. Geospatial and prediction maps were generated to show vector distribution. Species composition, spatio-temporal distribution, and population dynamic of both An. stephensi and An. arabiensis were observed. The current invasive vector’s population dynamic, establishment, the associated sociocultural factors, and the optimization of control measures will be discussed.

5204

CHANGES IN THE BITING BEHAVIOR OF ANOPHELES GAMBIAE S.L. FOLLOWING THE COMBINATION OF MASS-DISTRIBUTION CAMPAIGNS OF INSECTICIDE-TREATED NETS AND INDOOR RESIDUAL SPRAYING OVER FIVE YEARS IN KIREMBA, NORTHERN BURUNDI

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In Burundi, malaria remains a major public health problem, and was responsible for 48% of outpatient consultations and 59% of hospital deaths in 2021. Anopheles gambiae s.l. is the major malaria vector, and insecticide-treated nets (ITNs) and indoor residual spraying (IRS) are the core vector control interventions in the country. Unlike the other entomological monitoring sites in the country, Kiremba health district in northern Burundi received both ITNs, distributed between the 2017 and 2019 mass-campaigns, and yearly IRS implemented from 2017 to 2021.

Pyrethroid-treated nets were distributed in 2017 and 2019 whereas, for IRS a carbamate insecticide (bendiocarb) was sprayed in 2017 and 2019 followed by Fludora Fusion (a mixture of neonicotinoid and pyrethroid insecticides) up to 2021. As part of the regular monitoring on the impact of interventions, monthly entomological surveys were conducted using human landing catches to assess malaria vectors feeding behavior. Mosquitoes were collected both indoors and outdoors with four collectors for two consecutive nights in four selected houses. The indoor and outdoor human biting rates (HBRs) of the An. gambiae s.l. compared using the Kruskal-Wallis test. The mean HBR (bites/person/night) of An. gambiae s.l. was similar indoors and outdoors in 2017 (p = 0.27); in 2018 (p = 0.46), and in 2019 (p = 0.16). Contrary to the previous years, the biting activity was higher outdoors than indoor in 2020 (p = 0.04) and 2021 (p = 0.001). The results indicated the shift in biting behaviors of An. gambiae s.l., from equally feeding indoor and outdoor to predominantly outdoor feeding in Kiremba might be associated with continuous indoor vector control interventions in the area.

5205

SURVEILLANCE OF Aedes-Borne Arboviruses in Selected Sites in the Savanna Region of Ghana

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Aedes-borne arboviruses such as yellow fever (YFV), dengue (DEN), chikungunya (CHIKV), and zika (ZIKV) have increasingly become a critical public health concern worldwide. Many countries all over the world including Africa have experienced emergence and re-emergence of arboviruses in the past and in recent times. In October 2021, Ghana experienced yellow fever outbreak in the Savanna region. Recent studies showed antibodies to Dengue virus serotype-2 among febrile illness patients in some areas of the Greater Accra region although the virus has not been detected in vectors. Therefore, this study assessed the prevalence of Aedes mosquitoes in Sawla, Larabanga and the Mole National Park, as well as the arboviruses (YFV, DENV, CHIKV, and ZIKV) they may be harboring, as a follow up to the yellow fever outbreaks in these areas. A cross-sectional study was conducted in three study sites (Sawla, Larabanga and Mole National Park). Adult Aedes mosquitoes were collected using the Biogent sentinel traps and immature stages (eggs, larvae, and pupae) were sampled using ovitraps and dippers. The immature stages were raised to adults for identification using morphological keys/features and viral analyses using RT-PCR. Data analysis was performed using two-way ANOVA in excel. A significantly higher number of eggs were collected in Sawla as compared to Larabanga and the Mole National Park (P < 0.05). The positive ovitraps index (POI) was high (>10) in all three sites; Sawla: POI = 50%; Larabanga: POI = 50%; Mole National Park: POI = 60%. All the Aedes mosquitoes identified from the three sites were Ae. aegypti, specifically of the subspecies Ae. aegypti formosus. No arbovirus (YFV, DENV, CHIKV, and ZIKV) was detected from the mosquitoes collected after analysis by RT-PCR. The study found that there is a high-risk of Aedes-borne arboviruses in the study areas and regular surveillance is needed to prevent and contain outbreaks.

5206

PILOTING THE USE OF TRANSFLUTHRIN-TREATED EAVE RIBBONS AS A SUPPORTING VECTOR CONTROL TOOL IN A HIGH TRANSMISSION SETTING IN ZAMBIA

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Nchelenge District, Zambia still experiences holoendemic malaria transmission despite over ten years of annual indoor residual spray (IRS)
and intermittent insecticide treated net (ITN) distribution. High levels of resistance to pyrethroids have been demonstrated among the most prominent vector, Anopheles funestus, which may contribute to the lack of effect of these indoor interventions. We examined the insecticidal activity, protective effect, longevity, and acceptance of transfluthrin-treated eave ribbons in Nchelenge District, Zambia. Two household clusters were identified, and transfluthrin-treated eave ribbons were distributed to every household in cluster 1, while cluster 2 received no additional intervention beyond their existing ITNs and annual IRS. Anophelines were collected monthly from 40 households using indoor and outdoor CDC light traps and Prokopack aspirations indoors. Anophelines were morphologically identified and tested for the presence of Plasmodium falciparum sporozoites.

Household-level surveys identified key human behaviors that may play a role in the success or failure of the intervention, including evening outdoor activities, outdoor sleeping, and overall acceptance of the spatial repellent. We hypothesize that these ribbons will reduce overall indoor and peri-domestic abundance in treated households, leading to reduced contact with humans and reduced pathogen transmission.

5020
TREATED EAVE SCREENS IN COMBINATION WITH SCREENED DOORS AND WINDOWS, ARE MORE EFFECTIVE THAN UNTREATED EAVE SCREENS IN A SIMILAR COMBINATION IN REDUCING INDOOR AND OUTDOOR ANOPHELES POPULATIONS UNDER SEMI-FILED CONDITIONS IN WESTERN KENYA

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Human dwellings remain the main point of human-mosquito interaction leading to malaria transmission despite sustained use of insecticide-treated nets (ITNs) and indoor residual spraying (IRS). Simple structural modifications have great potential to prevent mosquito entry into houses and reduce malaria transmission. The study utilized four huts, each constructed inside a separate semi-field structure (SFS) for the experimental release of mosquitoes. Two huts had screened eaves, doors, and air cavities in place of windows while the other two were unscreened. In experiment 1, the eave screen was untreated while in Experiment 2, the eave screen was treated with Actellic® insecticide. The modification cost was less than 250USD/structure. First filial (F1) generation of Anopheles funestus from Siaya, F0 reared from An. arabiensis larvae collected from Ahero and An. arabiensis Dongola strain from the insectary were raised to 3-day-old adults and used in experiments. Two hundred, 3-day old adults of each species were released in each semi-field structure at dusk and recaptured the following morning. The presence of at least one cow (RR=2.682, 95% CI: 1.492 – 4.320, p = 0.001) and dog (RR=1.895, 95% CI: 1.531-2.346, p<0.001) were associated to the presence of at least one sheep (RR=0.345, 95% CI: 0.125-0.953, p=0.04). The outdoor catches, An. gambiae mosquitoes indoors was related to increased catches of An. gambiae mosquitoes indoors. The indoor catches of An. gambiae mosquitoes were negatively associated to the presence of at least one goat (RR=0.035, 95% CI: 0.125-0.953, p=0.04). The outdoor catches, An. gambiae mosquitoes indoors was related to increased catches of An. gambiae mosquitoes indoors. The indoor catches of An. gambiae mosquitoes were negatively associated to the presence of at least one goat (RR=0.709, 95% CI: 4.279-11.715, p<0.001) and chicken (RR=0.383, 95% CI:0.185-0.631, p<0.001). The number of An. funestus indoors was higher in houses with chicken (RR=11.627, 95% CI: 2.111-64.032, p = 0.005) than those with no chicken. The study shows that the association between livestock keeping and malaria transmission is controversial as there are livestock which increase malaria vectors while others decrease them indoors and outdoors. Thus, zooprophylaxis and other livestock-based malaria interventions should be used with other interventions for malaria control.

5208
IMPACT OF LIVESTOCK MANAGEMENT ON MALARIA TRANSMISSION RISKS IN RURAL TANZANIA

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Livestock keeping is one of potential factors associated with malaria transmission. To date, the impact of livestock keeping on malaria transmission is contradicting with some studies reporting a zooprophylaxis effect while others reporting zoopotentiation effect. This study aimed to assess the impact of livestock management on malaria transmission risks in a malaria endemic region in south-eastern Tanzania. A longitudinal study was done in Minepa village. Forty randomly selected houses were sampled, 20 had livestock and others had no livestock. Daily mosquito collection was done in 8 houses each day, to ensure each house was visited once per week from January to March 2023. Indoor collections used CDC-Light traps and Prokopack aspirators. Outdoor collections used human-baited double net traps and nesting buckets. Poisson GLMM was used to assess the influence of livestock on mosquito density. A total of 18,620 female Anopholes mosquitoes were collected. Out of these, 98% were An. gambiae s.l. while others were An. funestus, An. pharoensis, An. coustani and An. squamosus. The presence of at least one cow (RR = 2.682, 95% CI: 1.492 – 4.320, p = 0.001), dog (RR=1.895, 95% CI: 1.531-2.346, p<0.001) and chicken (RR=8.387, 95% CI: 4.667-15.073, p < 0.001) near houses was related to increased catches of An. gambiae mosquitoes indoors. The indoor catches of An. gambiae mosquitoes were negatively associated to the presence of at least one sheep (RR=0.345, 95% CI: 0.125-0.953, p=0.04). The outdoor catches, An. gambiae mosquitoes indoors was related to increased catches of An. gambiae mosquitoes indoors. The indoor catches of An. gambiae mosquitoes were negatively associated to the presence of at least one goat (RR=0.709, 95% CI: 4.279-11.715, p<0.001) and chicken (RR=0.383, 95% CI:0.185-0.631, p<0.001). The number of An. funestus indoors was higher in houses with chicken (RR=11.627, 95% CI: 2.111-64.032, p = 0.005) than those with no chicken. The study shows that the association between livestock keeping and malaria transmission is controversial as there are livestock which increase malaria vectors while others decrease them indoors and outdoors. Thus, zooprophylaxis and other livestock-based malaria interventions should be used with other interventions for malaria control.
Fertility, lifespan, fecundity, and egg viability were comparable with the local Dhaka mosquitoes, and wAbB-infected males were competitive with local males in mating experiments. Given the lack of substantial fitness cost to host mosquitoes, the insecticide resistance profile of its infected mosquito strain, and the potential for virus blocking, the Wolbachia wAbB strain is likely to offer a viable alternative to dengue control in Bangladesh.

3D-SCREENS FOR SUSTAINABLE MALARIA CONTROL: OUTCOMES OF PHASE II SEMI-FIELD EVALUATION AND STUDY DESIGN OF A LARGE-SCALE PHASE III EVALUATION IN NORTHEASTERN TANZANIA

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The increasing prevalence of insecticide-resistant malaria vectors has created a need for sustainable vector control alternatives that are non-reliant on insecticides. A novel insecticide-free window screen, called the 3D-Screen, has been developed to exploit mosquitoes’ innate attraction to humans. The screen is composed of 3D conical structures with a perforated tip of 5mm and fully open base of 5 cm diameter that are fitted on a traditional screen (100 cones/m2). When installed as a double screen setup in window openings, forming the 3D-Window Double Screen Trap (3D-WDST), its unidirectional function allows mosquitoes to pass from outside only, trapping them between the double screens. In phase I laboratory studies (2015), the 3D-Screens demonstrated a remarkable efficacy, capturing 92% of the host-seeking mosquitoes in a double screen setup. In phase II semi-field studies, we installed 3D-WDST in window openings of experimental huts and found that the 3D-Screens installed on both sides of the window openings (outside and inside) in huts with open eaves were more effective in trapping female Anophelines (FA) than other conditions (efficacy: 33.11%, CI: 7.399 – 58.81). The introduction of baffles in the huts with 3D-WDST resulted in higher trapping efficacy of 70.32% (CI: 56.87 – 83.77) for FA. Comparison of 3D-Screen with machine-made (MM) and handmade (HM) cones showed no significant difference in the weekly mean catch (P=0.1887), however the integral superiority of MM over HM made it the preferred choice for further testing. To elucidate the efficacy of 3D-Screens in community settings, we conducted a large-scale phase III studies in Muheza, Tanzania, enrolling 892 houses from 14 hamlets, 7 of which received the 3D-WDST and insecticidal nets (LLINs) as intervention and 7 received LLINs only as control in a two-arm cluster randomized controlled trial. Follow up studies on malaria infection, entomological parameters, ancillary social aspects, and cost-effectiveness were conducted during the 52 weeks follow up. Final findings is expected to be released this year addressing the research gap on sustainable vector control approaches.

3521

3520

EVIDENCE OF TRANSMISSION OF PLASMODIUM VIVAX 210 AND P. VIVAX 247 BY ANOPELES GAMBAE AND AN. COULUZII MAJOR MALARIA VECTORS IN BENIN

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Historically, malaria in sub-Saharan Africa has been almost exclusively attributed to Plasmodium falciparum (Pf). Current diagnostic and surveillance systems in Benin are not designed to accurately identify or report non-Pf human malaria infections, resulting in a dearth of routine epidemiological data on their significance. This study aims to assess and compare the prevalence of circumsporozoite protein (CSP) antibodies of Pf, P. vivax (Pv) 210 and Pv 247 in An. gambiae s.l. in 24 communes of Benin. For that, mosquito collections were performed through human landing catches (HLC) and pyrethrum spray catches (PSC). For HLC, 384 collectors were used overnight while 240 bedrooms were used for PSC. Collected mosquitoes were morphologically identified, and Pf, Pv210 and 247 CSP antibodies were sought in An. gambiae s.l through the ELISA and polymerase chain reaction (PCR). Of the 3273 collected mosquitoes, 20.9% (n=6857) were An. gambiae s.l. 3.9% (n=1292) An. funestus gr., and 0.6% (n=189) An. nil gr. Molecular species identification performed in An. gambiae s.l. revealed that, An. gambiae (53.68%) was the predominant species, followed by An. coluzzii (45.88%), and An. arabiensis (0.44%). In An. gambiae s.l., the sporozoite rate (SR) was 2.6% (95% CI: 2.1-3.1) for Pf, against 0.30% (95% CI: 0.1-0.5) and 0.2% (95% CI: 0.1-0.4) respectively for Pv 210 and Pv 247. Pf sporozoite positive mosquitoes were mostly An. gambiae (64.35%), followed by An. coluzzii (34.78%) and An. arabiensis (0.86%). At the opposite, the Pf 210 sporozoite positive mosquitoes, An. coluzzii and An. gambiae accounted respectively for 76.92% (10/13) and 23.08% (3/13). Only An. coluzzii was found infected to Pv247. Overall, the present study shows that P. f is not the sole Plasmodium species involved in malaria cases in Benin. In addition, An. gambiae and An. coluzzii have different ability to get infected to the different identified Plasmodium species. These results will help the National Malaria Control Program to better plan the therapeutic management strategy of malaria cases to move towards the pre-elimination of the disease.

MOSQUITOCIDAL ACTIVITY OF IVERMECTIN-TREATED NETTINGS AND SPRAYED WALLS ON ANOPELES GAMBAE


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Ivermectin (IVM) has been proposed as a new tool for malaria control due to its mosquitocidal effect on malaria vectors when they blood feed on treated humans or cattle. Nevertheless, IVM may have a direct insecticidal effect on vectors when applied on bed nets or sprayed walls. We conducted a pilot study to measure the direct mosquitocidal effect of IVM on Anopheles gambae. Laboratory-reared mosquitoes (Ksumu) were exposed to IVM on impregnated netting materials and sprayed walls (plastered and mud) at a determined discriminating dose using core bioassays. Mosquito survival was assessed at 24, 48 and 72 hour post-exposure and compared with positive (deltamethrin (DM) and pirimiphos methyl) and negative (no insecticide) controls. Mosquitoes were also blood-fed either 12 hours pre- or post-exposure and monitored for oviposition. Hazard rates for IVM mortality was modeled using Cox proportional hazards model. Over 850 mosquitoes exposed to IVM discriminating concentration of 28mg/ml (2.8% w/v) died within 6 hours when exposed to IVM-treated nettings, within 18 hours for sprayed walls. Mosquito survival on the IVM-treated nettings was significantly lower than that of DM-treated nettings. However, mosquitoes survived significantly longer on the IVM-sprayed walls (100% mortality at 18 hr) compared to positive control walls (100% mortality at 6 hr) (Log rank X²=14.03, p<0.001). The adjusted Cox model predicted a hazard rate of 0.24 (95% CI: 0.19-0.30; p<0.001) with IVM compared to DM or pirimiphos methyl. Moreover, a significant interaction was found between treatment and surface predicting a three-fold higher hazard with IVM-treated nettings [HR=3.1 (95% CI: 2.21-4.23); p<0.001] but not with IVM-treated plastered walls [HR=1.1 (95% CI: 0.7-1.7); p=0.077]. IVM also inhibited mosquito blood feeding and oviposition regardless of exposure being pre-or post-feeding. Our findings confirm the direct mosquitocidal effect of IVM on An.
gambiae and suggest that IVM could be deployed as a new insecticide against malaria vectors together with the other currently used insecticides for long-lasting insecticidal nets and indoor residual spraying.

5213

IN SILICO ANALYSIS AND DESIGN OF A MOLECULAR CONSTRUCT TO TARGET THE BETA TUBULIN2 GENE IN ANOPHELES GambiAE

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The increasing expansion of vector resistance to insecticides requires finding alternative control methods to achieve malaria elimination. Genetic control is one of the promising approaches to control malaria vectors. In the context of genetic control of malaria vectors, genes involved in reproduction are of crucial importance to replace or suppress a vector population. The search for male-specific transcripts and proteins could lead to a better understanding of testicular specificity signals, whether at the promoter level, at splicing, or during translation. The regulatory sequences of these genes can also be used in genetic control strategies to engineer gene drive systems to disseminate desired traits including pathogen resistance or sex distortion. Beta tubulin2, is one of the genes involved in gamete formation which could be further exploited to optimize the efficiency of genetic control methods under development. Here, we developed in silico, a molecular construct to target beta tubulin2 gene in Anopheles gambiae to interfere with mosquito fertility, basing on Drosophila beta tubulin2 gene which has three putative orthologs in An. gambiae (AGAP010929, AGAP008622 and AGAP008623). Specifically, this is to identify the ortholog of the beta tubulin2 gene in An. gambiae, and to design knockdown and knockout strategies of the beta tubulin2 gene with RNA interference and CRISPR Cas9 technology respectively. A double-stranded RNA of 154 nucleotides was generated for the knockdown and a guide RNA was designed to knockout AGAP008622 gene via the CRISPR/Cas9 strategy. All these designs will be introduced into the mosquito for an in vivo experimental study to characterize the beta tubulin2 gene function in Anopheles.

5214

IDENTIFICATION OF ODORANT CO-RECEPTOR GENE IN ANOPHELES GambiAE AND IN SILICO DESIGN OF STRATEGIES TO STUDY ITS FUNCTION IN A VECTOR CONTROL PERSPECTIVE

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The most effective strategies to control malaria aimed to prevent the mosquito from biting. The African malaria mosquito shows an incredible resistance to antimalarial drugs and insecticides. Target Malaria, is a research consortium that aims to develop and share new genetic mosquito control tools for integrated malaria control strategies. In July 2019, in Burkina Faso (BF), the consortium proceeded with the first release of a genetically modified (GM) strain of Anopheles coluzzii called Ac(DSM)2. The Ac(DSM)2, was created through backcrossing Ag(DSM)2 transgenic females from a previously-established early dominant embryo lethality-inducing strain. In June 2019, a large cohort of the strain was produced, sexed, and males dusted with fluorescent powder. A single release of 6,428 hemizygous Ac(DSM)2 males and 8,422 non-transgenic male siblings was conducted in the village of Bana (BF). After 17 days, 527 dusted males were collected from swarms and houses and Polymerase Chain Reaction analysis revealed 145 of these to be Ac(DSM)2 males. GM males were recaptured 50.8 - 497m and siblings 50.8 – 1,678m from the release point. A Bayesian approach showed, that GM males were found to have significantly shorter daily survival rates than their wild type siblings (0.55 - 0.63 vs 0.73-0.77 survival day-1) and were also less mobile (diffusion rates 11.200 - 20,100 m2/day-1 vs 28,800 - 75,700 m2/day-1). The male population size at the time of the release was estimated to be in the range 28,000-37,000. These results provide information about the fitness and behaviour of GM males released at the start of the rainy season. The first release of genetically modified mosquitoes in Sub-Saharan Africa is an important milestone towards future releases of more effective strains targeting the sibling species of the An. gambiae complex.

5216

DENGUE VECTOR HABITAT CREATION IN PUBLIC PLACES: AN UNINTENDED CONSEQUENCE OF THE INSTALLATION OF PUBLIC HANDWASHING STATIONS FOR COVID-19 PREVENTION IN OUAGADOUGOU, BURKINA FASO 2020

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The Ac(DSM)2, was created through backcrossing Ag(DSM)2 transgenic females from a previously-established early dominant embryo lethality-inducing strain. In June 2019, a large cohort of the strain was produced, sexed, and males dusted with fluorescent powder. A single release of 6,428 hemizygous Ac(DSM)2 males and 8,422 non-transgenic male siblings was conducted in the village of Bana (BF). After 17 days, 527 dusted males were collected from swarms and houses and Polymerase Chain Reaction analysis revealed 145 of these to be Ac(DSM)2 males. GM males were recaptured 50.8 - 497m and siblings 50.8 – 1,678m from the release point. A Bayesian approach showed, that GM males were found to have significantly shorter daily survival rates than their wild type siblings (0.55 - 0.63 vs 0.73-0.77 survival day-1) and were also less mobile (diffusion rates 11.200 - 20,100 m2/day-1 vs 28,800 - 75,700 m2/day-1). The male population size at the time of the release was estimated to be in the range 28,000-37,000. These results provide information about the fitness and behaviour of GM males released at the start of the rainy season. The first release of genetically modified mosquitoes in Sub-Saharan Africa is an important milestone towards future releases of more effective strains targeting the sibling species of the An. gambiae complex.
Public places can be an important source of breeding habitats of Aedes aegypti, the main dengue vector, but data to support this evidence are missing in Africa. This study conducted to fill this gap for Ouagadougou, coincided with the COVID-19 pandemic and the consequent installation of handwashing stations (HWS). We investigated the abundance and diversity of water holding containers in some public places of Ouagadougou, including HWS, and assessed their contribution to Ae. aegypti immature stages proliferation. Between September and October 2020, water-holding containers were systematically inspected for Aedes mosquito immature stages in 61 public places of Ouagadougou. All collected immature mosquitoes were counted, identified to genus. Breeding containers abundance and preference were estimated and generalised mixed models were fitted to larval and pupal densities. A total of 924 containers that were considered suitable for Ae. aegypti breeding habitat were identified, of which HWS and tires had the highest proportions of positive containers (37.42% and 37.27% respectively), followed by small containers (SC) (13.03%). At its peak, 44.73% and 41.02% of the total production (produced by all type of containers encountered in public places) of Ae. aegypti larvae and pupae, respectively were produced by HWS. Tires, bucket/can/pot (BCP) and SC followed in terms of larval and pupae productivity. Additionally, some containers were hosting immatures of non-Ae. aegypti mosquito species, including Anopheles gambiae s.l. and Culex species. In conclusion, containers in public places contribute to increase Ae. aegypti immatures densities in Ouagadougou and should be taken into account for dengue control. The major role of newly introduced HWS in Ae. aegypti immatures productivity recommends risk assessment prior to introduction of new tool in the public areas.

RETHINKING INSECTICIDE TREATED NET (ITN) DISTRIBUTION: A REVIEW OF CURRENT DISTRIBUTIONS SYSTEMS, COSTINGS AND CHALLENGES

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While malaria cases and deaths reduced steadily between 2000 and 2009, continued progress has stalled in parallel with annual funding gaps. To optimize the impact of continued limited resources, there is an even greater need to maximize the efficient, effective, and equitable targeting and use of malaria vector control strategies. Countries where malaria is endemic must be able to identify and deploy an optimal mix of vector control interventions suited to their local context, established priorities, and available resources. Insecticide Treated Net distribution through mass campaigns have been the mainstay of vector control interventions for over 20 years. Mass campaigns and continuous distribution channels have delivered over 2.2 billion nets in Sub-Saharan Africa and have been largely responsible for the impressive declines in malaria morbidity and mortality since 2000. However, many factors require new thinking and flexible approaches to ITN distribution including inadequate and inequitable targeting resulting in many households receiving nets they may not need or use while others remain without access, increasing insecticide resistance requiring more expensive nets with dual actives, challenges with durability with nets not lasting as long as expected, decreasing compliance with use particularly in urban areas and increasing fatigue from some partners for mass campaigns. These factors, combined with funding gaps and human resource constraints, necessitate a rethinking of ITN distribution through mass campaigns with a renewed focus on the alternative distribution mechanisms. Findings from a modelling study of continuous distribution versus mass distribution in Tanzania, costings studies of cost per net delivered (mean USD 6.29) from continuous distribution activities in four countries, durability monitoring data showing the period between net receipt and use, and findings from scaling up from pilots to national scale school-based distribution will be presented in order to highlight some of the factors to be considered in order to optimize ITN coverage, increase use, and maximize the impact of available funds.

ASSESSING INSECTICIDE RESISTANCE IN TWO MALE-BIASED ANOPHELES Gambiae S.L. TRANSGENIC STRAINS

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The transgenic (TG) mosquito insecticide susceptibility must be determined, in comparison to that of the wild-type (WT), as part of the risk analysis performed prior to their release into the natural environment. Increased resistance could make the TG mosquitoes more difficult to control or could introduce novel resistance levels or mechanisms into the wild mosquitoes. A strain of Anopheles coluzzii, BF_Ac(PMB)1, and one of Anopheles gambiae, UG_Ag(PMB)1, contain a transgene that biases the sex ratio of progeny from TG fathers to > 95% males. These strains were previously developed by repeatedly backcrossing the transgene from Ag(PMB)1 into a WT strain of A. coluzzii from Burkina Faso (BF_Ac(WT)) or a WT strain of A. gambiae from Uganda (UG_Ag(WT)). This study compared TG mosquitoes to their WT counterparts for each introgressed strain. The insecticide susceptibility of females was determined using the standard World Health Organization (WHO) adult insecticide exposure kits and discriminating doses recommended by the WHO. The panel of insecticides included four pyrethroids (alpha-cypermethrin, lambda-cyhalothrin, permethrin, and deltamethrin), a carbamate (bendiocarb), an organophosphate (fenitrothion), and an organochlorine (DDT). Exposures were for 1 h except for fenitrothion which was for 2 h, as recommended by WHO. Mortality was determined 24 h later. Larvae were also exposed to a larvicide that is widely used in Africa, temephos, to determine susceptibility to that insecticide. TG females showed no increases in resistance to eight different insecticides representing four different classes when compared with their WT controls for either strain. When any resistance to insecticides was observed, it was detected in both TG and WT individuals for either strain. Overall, our data demonstrate the transgene has no effect on insecticide resistance for the two transgenic strains tested under the study conditions tested. Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the U.S. Centers for Disease Control and Prevention.

ASSESSING VECTOR COMPETENCE FOR PLASMODIUM FALCIPARUM AND O’NYONG-NYONG VIRUS IN A MALE-BIASED ANOPHELES COLUZZII TRANSGENIC STRAIN

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Anopheles mosquitoes vector the etiological agents of important diseases including malaria and O’nyong-nyong virus (ONNV) fever. Because insecticide resistance levels are rising in vector populations, alternative control tools are urgently needed. A promising approach is the use of transgenic (TG) mosquitoes to suppress or modify wild populations. Before such TG mosquitoes can be considered for testing in the field, potential risks to the environment or health caused by their release must first be assessed. As part of a wider vector control development program,
we assessed the impact of a male-biasing transgene introgressed into an *Anopheles coluzzii* strain from Burkina Faso on vector competence for *Plasmodium falciparum* and ONNV. Previously, we have shown no significant difference in *P. falciparum* vector competence in TG females compared to their wild-type (WT) counterparts. In the current study, we determined if a potential reduction in egg production, a phenotype previously observed in females that mate with TG males, affects the *P. falciparum* infection rates by comparing infection rates in females from two crosses: 1) WT females x TG males, and 2) WT females x WT males. No significant differences were observed between groups (P > 0.05), in terms of oocyst counts 7 days post-infectious blood meal (d piBM) (median WT: 21, TG: 25), with a slight significant reduction in prevalence in cross 1 (1 = 91.3%, 2=97.5%, P= 0.047). Also, no significant increase in sporozoite intensity or prevalence at 11 and 15 d piBM was seen in either group. For ONNV, the infection, dissemination, and transmission potential rates of TG females were compared to that of WT females. No significant difference was found in the three parameters measured between the two groups. Overall, our data suggest no effect of the transgene, the genetic background or the reduced fecundity on *P. falciparum* infection rates or ONNV infections for any of the parameters analyzed. Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the U.S. Centers for Disease Control and Prevention.

**5220**

**MOLECULAR AND BIOINFORMATIC CHARACTERIZATION OF THE INTROGRESSION OF A MALE-BIASING TRANSGENE INTO AN UGANDAN LOCAL WILD-TYPE STRAIN**

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To ensure that a transgenic mosquito strain that may eventually be released into the field reflects that of the wild population, a transgene from a founding transgenic strain generated in a laboratory line, is then introgressed into genetic backgrounds of colonies recently established from the field?. However, alleles potentially associated with adaptations to laboratory rearing conditions and to the transgenic flanking regions may be inherited with the selected locus to[LaE(1) [BGA(2)] the introgressed strain, even when they are separable by recombination. Ag(PMB)1 is a transgenic *Anopheles* gambiæe strain that during male spermatogenesis expresses the l-Pol variant W124L fused to eGFP, leading to approximately 95% male offspring. The subsequent introgression of the PMB1 transgene into an *A. gambiae* s.s wild-type (WT) strain from Uganda (UG_Ag(WT)) led to the generation of the UG_Ag(PMB)1 strain. This study describes the introgression process and the molecular characterization of the introgression after six repeated backcrosses of Ag(PMB)1 transgenic females with UG_Ag(WT) males. Life-history traits (i.e. egg yield, hatching rates, etc.), SNP genotyping analysis via KASP assays, and 2La inversion analysis via PCR were conducted. The recorded life-history traits revealed that the newly introgressed strain UG_Ag(PMB)1 is robust and demonstrated sufficient rates in terms of egg yield, hatching, and pupal eclosion to maintain and amplify the colony under lab conditions. Preliminary SNP analyses suggest that the introgressed strain has a similar genotype profile to the UG_Ag(WT) strain for the X and 3L chromosomes, while chromosome 2L carries allelic forms from both parents. Finally, the 2La genotyping showed that both arrangements of the 2La inversion are present in the WT parental and the introgressed strain. Overall, this study presents a phenotypic and molecular characterization of the PMB1 transgene introgression process into the genetic background of a Ugandan WT strain after six backcrosses.

**5221**

**MARK, RELEASE AND RECAPTURE EXPERIMENT OF A LABORATORY STRAIN OF ANOPHELES COLUZZII IN TWO VILLAGES IN MALI**

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Regular surveys are usually carried out to understand the composition, structure, and abundance of vectors. However, these surveys do not make it possible to estimate the size of the vector population of the locality because the fraction of the people collected is not known. One of the most widely used methods of estimating population size is the “mark-release and recapture” method. Tagging, release, and recapture experiments were carried out in the villages of Tiéneguebougou and Ouassorola in Mali for two consecutive years (August 2016, July 2017, and November 2017). For each experiment, approximately 5,000 adult male mosquitoes (*Anopheles coluzzii*) were released. Swarming sampling was the most productive method for collecting male mosquitoes in the field. The size of the population was estimated in Tiéneguebougou in August 2016 (106,103) greater than that observed in July 2017 (11,546) and November 2017 (29,227), this same phenomenon was observed in Ouassorola, the size of the population observed in August 2016 (90,674) is higher than that observed in July 2017 (19,046) and in November 2017 (19,559). The results of the three mark, release, and recapture experiments show a statistically significant difference between the recapture rates obtained in August 2016 (0.52%), July 2017 (2.07%), and November 2017 (1.09%), this difference was also observed in the village of Ouassorola during August 2016 (1.76%), July 2017 (1.60%) and November 2017 (0.75%). The daily survival rate for Tiéneguebougou is 0.26 in August 2016; 0.35 in July 2017 and 0.22 in November 2017 and that of Ouassorola: 0.35 in August 2016; 0.38 in July 2017, and 0.29 in November 2017. The average distance covered by males varied from 40 m to 100 m.

**5222**

**MOLECULAR STRATEGIES TO DEPLOY SINGLET OXYGEN AS AN UNASSAILABLE BIOCIDE FOR DISEASE PREVENTION AND VECTOR CONTROL**

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Singlet oxygen (1O2) is a potent biocide potentially deployable for integrated control of tropical diseases and their insect vectors. This is a very short-lived oxidative radical, but is highly destructive of cellular molecules when generated intracellularly. Parasites and insects are defenseless against 1O2. Only plants have evolved specific mechanism to detoxify 1O2 by necessity, as it is produced abundantly during photosynthesis. In the presence of atmospheric O2, exposure of certain dyes, e.g., porphyrins and phthalocyanines (PC) to light produces 1O2. Its half-life is only in the order of µs, necessitating its intracellular generation to feasibly harness its biocidal necessity, as it is produced abundantly during photosynthesis. In the presence of atmospheric O2, exposure of certain dyes, e.g., porphyrins and phthalocyanines (PC) to light produces 1O2. Its half-life is only in the order of µs, necessitating its intracellular generation to feasibly harness its biocidal activity effectively. One example is genetic engineering of *Leishmania* to complement its inherent defects in porphyrin biosynthesis, resulting in cytotoxic accumulation of abundant uroporphyrin 1 (URO). Another example is chemical engineering of PC for hydrophilicity and cationicity, facilitating its endocytosis by cells. Leishmania doubly loaded with cytotoxic URO
and endosomal PC are inactivated by dim light to completion. These inactivated Leishmania preserved their natural vaccines and adjuvants with prophylactic activities against experimental leishmaniasis. Preliminary data further show the potential of 102 inactivated Leishmania as a platform for safe and effective delivery of transgenically add-on vaccines against malignant and viral diseases in mouse models. Hydrophobic and catonic PC were also shown experimentally to represent a new type of light-activated insecticides, i.e., their mosquito larvicidal activities, featuring the requirement of dim light with <1μM LD50 values. Similar results have been obtained by studying PC in additional laboratory insect models. A significant advantage has long been attributed to this type of insecticides, i.e., their aversion to selection of genetic variants for resistance. An additional advantage of PC is their excitability to produce insecticidal 1O2 by deep-penetrating red/IR light invisible to insects, thereby potentially increasing considerably the range and scope of targetable insect vectors.

5223

DISCOVERING NATURAL PRODUCT CHEMISTRIES FOR VECTOR CONTROL

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Natural products (NPs) represent diverse chemical structures and, potentially, modes of action of vector control importance. NPs have inspired the development of multiple synthetic insecticides, suggesting the discovery of novel NPs for the development of highly effective insecticides needed to control insecticide-resistant vector populations. Here, we report two independent studies performed to identify novel mosquito-active insecticides with modes of action distinct from existing insecticides used in mosquito control programs. In the first study, we performed a high-content larval phenotypic screening using first instar larvae of Aedes aegypti against 3,680 compounds from the AnalytiCon MEGx Natural Product Libraries and a screening platform developed, as reported previously. Screening revealed five chemistries that caused larval mortality, including rotenone, the detection of which confirmed the ability of the screen to detect mosquito-active NP chemicals. 140 chemicals that caused atypical larval phenotypes, including cuticular pigmentation and morphometric changes relative to negative controls, were also identified by the screen. Some of these chemistries may act via disruption of pathways regulating mosquito melanization, growth and development, including potentially unique targets in the insect nervous systems, thus representing important opportunities for novel insecticide development. In the second study, we performed electrophysiological recordings using the suction electrode technique and ganglia of Blattella germanica to investigate the mode of action and impact on the insect nervous systems of metergoline and NP-1, two chemistries identified by HTP screening. Results suggested metergoline and NP-1 may act via serotonic pathways and one or more conserved targets in the insect nervous systems, respectively, making them potential leads for the development of new insecticides that could be used to control insecticide-resistant populations. Results will be reviewed and future lines of research proposed in the context of new insecticide development.

5224

IMPACT OF USING DIFFERENT TYPES OF MOSQUITO TRAPS TO ASSESS ENTOMOLOGICAL EFFICACY OF DUAL-ACTIVE INGREDIENT LONG-LASTING INSECTICIDAL NETS (LLINS) IN BENIN

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Selection of mosquito sampling traps is of crucial importance to evaluate impact of vector control tools on entomological outcomes. During a cluster randomised control trial evaluating the relative efficacy of two dual-active ingredient (a.i.) nets compared to pyrethroid-only nets, we assessed performance of different mosquito trap types: Human Landing Catch (HLC), CDC light traps, and Pyrethrum Spray Catch (PSC). Anopheles mosquitoes were collected with the three trap types in 4 houses in each of the 60 trial clusters at baseline and every quarter for 24 months using PSC and HLC, while CDC light traps were performed during two quarter only. The density of An. gambiae s.s and its Plasmodium falciparum sporozoite infection were assessed. Mean density of vectors collected per trap per night was the highest with HLC (15.9), followed by CDC light trap (6.8), with the PSC (1.1) collecting 10 times less mosquitoes than HLC. All three trap types showed that the lowest mosquitoes density was collected in the Intecceptor G2® dual a.i. arm compared to the other arms, although only HLC and PSC demonstrated strong evidence of this due to a greater number of collection rounds than CDC light traps. Furthermore, CDC light traps and PSC measured similar reductions in SR and EIR (“CDC light trap only” as compared to HLC between study arms. The broadly similar results between trap types suggest that the more ethically acceptable, cheaper and logistically simpler methods such as CDC light traps could be prioritised for use in large community trials for measuring efficacy of vector control tools.

5225

EFFICACY OF PYRETHROID-PYRIPROXYFEN AND PYRETHROID-CHLORFENAPYR LONG-LASTING IMPregnATED NETS (LLINS) FOR THE CONTROL OF NON-ANOPHELES MOSQUITOES: SECONDARY ANALYSIS FROM A CLUSTER RANDOMIZED CONTROLLED TRIAL

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Failure to control nuisance of mosquitoes may potentially affect adherence to vector control tools. The present study compares the vector density of Culex spp and Mansonia spp across the two dual a.i. LLINs and the standard pyrethroid-only LLINs and assessing the seasonality of these mosquito species. 85,723 Culex spp and 144,025 Mansonia spp were collected with the three trap types in 4 houses in each of the 60 trial clusters at baseline and every quarter for 24 months using PSC and HLC, while CDC light traps were performed during two quarter only. The density of An. gambiae s.s and its Plasmodium falciparum sporozoite infection were assessed. Mean density of vectors collected per trap per night was the highest with HLC (15.9), followed by CDC light trap (6.8), with the PSC (1.1) collecting 10 times less mosquitoes than HLC. All three trap types showed that the lowest mosquitoes density was collected in the Intecceptor G2® dual a.i. arm compared to the other arms, although only HLC and PSC demonstrated strong evidence of this due to a greater number of collection rounds than CDC light traps. Furthermore, CDC light traps and PSC measured similar reductions in SR and EIR (“CDC light trap only” as compared to HLC between study arms. The broadly similar results between trap types suggest that the more ethically acceptable, cheaper and logistically simpler methods such as CDC light traps could be prioritised for use in large community trials for measuring efficacy of vector control tools.

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Efficacy of Pirikool® 300 CS Used for Indoor Residual Spraying on Three Different Substrates in Semi-Field Experimental Conditions

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Vector control using insecticides is a key prevention strategy against malaria. Unfortunately, insecticide resistance in mosquitoes threatens all progress in malaria control. In the perspective of managing this resistance, new insecticide formulations are being urged to improve the effectiveness of vector control tools. The efficacy and residual activity of Pirikool® 300CS was evaluated in comparison with Actellic® 300CS in experimental huts at the Tiassalé experimental station on three substrates including cement wood and mud. The mortality, blood-feeding inhibition, exiting behaviour and deterrence of free-flying wild mosquitoes was evaluated. Bioassay cone assays with susceptible and resistant mosquito strains were conducted in the huts to determine residual efficacy. A total of 20505 mosquitoes of which 10979 (53.5%) wild female Anopheles gambiae were collected for 112 nights. Residual efficacy obtained from monthly cone bioassay was higher than 80% with the susceptible, laboratory-maintained Anopheles gambiae Kismu strain from the first to the tenth study period on all three types of treated substrate for both Actellic® 300CS and Pirikool® 300CS. This residual efficacy on the wild Tiassalé strain was over 80% until the 4th month of study on all Pirikool® 300CS and Actellic® 300CS treated substrates. Overall 24-hour mortalities of wild free-flying An. gambiae s.l. which entered the experimental huts over the 8-months trial on Pirikool® 300CS treatment was 50.5%, 75.9% and 52.7% respectively on cement wall, wood wall and mud wall. The positive reference product Actellic® 300CS treatment induced mortalities of 42.0%, 51.8% and 41.8% on cement wall, wood wall and mud wall. Pirikool® 300CS has performed against resistant strains of An. gambiae s.l. using indoor residual spraying in experimental huts. It could be an alternative product for indoor residual spraying in response to the vectors’ resistance to insecticides.

Measurement of Oocyst and Sporozoite Infection Rates in Anopheles Gambiae S.l. Under Natural Conditions in Bancoumana, Mali

The dynamics of mosquito infections play a central role in Plasmodium 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 Anopheles 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 speciations). 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 (2.7%) with mean of 2.1 oocysts [range 0-15]. 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.

Evaluating Mosquito Behavior during Exposure to Different Insecticide-Treated Nets (ITNs) Using Video Cone Test

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The increasing resistance of mosquitoes to the active ingredients in Insecticide treated Nets (ITNs) has created a need for the development of effective vector control methods and testing protocols. The Video Cone Test (VCT) builds on the World Health Organization (WHO) cone bioassay by incorporating a behavioural record of mosquito interactions with ITNs. The objective of this study is to assess the behavioural traits of mosquitoes when exposed to various types of ITNs. To achieve this objective, the study involved exposing 2-5 day old Anopheles gambiae mosquitoes to different ITNs (PermaNet® 2.0, PermaNet® 3.0, and Olyset®) for varying lengths of time (1, 2, 3, 4, 5, and 6-minutes) using the WHO cone bioassay. During the process, mosquito behaviour was recorded using a smartphone, and the activities or movements of mosquitoes in different regions of the cone (Region-0, Region-1, Region-2 and Region-3) were depicted. After exposure, the mosquitoes were given a 10% sugar solution, and mortality was recorded 24 hours later. The study found significant differences in the number of activities observed in each cone region between the two mosquito strains (p<0.05), with Region-1 recording the highest activity in both strains. Olyset® did not exhibit any variation between the two strains in any of the regions (p>0.05), while PermaNet® 2.0 and PermaNet® 3.0 showed variations between the strains in Regions 1, 2 and 3 (p<0.05). Mortality was strongly associated with all regions of the cone (p<0.00), with Regions 2 and 3 showing the strongest associations (coefficients = 1.661457 and 1.35458, respectively). These results indicate that different types of ITNs have varying effects on mosquito behaviour. Furthermore, the WHO cone bioassay, with the addition of a camera component can provide valuable insights into mosquito behaviour.

Key Entomological and Malaria Indicators during the Periods of Indoor Residual Spraying with Pirimiphos-Methyl and Clothianidin-Based Products in Zambia

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Zambia deployed pirimiphos-methyl for IRS from 2013 -2018, before switching to clothianidin products from 2019 -2022, given its duration of efficacy was found to be >10 months compared to 4 -5 months for pirimiphos-methyl. The trends in entomological and malaria indicators were reviewed from 2015 -2018 and from 2019 -2022. This analysis investigates whether pirimiphos-methyl should be reintroduced for IRS in Zambia. We analyzed two available and complete entomological indicators— indoor density (ID) and human biting rate (HBR)—collected in 10 sentinel sites from 2015 -2022, as well as annual malaria incidence rates in 116 districts from 2013 -2022. Only one out of the 10 sites had entomological monitoring.
data covering the deployment periods of both insecticides; the rest of the sites covered only one of the periods. Mixed effect models were used with random intercept per site and insecticide as fixed effect controlling for the month of collection and rainfall. We found no significant differences in ID and HBR between the pirimiphos-methyl versus clothianidin periods for either An. gambiae s.l. (ID 0.45 vs 0.53, p=0.8141, HBR Indoor 1.31 vs 3.81 p=0.8696, HBR outdoor 1.71 vs 3.37 p=0.1612) or An. funestus s.l. (ID 2.70 vs 2.84 p=0.9817, HBR indoor 11.29 vs 19.09 p=0.7912, HBR Outdoor 8.37 vs 9.93 p=0.9664). The malaria incidence rate was significantly lower for pirimiphos-methyl (94.6 per 1.000) compared to clothianidin (613.2 per 1.000) (p<0.0001). This preliminary investigation indicates that the change from pirimiphos-methyl to clothianidin products did not result in a reduction in vector numbers despite the difference in duration of efficacy while malaria incidence rates increased during the period of clothianidin deployment. While these results suggest that pirimiphos-methyl could be safely reintroduced into the IRS insecticide rotation with no significant adverse effect on program outcomes, more analyses controlling for other confounders are still needed.

5230

MOSQUITO TRAPPING BEDNET (T-NET) FOR INSECTICIDE RESISTANCE MANAGEMENT AND MALARIA CONTROL

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Malaria burden is increasing. An estimated 241 million de cases and 627 000 deaths occurred worldwide in 2020. About 228 million cases were recorded in 2018 while in 2017, the WHo reported 219 million cases and 435,000 related deaths comparing to 216 million cases reported in 2016, which had already increased about 5 million cases over 2015. This situation is problematic and highlights the urgent need to develop new malaria control strategies. The widespread insecticide resistance in malaria vectors is considered as the principal reason why the LLINs along with IRS which have been critical to malaria prevention, are now failing to control the disease. To overcome this problem, we have developed a mosquito trapping bednet, the so-called T-Net which has the particularity to trap and kill mosquitoes regardless of their insecticide resistance status. Field testing in WHO-recommended experimental huts in Africa showed a 4.3-fold greater trap-kill rate of insecticide-free T-Net compared to Permanet 2.0, the most common bednet in Africa. A T-Net population model developed from field data to predict community-level mosquito control showed that the insecticide-free T-Net under field conditions against pyrethroid resistant mosquitoes was 12.7-fold more efficacious than single chemical, pyrethroid-treated nets. The current presentation covers recent findings obtained from ongoing studies on insecticide resistance and diversity. It is clear that the T-Nets are effective in reducing malaria incidence and transmission and can be used in conjunction with other interventions to control malaria.

5232

IN SILICO DESIGN OF MOLECULAR MODEL TO STUDY THE SIFAMIDE GENE FUNCTION IN ANOPHELES GAMBIAE OLFACTORY SYSTEM, IN A PERSPECTIVE OF GENETIC CONTROL OF THE VECTOR

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Despite decades of control effort, malaria still cause a public health problem. Fortunately, the main vector of the disease Anopheles gambiae has a weakness: it uses its sense of olfaction to target the human host on which feed. Several genes including SIFamide regulate this behaviour in the vector. The overall objective of this study is to develop in silico strategies to access the function of the SIFamide in An. gambiae. For this purpose, the EnsemblMetazoa database was used to identify the orthologue in the mosquito. Then, tools such as BLAST-NCBI, CD-Search, FlyAtlas2 and MozAtlas was used to confirm this orthologue using Drosophila melanogaster as reference. Also, a knockout model was generated in silico for the orthologue by using CRISPR-Cas9 technology with the CHOPCHOP and Benchling tools. The orthologue identified is AGAP007056. The similarity and identity percentages between the protein and nucleotide sequences, demonstrate the conservation of the gene in insects. Similar expression pattern of the gene was found in An. gambiae and Drosophila melanogaster. The orthologue is targets for the siRNA expression. For this purpose, the expression in An. gambiae and Drosophila melanogaster tissues but appears to be more expressed in the male than female. This could be explained by the fact that the male feeds exclusively on nectar and needs a regular supply of sugar for its survival. Otherwise, the absence of a protein domain could be due to a lack of annotation or that the gene really doesn’t have a domain that has remained conserved through evolution. For the knockout technology, the gRNA has 20 bp in size and an efficiency score of 66.62%. It targets a conserved region of exon 2 and [%GC] = 65. The plasmid used for homology repair contains basic features such as a green fluorescent protein flanked by homology arms (1500 bp) immediately upstream and downstream of the Cas 9 cleavage site. Our constructs show a high efficiency as demonstrated by the e-values less than 0.05. Better understand the phenotype associated to SIFamide expression will allow us to select this trait in a gene drive approach or other strategies targeting the same process in the vector.

5231

VALIDATION OF A METHOD FOR DRY PRESERVATION AND REHYDRATION OF ANOPELEHS GAMBIAE SENSE LATO FOR PARITY ANALYSIS TO ASSESS IMPACT OF VECTOR CONTROL MEASURES IN THE FIELD

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The control of malaria is still heavily dependent on mosquito control interventions. With progress in malaria control stalling in recent years, it is essential to understand the impact of interventions on vector populations. Age grading is a valuable method for determining whether interventions alter the age structure of target mosquito populations, but current methodologies are logistically challenging to incorporate into clinical trials and routine surveillance. We validated a method for dry preserving mosquitoes using silica gel and rehydrating prior to parity assessment using the ovarian tracheation method. Lab-reared Anopheles coluzzi mosquitoes with known parity-status were dry-preserved in silica gel for 1, 2, 6, 9 and 12 weeks, and rehydrated prior to parity assessment. Results were compared to parity results from freshly-killed mosquitoes from the same colony. Following lab validation, field-caught An. gambiae s.l. from the Bijagós Archipelago, Guinea-Bissau, were assessed by three different assessors who were blinded to each other’s scores. Inter-rater reliability (IRR) was calculated for all assessor-pairings, and an overall index of agreement was calculated using the arithmetic mean of these IRRs. The impact of time preserved was investigated using a one-way ANOVA to look for differences in assessor agreement over three timeframes; (1) 16-70 days (2) 71-90 days and (3) 91-110 days. When dry-preserved and rehydrated, the parity status of 90.1% of insectory-reared An. coluzzi were correctly identified compared to 97.8% in freshly-killed mosquitoes. IRR of freshly-killed An. coluzzi was highest (0.94). Results at all time points showed excellent strength of agreement between assessors. For field-caught An. gambiae s.l., the overall index of agreement between all three assessors was 0.86 (95% CIs 0.78-0.93) indicating an almost perfect agreement. Dry preserving and rehydrating Anopheles mosquitoes to assess the efficacy of a control intervention provides an excellent and feasible alternative to using freshly-killed mosquitoes in remote settings where standard methodologies are logistically infeasible.
A SEMI-FIELD EVALUATION OF THE USE OF HUMAN LANDING CATCHES VS HUMAN-BAITED DOUBLE NET TRAPS FOR ASSESSING THE IMPACT OF A VOLATILE PYRETHROID SPATIAL REPELLENT AND PYRETHROID-TREATED CLOTHING ON ANOPELEHS MINIMUS LANDING

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The mosquito landing rate measured by human landing catches (HLC) is the conventional endpoint used to evaluate the impact of vector control interventions on human-vector exposure. Non-exposure based alternatives to the HLC are desirable to minimize the risk of accidental mosquito bites during evaluations. One such alternative is the human-baited double net trap (HDN), but the estimated personal protection of interventions using the HDN has not been compared to the efficacy estimated using HLC. This study evaluates the performance of the HLC and the HDN for estimating the effect on mosquito landing rates of two intervention types characterized by contrasting modes of action, a volatile pyrethroid spatial repellent (VSPR) and insecticide-treated clothing (ITC). Experiments were performed to estimate the impact of both interventions on mosquito landing using the two methods. A block randomized cross-over design was carried out over 32 nights with both the HLC and HDN. Eight replicates per combination of collection method and intervention or control arm were conducted. For each replicate, 100 Anopheles minimus were released and were collected for 6 hours. For the VPSR, the estimated effect was similar for both methods; when measured by HLC (OR 0.007 95% CI (0.005, 0.001) p<0.001) and by HDN (OR 8.72e14 (0.0, Inf) p = 0.99), and no mosquitoes were recaptured by HDN. For the ITC, the protective efficacy was measured by HLC (OR 0.30 (0.23, 0.40) p<0.001), but there was no evidence of protection when measured by HDN (OR 1.04 (0.85, 1.27) p=0.69). Interplay between mosquitoes, bite prevention tools, and the sampling method result in vector behaviour changes which may impact the estimated protective efficacy. Consequently, sampling method must be considered when evaluating these interventions. The HDN is a valid alternative trapping method (relative to the HLC) for evaluating the impact of bite prevention methods that affect mosquito behaviour at a distance (e.g., VPSR), but not for interventions that operate through tarsal contact (e.g., ITC).

FIRST EVIDENCE OF THE PRESENCE OF THE WOLBACHIA AND MICROSPORIDIA MBITA IN NATURALS POPULATIONS OF ANOPELEHS GAMBIAE IN SOUTH OF BENIN

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The Wolbachia endosymbiont can have major effects on the reproductive capacity and vectorial capacity of host insects and can be tools to control mosquito-borne pathogens. Anopheles gambiae s.s is the main vector of malaria in Africa, but the use of Wolbachia in this species has been limited by difficulties in establishing stable transinfected lines and uncertainty surrounding native infections. The presence of Wolbachia and microsporidia have never been reported in An. gambiae s.s in Benin. For this study, we searched for the presence of Wolbachia and Mbita(MB) microsporidia in natural samples of An. gambiae s.l infected or not with Plasmodium falciparum collected in southern Benin (Cotonou, Porto-Novo, Calavi) over a period of 6 years. Our results showed that Wolbachia is present at a low prevalence in the natural population of An. gambiae s.l. Of 8435 samples analyzed, only 29 were positive for Wolbachia by nested PCR representing 0.34% prevalence. No positive samples were found with regular PCR. However, MB microsporidia were present at a high proportion of 20.62% or 1740 positives out of 8435. The results also showed a very low prevalence of Wolbachia and microsporidia on samples positive for Plasmodium falciparum, respectively 0.23% (1/296) and 1.68% (5/296). The absence of a positive sample with regular PCR is encouraging for applications using Wolbachia-transinfected mosquitoes for malaria control. These results will also enable the National Malaria Control Program to diversify the methods of combating malaria.

LABORATORY AND SEMI-FIELD EVALUATION OF BIO-EFFICACY AND PHYSICAL INTEGRITY OF OLYSET PLUS AND INTERCEPTOR G2 NETS AFTER THREE YEARS OF FIELD USE IN TANZANIA

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Long-lasting insecticidal nets (LLINs) provide protection against malaria vectors by its residual insecticidal activity even when its fabric is torn. Usage and washes during the LLIN's lifetime could result into loss of insecticidal component to an extent where users can be subjected to high risk of malaria transmission. To sustain gains in malaria control, LLINs should maintain high bio-efficacy and fabric strength for at least 3 years post distribution. Mortality and blood feeding inhibition (BFI) induced by 3 years old used Interceptor G2 (IG2) and Olyset plus (OP) LLINs were assessed following WHO guidelines at both laboratory and semi-field experimental hut trial. Both IG2 and OP LLINs were able to induce significant mortality and BFI to mosquitoes compared to the untreated LLIN (negative control). In cone bioassay, mortality induced by OP nets decreased significantly (50%, 46.3%) with washes and community usage respectively against susceptible KisuM strain, compared to IG2 (10%, 11.25%). Higher mortality and BFI were induced by IG2 LLIN than OP LLIN in laboratory tunnel tests (against KisuM strain and pyrethroid resistant Anopheles gambiae Mulaea-Kis), and semi-field experimental hut trial against pyrethroid-resistant An. arabiensis. However, the difference was not statistically significant. Similarly the bursting and tensile strengths, mesh size and fabric weight of the IG2 LLIN were higher than that of the OP LLIN with a decreasing trend from unwashed, laboratory washed to community usage. In general bio efficacy and fabric strength of IG2 LLIN was higher than that of OP LLIN. National malaria control programs should consider both bio-efficacy and fabric integrity of different types LLINs prior to LLINs procurement and LLIN replacement.

CRYOPRESERVATION AND THE OPTIMIZATION OF THE DEVELOPMENT OF WOLBACHIA IN THE CULEX PIPIENS MOSQUITO CELLS

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Wolbachia is common in insects and is an intracellular, maternally transmitted bacterium symbiont. These endosymbiotic bacteria can be mutualistic by, for example, enhancing their host’s nutrition and modifying immune responses to improve survival rates. Wolbachia can also act as a parasite by taking resources from their host to boost survival or by influencing host reproduction to increase their potential to proliferate in the host population. Wolbachia has recently been utilized to sterilize male mosquitoes for population control, but little is known about how it interacts with certain traits like diapause. An essential biotechnological tool for both fundamental and practical research is the insect cell culture system. This work’s goal was to demonstrate the usage of a Culex cell line for multiplying
The mean number of Anopheles specimens per swarm was 31.5 (Min = 5, Max = 120). Most of the swarms were located outside human settlements. The mean height of swarming was 2.0 meters (Min = 1m, Max = 3.5m) above the ground. This study showed that most of the swarming markers were created by anthropogenic activities and were located outside of human dwellings making them easily accessible for destruction.

HOST-FEEDING PREFERENCES AND TEMPERATURE SHAPE THE DYNAMICS OF WEST NILE VIRUS: A MATHEMATICAL MODEL ENDEAVOR

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Host-feeding preferences and temperature shape the dynamics of West Nile virus: a mathematical model endeavour. Suman Bhowmick,1 Rebecca Lee Smith1,2,3,4 1Department of Pathobiology, University of Illinois, Urbana-Champaign, Urbana, Illinois, USA; 2Sumanb@illinois.edu; 3Carl R. Woese Institute for Genomic Biology, University of Illinois, Urbana-Champaign, Urbana, Illinois, USA; 4Illinois College of Medicine, University of Illinois, Urbana-Champaign, Urbana, Illinois, USA

West Nile virus (WNV) is the leading cause of mosquito-borne disease in the United States. It is most commonly spread to people by the bite of an infected mosquito. The impact of WNV on human health is widely predicted to increase in coming years as the temperature warms, since mosquito biology and disease ecology are strongly linked to environmental conditions. However, direct evidence linking these changes to the traits of mosquito and the ecological mechanisms that may underpin such changes are poorly understood topics. Transmission of WNV within the host community primarily is predicted by the relative abilities of the host to maintain and disseminate the virus and different eco-environmental factors. Related to that ability, there is an increase of evidence that shows strong preferences by mosquitoes for certain host species can dictate the dynamics of WNV and potentially govern the spill-over into mammals, such as humans, horses, and dogs. We have developed a mechanistic transmission model for WNV in one vector species (Culex pipiens) and preferred avian hosts based weather driven mosquito traits. Sensitivity analysis has revealed that feeding preference is one of the most influential parameters on intensity and timing of peak WNV infection. Our studies show that heterogeneous contact rates induced by host preference are a key factor in the WNV epizootics in multi-species host communities.

CHARACTERIZATION OF ANOPHELINE SWARMS DURING THE DRY SEASON ALONG THE NIGER RIVER, MALI

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Previous studies in Mali have implicated riverbeds as malaria hotspots during the prolonged dry season. Those Anophele populations found on riverbeds sustain malaria transmission throughout the dry season. They also serve as inoculums for both the transmission and the spread of insecticide resistance in surrounding areas at the onset of the rainy season. Mosquito swarm physical destruction is an alternative control intervention to reduce insecticide-resistant vector population density. This study aims to characterize the swarming behavior of Anophele populations during the dry season. This is in the prelude to their physical destruction as a control intervention along the Niger River in Mali. We conducted an active search for Anophele swarms, starting 30 minutes before sunset during 3 successive days in and around each fishing hamlet located along the Niger River. For each detected swarm, the following characteristics were recorded: type of marker, height, size, and coordinates of the markers. In the fishing hamlets along the river, there were 84 swarming places. The main type of swarm markers was related to anthropogenic activities and included bundles of wood for cooking (30.8%), bare ground (29.1%), piles of garbage (12.8%), walls (12.8%), latrines (5.1%), and brick (4.3%). The mean number of Anopheles specimens per swarm was 31.5 (Min = 5, Max = 120). Most of the swarms were located outside human settlements. The mean height of swarming was 2.0 meters (Min = 1m, Max = 3.5m) above the ground. This study showed that most of the swarming markers were created by anthropogenic activities and were located outside of human dwellings making them easily accessible for destruction.

PREVALENCE OF BORRELIA BURGDORFERI SENSU LATO-INFECTED IXODES SCAPULARIS TICKS IN THE UNITED STATES AND CANADA: A COMPREHENSIVE REVIEW

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Increasing densities and geographic expansion of ixodes scapularis, the primary vector of Borrelia burgdorferi sensu lato (Bbssl), poses new risks for Lyme disease (LD). The objective of this review was to summarize tick surveillance data reported in high LD endemic areas in the US and Canada with a focus on the prevalence of Bbssl-infection in I. scapularis ticks. We conducted a literature search in PubMed from 2006 to 2023 to identify studies reporting on Bbssl-infection prevalence in questing I. scapularis ticks. Data were excluded if they were from low endemic regions (western and southern US, and western Canada), larvae, non-l. scapularis species, and studies collecting less than 50 ticks. Bbssl-infection prevalence was calculated as the number of ticks infected divided by the number of ticks tested. Descriptive analyses were performed to estimate the prevalence of Bbssl-infected I. scapularis ticks, stratified by county, surveillance type, tick life stage, and region. Of 3169 articles identified, 76 met inclusion criteria. A total of 239,161 ticks (n=156,107 in US; n=83,054 in Canada) collected between 1998-2019 were included in the analyses from 81 datasets (US=48; Canada=33). Overall, the mean prevalence of Bbssl-infected questing ticks was higher in the US than Canada (25.4% vs 22.3%; p<0.0001). Comparing regions and tick life stages, the mean prevalence of Bbssl-infected questing nymphs (21.1%) and adults (50.2%) in the US Northeast were significantly higher compared to those in the US Midwest (nymphs=17.7%; adults=34.7%) or Canada (nymphs=10.7%; adults=32.0%) (nymphs p=0.013; adults p=0.001). Increasing trends in the prevalence of Bbssl-infected questing ticks were observed from longitudinal data in both countries. Overall, the data reveal that the prevalence of Bbssl-infected I. scapularis ticks is high throughout high-endemic LD areas in the US and Canada. Regular assessment of densities of Bbssl-infected ticks in these areas is useful to understand the changing trends of tick infection prevalence and increasing LD incidence.

THE POSSIBLE MICROBIAL ETIOLOGY OF ALZHEIMER’S DISEASE AND RELATED DEMENTIA

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Neurocognitive disorders are sporadic, age-related, and hereditary conditions that can lead to multiple outcomes such as progressive cognitive impairment, psychiatric and behavioral disorders, and declines in daily life functions. Observational, epidemiological, experimental, and pathological studies have generated evidence for the possible polymicrobial causality in dementia-inducing diseases. The microbial hypothesis states that pathogens and microbes act as triggers, interacting with genetic factors to initiate the accumulation of beta-amyloid plaques, hyperphosphorylated tau protein, immunosuppression, and inflammation in the brain. Evidence indicates that Borrelia spp., HSV-1, treponemal spp., Chlamydia pneumoniae, Candida albicans, and others can lead to cytokine dysregulation, alterations in brain biochemistry and neurotransmission, neuronal degeneration, and neural death. These effects, usually manifested during aging, accumulate over time and ultimately result in neurodegeneration and dementia. In the present study, we aim to...
test the possibility that polymicrobial infections exist in post-mortem brain tissue samples from patients with Alzheimer’s disease using overlapping molecular methods. To establish a link between neurocognitive disorders and microbial or polymicrobial infections, autopsy samples will be examined with immunohistochemistry, highly sensitive polymerase chain reaction, and FISH in situ hybridization. The aforementioned methods were used to successfully detect Borrelia burgdorferi, Bartonella henselae, Treponema denticola, and Candida albicans in neural tissue. These findings further indicate that microbes should be considered in the etiology of neurocognitive disease. Though the etiopathogenesis of Alzheimer’s disease remains controversial, this study seeks to better elucidate the multifactorial neuropathology associated with dementia-inducing disorders as well as provide compelling evidence for the existence of persistent infection in brain tissue.

5241

EXAMINING THE ROLE OF NYMPHAL IXODES IN THE TRANSMISSION OF BORRELIA BURGDORFERI TO DOGS

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In the United States, Lyme disease (LD) is the most commonly reported vector-borne disease among humans. LD is caused by Borrelia burgdorferi which is transmitted to mammals through the bite of infected ixodes ticks. Nymphal ixodes, which quest in late spring and early summer, are responsible for the majority of transmission to humans; adult ticks are responsible for a subtler peak of cases in the fall. B. burgdorferi seroprevalence among dogs has been used to estimate LD risk among humans; however, past research has suggested that dogs are less susceptible to infection from nymphal ixodes, possibly indicating a different host preference between dogs and humans for these different life stages. To evaluate this, we reviewed LD serology results from serially tested dogs over a 9-month period to better understand timing of seroconversion and frequency of transmission by ixodes nymphs versus adults ticks to canine hosts. In 2016, blood was collected from 215 dogs at 3 timepoints (February, August, and November). In February, blood was tested by SNAP 4Dx Plus Test and Borrelia burgdorferi C6 ELISA. Testing was limited to C6 ELISA for later timepoints. Dogs were considered serologically positive for LD if they were positive by SNAP 4Dx Plus Test or Borrelia burgdorferi C6 ELISA. Of 161 dogs serologically negative for B. burgdorferi in February, 4 seroconverted by August. All 4 dogs resided in the East region. Of 146 dogs that were serologically negative in August, 15 seroconverted by November; dogs resided in the East (10), Mid-west (3), South (1) and West (1) regions. Although the majority of canine incident cases as indicated via seroconversion occurred during adult tick season, results indicate that four dogs became infected prior to adult tick season. Future studies may be warranted to better understand the role of nymphal ixodes ticks and B. burgdorferi transmission in dogs. Nymphal transmission of B. burgdorferi to dogs has important implications for dog-owners, veterinarians and researchers studying LD among dogs, especially in circumstances where dogs are used as proxies for human risk.

5242

MULTI-DRUG THERAPY IS REQUIRED TO EFFECTIVELY TREAT BARTONELLA INFECTION IN DIFFERENT ENVIRONMENTS

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Bartonella is a gram negative, facultative intracellular bacterium that manifests as different clinical syndromes collectively known as bartonellosis. The well-known diseases caused by these bacteria are cat scratch disease (B. henselae), trench fever (B. quintana) and Carrion’s disease (B. bacilliformis). Excluding B. bacilliformis, which is evolutionarily more distinct than the 30+ other species, Bartonella infections result in self-limiting disease that is often undiagnosed and untreated. However, individuals with compromised immune systems may experience clinical manifestations, which can become life threatening and need to be treated with effective antibiotics. To date, there is no standard treatment course for these infections and many doctors prescribe antibiotics based on limited case studies. It has been shown that Bartonella can grow extracellularly, intracellularly, and in biofilms. To determine an effective antibiotic strategy, it is important to understand Bartonella susceptibility in each of these growth conditions. We hypothesize that combination antibiotic treatments are required to effectively eliminate B. quintana and B. henselae growth, particularly in biofilm and intracellular environments. In previous studies, B. henselae treatment with single antibiotics in different media, as well as in DH121 canine macrophages, was ineffective in preventing growth. We plan to expand this work with different antibiotics supported by case reports, as well as double and triple combination therapy in erythrocytes and biofilms. Antibiotics tested were the following: doxycycline, gentamicin, azithromycin, azlocillin, rifampin, and clarithromycin. The effectiveness of combination therapy supports the notion that Bartonella species utilize target cells and biofilms as an antibiotic evasion strategy in the treatment of bartonellosis.

5243

DIVERSITY AND DNA BARCODING OF IXODIDAE AND ARGASIDAE TICKS IN THE US-MEXICO BORDER REGION OF THE MUNICIPALITY OF JUAREZ, CHIHUAHUA

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Ticks are the most important arachnids for human and animal health because they are vectors of a plethora of disease-causal agents, including bacteria and arboviruses. In the border municipality of Juarez, Chihuahua, severe cases of human rickettsiosis and a high prevalence of bacteria transmitted by ticks in domestic animals have been reported in recent years. Inadequate morphological identification due to similarity between morphospecies or identification of juvenile stages is a common problem of the prevention and control programs. As a result, DNA barcode technology is a reliable support tool for completing the morphological identification of ticks at the border between Mexico and the United States. This study reports the diversity and DNA barcode of ixodidae (hard) and argasidae (soft) ticks collected between 2018-2022 along this Mexican border municipality. Based on morphology and confirmed species identity using DNA barcoding, 3245 ticks belonging to four species were collected along Juarez municipality in Chihuahua, state. Ticks were identified as Rhipicephalus sanguineus, Demacentor albipictus, Obitius megnini, and Argas persicus. A Bayesian analysis was constructed with a sample of 65 Cytochrome Oxidase subunit I mitochondrial sequences of the ticks collected. The topology of Bayesian tree displayed three groups of R. sanguineus, whereas the clades of other species were well-defined. The Markov model of nucleotide substitution for distance estimation show a mean (±SE) of 18 (±1.0) %. The intraspecific distance ranged between 0.02 %, whereas the interspecific distance was reached 11.9 to 29.5 %. Dogs were the major host of Rh. sanguineus, whereas O. megnini were prevalent mostly on cows and horses. A. persicus were collected on soil, and D. albipictus were collected only on one deer. Finally, the public health importance of these species from the perspective of the public health of transboundary United States-Mexico region is also discussed.
3rd instar pools to 4th instar pools by 1.2. Pools from female adult louse for R. prowazekii and C. burnetii. Average B. quintana Ct (combined and IS1111a (Coxiella burnetii). 7 individuals submitted ectoparasites. All (B. genus), yopP and fabB (B. quintana), ompB (Rickettsia prowazekii) louse mitochondrial cytochrome b (cytB) and Phum_PHUM540560 genes, using real-time PCR. Louse species and ecotype were identified using the discarded infested clothing and separated into pools based on instar and adults seeking medical care in Winnipeg. Ectoparasites were collected from louse instar and sex. Ectoparasites were collected among consenting first on body lice, correlates B. quintana gene cycle threshold (Ct) with species and identify B. quintana and other pathogens. This study, Canada's largest cluster of Bartonella quintana endocarditis was described among individuals experiencing homelessness in Winnipeg, Canada. The aim of this study was to analyze ectoparasites collected between March 2023 and June 2023 among para-vertebrate workers of Pakistan to assess their awareness and response toward tick-borne disease management in animals. We will then analyze the survey to identify areas of low knowledge or ineffective practice in smallholder livestock settings. The survey has been designed and approved for dissemination, but most recruitment will be centered in May. We will use the results of this survey to design outreach and education materials for para-vertebrate workers to improve their understanding and practices for managing and controlling tick-borne diseases.

In 2020, Canada’s largest cluster of Bartonella quintana endocarditis was described among individuals experiencing homelessness in Winnipeg, Canada. The aim of this study was to analyze ectoparasites collected from individuals experiencing homelessness in Winnipeg to confirm vector species and identify B. quintana and other pathogens. This study, Canada’s first on body lice, correlates B. quintana gene cycle threshold (Ct) with louse instar and sex. Ectoparasites were collected from consenting adults seeking medical care in Winnipeg. Ectoparasites were collected from discarded infested clothing and separated into pools based on instar and sex. Ectoparasite pools were decontaminated and homogenized. DNA was extracted. Vector species, louse ecotype and pathogens were identified using real-time PCR. Louse species and ecotype were identified using the louse mitochondrial cytchrome b (cytB) and Phum_PHUM540560 genes, respectively. Pathogens were identified using the following targets: ITS3 (B. genus), ypP and fabB (Rickettsia prowazekii) and IS1111a (Coxiella burnetii). 7 individuals submitted ectoparasites. All ectoparasites were confirmed to be Pediculus humanus corporis using real-time PCR. Lice from one individual (14%) demonstrated B. quintana positivity: ITS3, ypP and fabB. Lice from all individuals were negative for R. prowazekii and C. burnetii. Average B. quintana Ct (combined average of ITS3, YopP and FabB) decreased from 1st and 2nd instar pools to 3rd instar pools by 6.5. Average B. quintana Ct decreased from 3rd instar pools to 4th instar pools by 1.2. Pools from female adult louse pools demonstrated lower Ct values than male pools. A minority of body lice collected from individuals experiencing homelessness in Winnipeg demonstrated molecular positivity for B. quintana. Body lice in Winnipeg do not appear be significant vectors for R. prowazekii or C. burnetii. Ct on B. quintana genes decreases with each advancing instar. Manitoban individuals with pediculosis should be evaluated for B. quintana infection.

Prevention of tick bites and tick-borne diseases (TBDs) is reliant on individual-level protection measures, such as use of repellants and frequent tick checks in high-risk areas. However, these require knowledge of local spatiotemporal risk, awareness of basic prevention measures, and concern sufficient to implement protection measures. Since 2018, we have conducted Knowledge, Attitudes, and Practices surveys within multiple populations for which ticks and TBDs are a concern in the state of Illinois, USA. These populations include employees of local public health departments (n=42), veterinary professionals (n=72), and medical professionals (n=346). We will compare knowledge scores, subscores and TBD concern levels among these different populations, and compare their responses to existing tick presence and abundance and TBD prevalence data at the county and regional level. For instance, medical professionals had a higher median tick knowledge score (69%) than veterinarians (59%) or public health officials (52%), but veterinarians had a higher median disease score (47%) than the other two groups (30%). These scores varied significantly by time since last training for both veterinarians and medical professionals. These findings can serve as the basis of a One Health approach to tick prevention outreach and training for those at the front lines of TBD prevention.

Plague is a zoonotic disease, transmitted to humans by flea bites infected with the Yersinia pestis bacterium. The plague transmission cycle is complex and can involve several mammal hosts with various susceptibilities to the disease, including humans. Fleas acquire the infection while feeding on the septicemic host and transmit it upon the next feeding. Thus, host specificity and preference determine the flea’s role in pathogen transmission, for the host blood can affect the flea’s ability to transmit Y. pestis. Puliculus irritans, the “human flea,” is one of the probable vectors in Madagascar where plague is endemic. Previous investigations demonstrated that P. irritans is the most abundant species in households and it has been found infected with Y. pestis during epidemics. Identifying P. irritans host blood source will give a new insight into better understanding the plague transmission cycle in Madagascar. Here we report the preliminary results from a study aiming to identify the host blood source of P. irritans collected from households in a plague-endemic area of Madagascar. The DNA of individual blood-engorged P. irritans was extracted and amplified using conventional multiplex PCR, with a primer set that can amplify DNA from humans, birds, and non-human mammals DNA. The amplified DNA was visualized using gel electrophoresis to identify which P. irritans samples were positive for the three types of hosts. Our results showed that from 376 individual P. irritans, 7.25% fed on human hosts, 6% fed on avian hosts, and 1% fed on

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non-human mammal hosts. All avian DNA-positive samples were positive with human DNA, and all non-human mammal-positive samples were positive with human and avian DNA. We could not detect host DNA from the remaining 20.75% of the fleas. We demonstrated that although this flea species is mostly anthropophilic, it can also feed on other hosts such as birds and mammals in Madagascar. Our finding raises concern about plague transmission, especially if P. irritans would take a blood meal from rodents, the main reservoir of plague in Madagascar. Our next step will be to identify the species involved for each non-human host.

5248

SPATIAL DISTRIBUTION AND MOLECULAR DETECTION OF RICKETTSIA SPP. IN RAT FLEAS IN MADAGASCAR
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Rickettsioses are infectious diseases caused by bacteria of the genus Rickettsia and are mainly transmitted by arthropod vectors. Most of them are transmitted by ticks but R. typhi (murine typhus) and R. felis (flea-borne spotted fever) are transmitted by fleas and can cause severe illness and death. The aim of this study was to detect Rickettsia spp. from the fleas infesting small mammals in areas with different bioclimatic conditions in Madagascar. The study was conducted in 28 districts across Madagascar. Urban and rural areas are randomly chosen for each district and small mammal traps were set across different habitats including house, vegetation, field, market and abattoir. Fleas were collected from small mammals and identified. The presence of Rickettsia spp. was assessed by qPCR specific for R. typhi and R. felis. A total of 3694 fleas belonging to Xenopsylla cheopis (97.0%) and an endemic flea Synopsyllus fonquerniei (3.0%) were collected from 2046 small mammals captured from 56 localities. Rattus rattus represented 71.3% of the total animals captured and R. norvegicus had high flea infestation of X. cheopis (61.2%). In this study, 1323 oral fleas X. cheopis and 100 endemic fleas S. fonquerniei were randomly tested. Rickettsia spp. was identified in 16.4% of X. cheopis (21/1323) and 5% of S. fonquerniei (5/100). For Rickettsia species, 5.6% and 3.9% of X. cheopis were positive for R. typhi and R. felis respectively. For the endemic flea S. fonquerniei, 4% were positive for R. felis and none for R. typhi. Confinement was found in 2 small mammals. Sixteen districts of the 28 were found infested with Rickettsia spp.. Infected fleas were found in all habitats mainly inside houses and in market place and the prevalence was higher in urban areas. Although no clinical case has been described in Madagascar, R. typhi was detected in naturally infected fleas from rats in Madagascar. The geographic distribution of this pathogen emphasizes the potential risk of flea-transmitted infections and the risk is high when animal carries many fleas. Investigations are needed to further understand the ecology of Rickettsia in fleas and their implications for human health.

5249

STATUS EPILEPTICUS AND MULTIORGAN INJURY IN A PATIENT WITH MURINE TYPHUS
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A 40 year old man with a history of epilepsy, well controlled on valproic acid for over a decade, presented with 2 weeks of fevers, headaches, and cough. Initial lab results showed leukocytosis, thrombocytopenia, and elevated transaminase, alkaline phosphatase, and creatinine levels. The patient developed generalized tonic-clonic seizures in the emergency room that progressed to status epilepticus, necessitating intubation and mechanical ventilation. A lumbar puncture (LP) was performed, showing an elevated opening pressure, a decreased cerebrospinal fluid (CSF) glucose level, elevated CSF protein, and an elevated CSF pleocytosis with neutrophil predominance. CSF cytologic exam showed plasma cells and plasmacytoid lymphocytes. MRI brain and CT chest returned unremarkable.

5250

MOLECULAR DETECTION, CYTLOGICAL CHARACTERIZATION, AND GENETIC HETEROGENEITY OF 16S RDNA OF HEMOTROPIC MYCOPLASMAS IN POPULATIONS OF SMALL MAMMALS IN TWO STATES OF BRAZIL
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Hemotropic mycoplasmoses are pleomorphic, non-cultivable bacteria attached to the red blood cell surface. They have been detected in human patients with immunodeficiency conditions or co-infected with other infectious agents. New species and genotypes have been described in wild animals. This study aimed to perform molecular detection, cytological characterization, and genetic heterogeneity of 16S rDNA of hemotropic mycoplasmas in small mammals from Rio de Janeiro and Parana, Brazil. A total of 258 small mammals were captured. The cytological analysis was performed using a blood smear stained with Gimsa solution. Molecular detection of agents from the Family Mycoplasmataceae was based on the 16S rRNA gene. The products amplified in the polymerase chain reaction (PCR) were selected and purified for subsequent sequencing and construction of the phylogenetic tree. There were 11 Mycoplasma spp. of small wild mammals from this study. They were joined to another 32 sequences, with Mycoplasmaoides fastidiosum as an outgroup, in a dataset of 1050 positions. Among the 258 samples of small wild mammals analyzed, 23.2% (n=60) presented structures compatible with Mycoplasma sp. in erythrocytes. 33.7% (n=87) samples amplified Mycoplasma sp. in conventional PCR. The region with the highest frequency of positivity was Cruz Machado (46.15%, n= 24/52), followed by Ponta Grossa (43.10%, n=25/58), Nova Friburgo (30.56%, n=33/108), and Lidianopolis (12.50%, n=5/40). Ogilvyzymys had the highest percentage of positivity (78.05%), statistically differing from Oxymyxterus spp. (42.11%), Akodon spp. (27.59%) and Sooretamys (9.09%). Males were more frequently parasitized with Mycoplasma spp. than females (p<0.001). Regarding the phylogenetic analysis, Mycoplasma spp. from this study grouped together with Ogilvyzymys nigripes from São Paulo and Minas Gerais, forming a clade with these sequences. This study revealed the morphological, eco-epidemiological, and phylogenetic aspects of Mycoplasma sp. in small non-flying wild mammals in regions of the states of Parana and Rio de Janeiro, Brazil.
SPOTLIGHT REPORT: HISTORIC TICK SURVEILLANCE OF SIERRA LEONE

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Using a systematic review, the goal of this study was to better characterize the distribution of ticks and the microbial species they carry within Sierra Leone. Nineteen search terms, and their corresponding MeSH terms, were used to compile scientific literature from the PubMed, Scopus, and WOS search engines published between 1901–2022, resulting in an initial capture of 109 articles. These results were screened for relevance according to title and abstract, after which only six articles met the final inclusion criteria. Two additional articles were captured from searches of the reference sections of included articles as well as from the search results of another West African country, for a total of eight articles selected for data extraction. Information captured during the data extraction process included tick species, collection locality, collection host, and pathogen detections. A total of seven genera of ticks were reported from the articles, including Amblyomma, Dermacentor, Haemaphysalis, Hyalomma, Ixodes, Ornithodoros, and Rhipicephalus. Most ticks were collected feeding on host animals, of which 66.2% (51/77) were collected from domestic animals. Wildlife hosts included African buffalo, ball pythons, ball python, and Paravovirus B 19 infections in our cohort. 16S sequencing revealed a high prevalence of Borrelia spp. and Rickettsia spp. in febrile patients, but not in controls. As compared to qPCR, 16S sequencing was sensitive and specific for detection of bacterial infections. Patients infected with Borrelia spp. experienced a range of symptoms, many of which overlapped with common symptoms of malaria, and had a unique immune response, characterized by decreased lymphocyte count and increased granulocyte count. These data demonstrate that 16S sequencing is a useful tool for detecting bacterial pathogens across a large number of plasma samples and could be employed for future surveillance. Further, the results indicate that arthropod-borne bacterial pathogens are a significant contributor to NMFI in Senegal, suggesting the need for improved diagnostics, increased access to treatment, and vector control efforts in the region.
COVID-19 patients (n = 188) at the Dermatology Hospital of Banako. Pre-COVID-19 sera from Nicro de Sahel (clinical malariaicarias, n = 51) and Dangaissa (asymptomatic and healthy donors, n = 157), were collected in 2013and 2018, respectively, before the onset of COVID-19 in Mali. Samples were tested using ELISA assay to assess IgG antibodies level against each antigen. Overall, seroprevalence was higher including malaria samples compared to COVID-19 samples for Spike (65.9% vs. 53.19%, p = 0.01), RBD (61.1% vs 23.9%, p = 0.001), RBM (29.3% vs 37.5%, p = 0.08), LRR25 (71.1% vs 32.4%, p = 0.001), and P27 (38.5% vs 22.2%, p = 0.03). A significant low correlation was found between antibodies anti-P27 and anti-Spike (r = 0.27, p = 0.002), anti-P27 and anti-RBD (r = 0.34, p = 0.001), anti-P27 and RBM (r = 0.26, p = 0.003) in pre-COVID-19 samples. The highseroprevalence of antibodies against SARS-CoV-2 proteins in malaria endemic areas may suggestcross-reactivity with malaria parasite and further investigation is needed to better understand the role of Plasmodium in COVID-19 spreading in these areas.

5255

DEVELOPMENT OF A DIAGNOSTIC IGM-ANTIBODY CAPTURE ELISA FOR DETECTION OF ANTI-CACHE VALLEY VIRUS HUMAN IGM

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Cache Valley virus (CVV) is a mosquito-borne virus in the genus Orthobunyavirus, family Peribunyaviridae that has been identified as a viral pathogen causing severe disease in humans. Limited information exists on its potential as a human teratogen in ruminants causing fetal death and severe malformations during epizootics in the United States. CVV has recently emerged as a potential teratogen in ruminants resulting in fetal death and severe malformations during outbreaks in the United States. CVV is currently considered to be a potential agent for SARS-CoV-2 serodiagnostics. These results will be summarized and discussed.

5256

USING REGIONAL SERO-EPIDEMIOLOGY SARS-COV-2 ANTI-S ANTIBODIES IN THE DOMINICAN REPUBLIC TO INFORM TARGETED PUBLIC HEALTH RESPONSE

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Higher incidence of COVID-19 has been associated with sociodemographic factors such as living in an urban setting, high population density, and household crowding. In this study, we investigated variations in SARS-CoV-2 seroprevalence at regional and cluster levels in the Dominican Republic (DR) and assessed potential sociodemographic factors influencing the geographical distribution of COVID-19 at the regional level. Data were collected in a three-stage cross-sectional national serosurvey conducted in the DR from June to October 2021. Seroprevalence of antibodies against the SARS-CoV-2 spike protein (anti-S) was estimated and adjusted for selection probability, age, and sex. Multilevel logistic regression was used to estimate the effect of covariates on seropositivity for anti-S and correlates of 80% protection against symptomatic infection (PT80) for ancestral and Delta strains. A total of 6,683 participants from 134 clusters in all 10 administrative regions of the DR were enrolled in the survey. The adjusted anti-S prevalence ranged from 80.5% (95%CI 78.1-82.9) to 89.8% (95%CI 88.8-98.8) between regions, and from 25.7% (95%CI 24.3-27.1) to 100% (95%CI 91.2-100.0) between clusters. At the national level, Enríquillo and El Valle had the highest odds ratios (OR) for anti-S positivity (OR of 1.86 for both with 95%CI 1.24-2.80 and 1.14-2.30, respectively). Also, receiving three doses of COVID-19 vaccine was associated with anti-S positivity (OR of 1.56 (95%CI 1.66-1.85) for ancestral and Delta strains (OR of 1.76, 95%CI 1.0.12-2.42) and Delta strains (OR of 19.59, 95%CI 14.22-27.01). At the regional level, models identified that associations between covariates and outcomes varied between regions. However, vaccination was consistently associated with highest odds of seropositivity and correlates of protection in most regions. Our results can help inform more targeted regional-level public health response such as strategies to increase vaccination coverage in areas with low population immunity.

5257

DENGUE FEVER OUTBREAK AT THE KENYAN SOUTH COAST INVOLVING SEROTYPE 3, GENOTYPES III AND V

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Dengue fever (DF), caused by four distinct serotypes of dengue virus (DENV-1-4) is transmitted by the Aedes mosquito. There have been increasing reports of DF outbreaks in Africa, including Kenya, yet very little information is available on the virus, for instance diversity of circulating genotypes. The study aimed at characterizing DENV-3 strains from the March 2019 DF outbreak at the Kenyan south Coast. RNA was isolated from 37 human plasma samples and screened for the presence of DENV serotypes by RT-PCR. Only DENV-3 was identified. RNA from DENV-3 positive samples was used for cDNA synthesis using sequence-independent single-primer amplification (SISPA). Libraries were prepared using the NexteraXT kit, and sequenced on the Miseq. Quality filtering, sequence assembly and annotation was done using CLC Genomics v.8.5, while phylogenetic analysis was conducted in MEGA v10. A targeted sequencing approach for envelop gene was used on samples that failed to yield complete genomes. 21/37 samples tested positive for DENV-3. On sequencing, 4 samples produced complete genome (10.173 bp) and 3 had partial sequences with complete env genes (1479 bp). Partial sequences with incomplete env genes were generated from 14 samples which were re-sequenced by targeted amplification. This approach produced ten complete env genes (1439-1479 bp). Maximum likelihood analysis of the 4 complete genomes and the 17 env genes confirmed DENV-3 (Clade-I and V=2) as the cause of the March 2019. The estimated time-to-most-common recent ancestor for the two genotypes was in 2015. Genotype III's origin was estimated to have been introduced from Pakistan. The origin of genotype V could not be ascertained due to rarity of these sequences globally, but was related to 2006 Brazilian isolate. Unlike genotype III that is widely distributed, genotype V is associated with higher disease severity and mortality in most regions. Our results can help inform more targeted regional-level public health response such as strategies to increase vaccination coverage in areas with low population immunity.
**DEVELOPMENT OF A DNA HYBRIDIZATION PROBE-BASED SURVEILLANCE ASSAY FOR DETECTION OF ARBOVIRUSES IN ARTHROPOD POOLS**

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Vector-borne pathogens continue to burden public health, thus advanced detection and surveillance methods are needed. Sensitivity and specificity constraints have limited development of multi-pathogen detection assays. Metagenomic sequencing has been used for microbial detection, but often lacks sensitivity for detection of low-abundance nucleic acid species. Here we present Comprehensive Molecular Entomological Surveillance (CMES)-viral, a method for enhanced detection of nucleic acids through probe-based enrichment. DNA hybridization probes were designed to cover full genomes and account for genetic diversity of 24 arboviruses of concern in CONUS. We developed a method for next generation sequencing detection of low quantity viral nucleic acids from arthropod pools. We optimized library preparation protocols and probe panel design using spiked arthropod pools for each target virus and assessed sensitivity compared with qRT-PCR and plaque titration. Additional efforts focused on creation of a simplified and standardized pipeline using the free and publicly available genetic data analysis platform, Galaxy. CMES-viral demonstrated higher sensitivity than viral titration and could detect viral genetic diversity. This assay demonstrates the ability to use NGS as a method of surveillance to successfully detect multiple arboviruses within vector samples in a single run. This approach supports the goal of reducing the global burden of arboviruses by providing an additional tool for comprehensive surveillance of arboviral vectors.

**COLORIMETRIC RT-LAMP ASSAY FOR DETECTION OF LA CROSSE VIRUS IN ARTHROPOD POOLS**

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La Crosse virus (LACV) is the leading cause of arboviral pediatric encephalitis and is likely underdiagnosed. Due to the nonspecific symptoms early diagnosis is lacking, as are available treatments. The virus is primarily transmitted by Aedes triseriatus; though invasive container inhabiting mosquitoes, Ae. japonicus and As. albopictus, are also recognized vectors. Due to focal circulation of this virus, surveillance in mosquitoes is not consistently performed across the geographic range of LACV. Technical expertise and funding required for qRT-PCR testing is often not available in locales where transmission foci exist. To bridge the gap between field personnel collecting vector mosquitoes and state health departments with the capacity for qRT-PCR testing, we developed a reverse transcription loop-mediated isothermal amplification assay (RT-LAMP) for detection of LACV using a colorimetric indicator. Five sets of four primers were designed to the LACV S segment utilizing the NEB® LAMP Primer Design Tool and initial analysis in silica indicated two of the five sets to be ideal candidates. Primer sets were assessed for sensitivity and specificity in comparison to qRT-PCR utilizing multiple LACV strains and other orthobunyavirids spiked into mosquito pools. In summary, we developed a sensitive colorimetric assay that requires minimal technical experience or advanced machinery and can be easily deployed in local settings for arthropod surveillance of LACV. By making surveillance more accessible, these transferable assays have the potential to improve early recognition of circulating LACV and improve human health outcomes.

**HIGH TRANSMISSION OF ENDEMIC HUMAN CORONAVIRUSES DURING THE COVID-19 PANDEMIC IN ADOLESCENTS IN CEBU, PHILIPPINES**

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SARS-CoV-2, the causative agent of COVID-19, is a betacoronavirus closely related to human endemic coronaviruses (hCoVs) OC43 and HKU1 and more distantly related to alphacoronaviruses 229E and NL63. For other pandemic respiratory pathogens, the emergence of novel subtypes leads to the extinction of others; it is unknown whether the same phenomenon may occur for hCoVs. We evaluated if pre-pandemic hCoV immunity affected the risk of contracting SARS-CoV-2 and whether SARS-CoV-2 infection affected the transmission of hCoVs among adolescents in the Philippines participating in a longitudinal dengue cohort study. We tested a random set of 499 out of 2035 study participants for positivity to SARS-CoV-2 receptor binding domain (RBD) by enzyme-linked immunosorbent assay (ELISA) in 2021. From this group, we randomly selected n=120 SARS-CoV-2 RBD negative and positive individuals for further study. ELISAs were used to measure binding antibodies (optical densities, OD) to RBD and spike proteins for all four hCoVs and SARS-CoV-2 for samples collected before the COVID-19 pandemic and after the spread of COVID-19 but before vaccination. We observed 79 to 91% seropositivity to the four hCoVs before the pandemic. ELISA ODs increased with age for 229E and OC43, suggesting endemic circulation, while immunity was flat across ages for HKU1 and NL63. High alphacoronavirus immunity at baseline correlated with an increased probability of SARS-CoV-2 infection, possibly indicating greater exposure to coronaviruses in general. Antibodies increased significantly to the RBDs of OC43, NL63, and 229E and spikes of all four hCoVs in both SARS-CoV-2 negative and positive adolescents. Those aged 13-15 years old in 2021 had higher antibodies to RBD and spike of OC43, NL63, and 229E than children the same age in 2019, further indicating intense transmission. Overall, we observe a limited effect of the COVID-19 pandemic on SARS-CoV-2 infection on endemic hCoV transmission. This study provides insight into the co-circulation of hCoVs as SARS-CoV-2 becomes an endemic pathogen.

**IDENTIFICATION OF IMMUNODOMINANT B AND T-CELL EPITOPES OF KYASANUR FOREST DISEASE VIRUS AND THEIR EXPRESSION FOR DEVELOPING RAPID DIAGNOSTICS AND POTENT SUBUNIT VACCINE**

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The Kyasanur Forest Disease (KFD), which is also known as ‘Monkey Fever’ is caused by KFD Virus (KFDV) that belongs to the flaviviridae, KFD is a highly neglected and emerging tropical disease endemic to Western Ghat region of Karnataka, India, which is fatal with a mortality rate of 2-10%. Recently, KFD has been alarmingly spreading from its epicenter to neighboring districts and states also. The current ELISA based KFD diagnosis involves the detection of antibody in the patient’s blood which is relevant only after the development of antibody following the infection and is non-specific due to cross-reactivity with other flaviviruses. Further, currently available formalin-inactivated vaccine developed in the 1970s has now been found to be less effective leading to increased disease susceptibility and...
Severe fever with thrombocytopenia syndrome virus (SFTSV) is an emerging tick-borne viral infection in China and other Asian countries. Due to its high fatality rate and its pandemic potential, SFTSV is listed among the top priority infectious diseases requiring urgent research by the World Health Organization. Four cases of SFTSV were recently reported in Thailand. We performed unbiased metagenomic next generation sequencing (mNGS) coupled to viral target enrichment to identify known and novel viruses from 1,000 patients with undiagnosed, acute undifferentiated febrile illness (AUF), hospitalized between 2014 and 2021 at Siriraj Hospital, Bangkok, Thailand. Three patients with SFTSV were detected, including a 61-year female, 75-year male and 77-year male with durations of illnesses of 7, 6 and 7 days, respectively. They presented with non-specific symptoms such as fever, myalgia, and their complete blood count (CBC) revealed marked leukopenia and thrombocytopenia. The initial diagnosis was dengue hemorrhagic fever in two individuals and sepsis with septic encephalopathy in the other who also presented with alteration of consciousness. Dengue NS1, IgM, IgG markers and antibodies against Rickettsia typhi and Orientia tsutsugamushi were not detected. All cases were treated with empirical ceftriaxone and azithromycin for 7 days. Two of them developed severe complications, including gastrointestinal bleeding, acute kidney injury and hospital acquired pneumonia. Their WBC count and platelets were normal prior to discharge. All cases of SFTSV (4 cases previously reported and 3 cases in this study) in Thailand were diagnosed between 2019 and 2020. We are conducting additional studies with AUF specimens from as early as 2001 to determine if there has been an earlier introduction of SFTSV into Thailand. Our findings reinforce the need for rapid and accurate laboratory tests for the diagnosis of SFTVS in Thailand among febrile patients presenting with thrombocytopenia after dengue infection has been excluded.
obtained. Dengue seropositivity was defined as a positive IgM, IgG, or NS1. The mean age of the participants was 39.3 ± 16.4 and 48/90 (53.3%) were females. Dengue seropositivity was observed in 65/90 (72.2%) participants. The pattern of positivity for dengue markers comprised IgG only (38.9%), IgM only (10%), IgG and IgM (23.3%), and IgM and NS1 (1.1%). There was no statistically significant difference in the frequently reported symptoms/signs between dengue seropositive and seronegative febrile adults: headache (71.9% vs. 28.1%), muscle pain (73.7% vs. 26.3%), nausea/vomiting (68.4% vs. 31.6%), joint pain (73.5% vs. 26.5%), fatigue (88.9% vs. 11.1%), pallor (66.7% vs. 33.3%) and abdominal tenderness (75.0% vs. 25.0%), all p>0.05. Dengue seropositive and seronegative febrile adults had comparable laboratory parameters. The proportion of seropositive adults with hematological abnormalities compared to seronegative were: anemia (66.7% vs. 33.3%), leukocytosis (73.7% vs. 26.3%), and thrombocytopenia (69.6% vs. 30.4%), all p>0.05. We found a high prevalence of dengue seropositivity in this febrile population. Constitutional symptoms and laboratory investigations were comparable between dengue seropositive and seronegative participants. Our findings suggest the limited value of clinical and ancillary laboratory parameters in dengue surveillance which justifies a call for improved access to dengue diagnostic assays in endemic regions.

5266

OROPOUCHE VIRUS AS AN EMERGING CAUSE OF ACUTE FEBRILE ILLNESS IN COLOMBIA

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Arbovirus infections are frequent causes of acute febrile illness (AFI) in tropical countries. We conducted health facility based AFI surveillance at four sites in Colombia (Cucuta, Cali, Villavicencio, Leticia) during 2019-2022. Demographic, clinical and risk factor data were collected from persons with AFI that consented to participate in the study (n=2,967). Serologic specimens were obtained and tested for multiple pathogens by RT-qPCR and rapid test (Antigen/IgM), with 20.7% identified as dengue positive from combined testing. Oropouche virus (OROV) was initially detected in serum by metagenomic next generation sequencing(mNGS) and virus target capture in a patient from Cúcuta. Three additional infections from Leticia were confirmed by conventional PCR, sequenced, and isolated in tissue culture. Phylogenetic analysis determined there have been at least two independent OROV introductions into Colombia. To assess OROV spread, a RT-qPCR dual-target assay was developed which identified 87/791 (10.9%) viremic cases in AFI specimens from Cali (3/53), Cucuta (3/19), Villavicencio (38/566), and Leticia (43/153). In parallel, an automated anti-nucleocapsid antibody assay detected IgM in 27/503 (5.4%) and IgG in 92/568 (16.2%) patients screened, for which 24/68 (35.3%) of PCR positives had antibodies. Dengue was found primarily in children (<18 yr) and linked to several clinical manifestations (weakness, skin rash and petechiae), whereas Oropouche cases were associated with the location, climate phase, and odynophagia symptom. Our results confirm OROV as an emerging pathogen and recommend increased surveillance to determine its burden as a cause of AFI in Colombia.
controls to ensure expected extraction and amplification efficiency. In silico analysis of the MPXV+ oligos predicted 100% sensitivity for both mpox clades and detection of smallpox and vaccinia viruses. Exclusivity analysis at the time of writing predicted no cross-reactivity for 60 other organisms. Two commercially available mpox cultures (NR-2500 and NR-27, BEI) were serially diluted and tested in triplicate to estimate assay limits of detection in pfu/mL and TCID50/mL. Virus cultures were spiked into either UTM or a 1:1 mixture of UTM:saliva to confirm matrix inclusivity. UTM samples from lesions of 35 US CDC confirmed cases and a commercially available longitudinal mpox lesion panel (SLR) were diluted in UTM and tested to assess assay performance against known positive patient samples. Molecular detection of virus culture dilutions showed comparable performance of the MPXV+ assay in both UTM and UTM:saliva. Assay sensitivity was determined to be 1 TCID50/mL, suggesting infectious levels of virus are detected. 1:50 dilutions of 35 CDC confirmed mpox lesion swab samples were detected and 1:100 dilutions of commercially sourced samples were detected with the MPXV+ assay. Here we demonstrated the development of a fully automated assay for the detection of mpox virus with sufficient sensitivity to detect infectious levels of virus and 100% concordance to CDC confirmed infections, confirming robust assay performance.

5269
THE ECONOMIC BURDEN OF ILLNESS OF THE GLOBALLY SPREADING CHIKUNGUNYA VIRUS (CHIKV): A SYSTEMATIC LITERATURE REVIEW
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Chikungunya is a disease caused by the arbovirus Chikungunya Virus (CHIKV), which is transmitted to humans by the mosquito species Aedes albopictus and Ae. aegypti. Over the last decades, the geographic spread of these vectors, and therefore the dissemination of CHIKV, has increased due to climate change-related factors. The clinical development of chikungunya has been shown to heavily impact the life quality of the patients, which has led to raised public health concerns. An SLR was performed to identify the evidence of the costs and resource use associated with chikungunya as the impact of the disease remains unclear. The search was conducted on the electronic databases Medline and Embase, and congress abstract repositories. Of the 1,140 records identified, 33 studies reporting outcomes from eleven world regions were included. The most reported study sites were Reunion Island, Colombia, and India. Estimated costs for chikungunya’s direct and indirect effects varied greatly between studies and countries. Consultation costs, followed by hospitalization expenditures, were found to constitute the largest proportion of the total direct chikungunya costs. The frequency and duration of hospitalization also ranged significantly across studies. Indirect costs were primarily linked to absenteeism: The highest reported absenteeism rate for a CHIKV-positive population was 62.9%, with the longest median number of days patients were absent from work being 35. In conclusion, chikungunya was found to be associated with a substantial economic burden when considering the costs and frequency of inpatient and outpatient care—as well as absenteeism—for patients reporting symptoms of acute and chronic chikungunya. Moreover, misdiagnosis and mistreatment were identified as confounders to measure the economic disease burden. This highlights the need for more standardized approaches to diagnosing and treating chikungunya.

5270
DEVELOPMENT OF AN INTERDISCIPLINARY, MULTIAGENCY COLLABORATION TO COORDINATE LOCAL RAPID RESPONSES TO DENGUE CASE CLUSTERS IDENTIFIED AND MONITORED THROUGH UNIFIED VECTOR AND HUMAN SURVEILLANCE — PUERTO RICO, JANUARY 2021-2023

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In 2021, the Puerto Rico Dep of Health (PRDH), the Puerto Rico Vector Control Unit, and the CDC Dengue Branch established a collaborative process for identifying and mapping dengue case clusters in Puerto Rico (PR) to develop coordinated local responses aimed at interrupting transmission before an outbreak occurred. This group identified clusters based on confirmed or probable dengue cases (2015 CSTE case definition) reported to PRDH or mosquito pools positive by RT-PCR for dengue virus (DENV) 1-4. We defined clusters as 3 DENV detections (including ≥1 case) with disease onset or trap collection date within 21 days and located within 500 meters. Clusters were inactivated when no new detections occurred 6 weeks after the last detection. An analysis of January 2021-2023 identified 63 clusters comprising 409 cases and 97 positive pools. Twenty-six clusters (41%) started in 2021 and 37 (59%) in 2022, with 47 (75%) beginning during June-December. The median size and duration were 5 persons or mosquito pools (IQR 3-8) and 23 days (IQR 13-62). Fifteen clusters (24%) occurred in high-density public housing. Of 78 municipalities, 15 (19%) had ≥1 cluster. As cluster frequency increased during peak months, the group reached a consensus on prioritizing clusters based on size, newly circulating serotypes, public housing, minimal reporting delay, locations with historically high cases, and high-risk neighboring communities. Interventions developed and piloted included placement of autocidal gravid ovitraps, wide-area larvicide spraying, debris clean-up, home inspections, removal of mosquito egg-laying sites, and risk messaging to local authorities. Twenty-seven (43%) clusters received ≥1 intervention. Analyses of mosquito populations in intervention clusters are ongoing and will be included at the time of presentation. This collaboration facilitated rapid response to dengue clusters and highlights the disproportionate burden in public housing. Next steps include automating cluster identification and reporting, standardizing interventions, and critically evaluating unified surveillance and integrated intervention effectiveness.

5271
CORRELATION OF DENGUE TRENDS BETWEEN SENTINEL AND PASSIVE SURVEILLANCE SYSTEMS IN PUERTO RICO, 2012 - 2022
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The Sentinel Enhanced Dengue Surveillance System (SEDSS) is an ongoing hospital-based active surveillance program for acute febrile illness implemented in 2012 in Southern Puerto Rico and 2019 in the San Juan metropolitan area; however, the representativeness of sentinel surveillance for broader trends is often unknown. We compared laboratory-confirmed dengue cases identified in SEDSS to those reported in the passive arboviral disease surveillance system (PADSS) to determine whether SEDSS could serve as an early warning system and whether dengue trends were similar for both surveillance systems. We analyzed data from dengue epidemic (2012-2014) and non-epidemic (2019-2021) periods to determine the utility of SEDSS under different transmission scenarios. To assess the utility of SEDSS as an early warning system we 1) tested whether the distribution of SEDSS cases was shifted earlier in time relative to PADSS using Cramer-von Mises tests of equality (CvM), and 2) used cross-correlations of lagged SEDSS data relative to PADSS to examine whether cases reported to SEDSS could anticipate those reported to PADSS. During the epidemic period, 738 SEDSS and 10,592 PADSS laboratory-confirmed dengue cases were reported. We observed a trend towards earlier reporting in SEDSS compared to PADSS (CvM p=0.06). The highest cross-correlations in case counts between the two systems were at SEDSS lags of -2, -1, and 0 weeks. During the non-epidemic period, 179 and 1,348 laboratory-confirmed dengue cases were reported to SEDSS and PADSS, respectively. The cumulative distribution of cases in SEDSS was reported earlier compared to PADSS (CvM p=0.02). The highest cross-correlations between counts were at SEDSS lags of -2, -1, and 0 weeks. Plotted together, SEDSS and PADSS followed the same peaks and troughs in dengue cases in both study periods. During epidemic and non-epidemic periods, dengue trends in SEDSS were representative of island-wide trends. SEDSS may serve as an early warning system by detecting increases in incidence up to two weeks before passive surveillance.

COMMUNITY-BASED SERO-PREVALENCE OF CHIKUNGUNYA AND YELLOW FEVER IN THE SOUTH OMO VALLEY OF SOUTHERN ETHIOPIA

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Chikungunya (CHIK) and yellow fever (YF) are becoming major public health threats in East African countries including Ethiopia. This study aimed to assess a community-based sero-prevalence of CHIK and YF in the South Omo Valley of Ethiopia, an endemic area for YF. Between February and June 2018, blood samples were collected from study participants and screened for IgG antibody against CHIK virus (CHIKV) and YF virus (YFV) infections using ELISA. Data were computerized using Epi Data Software v.3.1 and analyzed using SPSS. A total of 360 participants (51.7% males, age range from 6 to 80) participated in this study. The overall sero-prevalence of IgG antibody was 43.6% (157/360) against CHIKV, while it was 49.5% (155/313) against YFV. There was a significant positive correlation between IgG antibodies to CHIKV and YFV (r=0.88; P<0.01). Residency in the Debub Ari district (AOR = 8.47; 95% CI: 1.50, 47.74) and travel history to sylvatic areas (AOR = 2.21; 95% CI: 1.02, 4.81) were significantly and positively associated with high sero-prevalence of IgG antibody to CHIKV and YFV, respectively. High sero-prevalence of IgG antibody to CHIKV shows circulation of the virus in the present study area. A low sero-prevalence of IgG antibody to YFV in YF vaccine received individuals is highly concerning from a public health point of view as waning of immune response to YFV infection could result in a periodic outbreak of YF in endemic areas. Nevertheless, the present study has not investigated for possible cross-reactivity of antibody to CHIKV with other alphaviruses and YFV with other flaviviruses and these warrants further studies in the present study area.

SUPERSPREADING OF SARS-COV-2: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Superspreading events have been a prominent driver of the COVID-19 pandemic, contributing to rapid spread and outbreaks of SARS-CoV-2. Superspreading occurs in settings where transmission is highly efficient and/or when an individual infects many others. We have limited knowledge on the epidemiological contexts and individual-level factors that contribute to SARS-CoV-2 superspreading. To better quantify heterogeneity in SARS-CoV-2 transmission, we performed a systematic review and meta-analysis of transmission events with data on secondary attack rates or contact tracing data for individual index cases published before 9 September 2021, prior to the emergence of variants of concern and widespread vaccination. We reviewed 592 distinct events and 9,883 index cases from 491 papers. "Superspreaders" were identified as index cases causing more than five secondary cases. Meta-analysis of secondary attack rates identified substantial variation across 12 event types/settings: the highest rates were estimated for co-living situations including congregate housing (35%), households (29%), and nursing homes (25%); the lowest rates were estimated for schools (9%), hospital/healthcare (8%), and shopping (1%). There was also substantial variation in attack rates within event types.

VIRAL ETIOLOGY OF LOWER RESPIRATORY TRACT INFECTIONS IN CHILDREN <5 YEARS OF AGE IN ETHIOPIA: A PROSPECTIVE CASE-CONTROL STUDY

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Lower respiratory tract infections (LRTIs) are a major cause of morbidity and mortality in children worldwide and disproportionately affects Sub-Saharan Africa. Despite the heaviest burden of LRIs in Ethiopia, to date, we have found no published studies reporting comprehensive viral etiology of LRTIs among children in Ethiopia. The objective of this study is to estimate the etiological contribution of respiratory viruses for LRTIs in < 5 years children in Ethiopia. A hospital based prospective case-control study was conducted from September 2019 to May 2022. A one-step Multiplex real-time PCR (Alpex™ Respiratory Panel Assays 1-3) was done to detect respiratory viruses from Naso/Oropharyngeal (NP/OP) swab samples. STATA software version 15 was used for the data analysis. We perform Odds ratio (OR), Attributable fraction among exposed (AFE) and population attributable fraction (PAF) analysis to measure the association of the detected viruses with LRTIs. A total of 210 LRTIs cases and 210 non-LRTI controls were included in the study. The likelihood of detecting one or more viruses from NP/OP was higher among cases than controls (83.8% vs. 50.3%). The multivariate logistic regression showed significantly higher detection rate for RSV A (OR: 14.6, 95% CI: 4.1-52.3), RSV B (OR: 8.1, 95% CI: 2.3-29.1), influenza A virus (OR: 5.8, 95% CI: 1.5-22.9), and PIV 1 (OR:...
4.3, 95% CI: 1.1-16.4), among cases when compared with controls. The overall AFE and PAF for RSV A were (93.2% and 17.3%), RSV B (87.7% and 10.4%) and Influenza A virus (82.8% and 6.3%), respectively. Only 2 children were positive for SARS-CoV-2. The mean CT values were lower for all the viruses detected in the cases group with the exception of corona viruses and human rhino viruses. In conclusion, based on our finding, 27.7% LRIs could be eliminated from children in Ethiopia if RSV were eliminated. Regarding SARS-CoV-2, Children are less likely to get infected by it; therefore in resource limited countries like Ethiopia, the cost-benefit balance of vaccinating under 5 years children against SARS-CoV-2 should be carefully done before launching a vaccination campaign.

5275

SEROPREVALENCE OF DENGUE, CHIKUNGUNYA AND ZIKA AT THE EPICENTER OF THE CONGENITAL MICROCEPHALY EPIDEMIC IN NORTHEAST BRAZIL: A POPULATION-BASED SURVEY

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The Dengue viruses (DENV) serotypes 1, 2, 3 and 4 were re-introduced in the Northeast Brazil one by one from the 1980’s until 2010’s. Zika (ZIKV) and Chikungunya (CHIKV) viruses were introduced early 2010’s and caused large outbreaks in 2015 and 2016. However, the extent of the ZIKV and CHIKV outbreaks and the risk factors associated with exposure remains vague. We conducted a stratified multistage household serosurvey among residents aged between 5 and 65 years, in the city of Recife, Northeastern Brazil, from August 2018 to February 2019. The city neighborhoods were stratified according to high, intermediate, and low socioeconomic strata (SES). Previous infections (ZIKV, DENV and CHIKV IgG) were detected by enzyme linked immunosorbent assay (ELISA). Recent ZIKV and CHIKV infections were assessed through IgG and IgM ELISA, respectively. Design-adjusted seroprevalence were estimated by age group, sex, and SES. The ZIKV seroprevalences were adjusted according to the accuracy of the tests. Individual and household-related risk factors were analyzed through regression models to calculate the force of infection . Odds Ratio (OR) were estimated as measure of effect. A total of 2,070 residents were investigated. The forces of infection for high SES were lower for all three viruses as compared to intermediary and low SES. Overall DENV seroprevalence was 88.7%. The overall adjusted Zika seroprevalence was 35.6%, 47.4% in the low SES and 23.4% in the high. The overall CHIKV seroprevalence was 35.7 %, with the low SES with a seroprevalence of 38.6% and the high SES with 22.3%. ZIKV seroprevalence increases fast with age while CHIKV seroprevalence almost constant through all ages. The serological markers of recent infections for ZIKV and CHIKV were 5% and 3.5 % respectively. In conclusion, our results confirmed continued DENV transmission and intense ZIKV and CHIKV transmission during the 2015/2016 epidemics followed by continued baseline transmission. The study also shows that there is a significant proportion of the population that still susceptible to infections by ZIKV and CHIKV in the region.

5276

INVESTIGATION OF THE MEASLES OUTBREAK IN DJIBOUTI

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Measles is one of the vaccine-preventable diseases. There are an estimated 9,484,000 cases with 128,000 deaths worldwide in 2021 of which nearly half have been reported in Africa. In addition to this morbidity and mortality, the total cost of a measles epidemic is estimated on average between $9862 and $1,063,936 per outbreak, thus constituting a significant economic burden. The aim of our study was therefore to describe the outbreak of cases and to analyse the epidemiological and spatio-temporal characteristics in order to be able to propose recommendations for measles surveillance and prevention. We therefore undertook a retrospective descriptive study that was conducted on data from measles cases investigated in Djibouti during the period February 2022 to December 2022. A total of 603 cases have been reported throughout Djibouti, of which 326 have been investigated. Of these 326, 204 (63.1%) were biologically confirmed by IgM serology and 2 (0.6%) cases by epidemiological link. The annual incidence of the disease cases per 100,000 population is 326 have been investigated. Of these 326, 204 (63.1%) were biologically confirmed by IgM serology and 2 (0.6%) cases by epidemiological link. The annual incidence of the disease cases per 100,000 population.

5277

MOLECULAR EPIDEMIOLOGY OF ACUTE DENGUE AND CHIKUNGUNYA INFECTIONS AMONG FEBRILE PATIENTS VISITING FOUR HOSPITALS IN BOTH URBAN (YAOUNDE) AND RURAL (DIZANGUE) SETTINGS FROM CAMEROON

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Dengue and chikungunya are widely distributed in Cameroon but there information on their prevalence in different epidemiological settings are still lacking. This study was undertaken to assess dengue and chikungunya prevalence among febrile patients in urban and rural settings. From December 2019 to September 2021, willing febrile (axillary temperature >38°C) outpatients visiting 4 healthcare facilities in the cities of Yaoundé and Dizangou were screened for malaria, dengue and chikungunya. Patients’ clinical symptoms were recorded and their blood samples collected in EDTA tubes were centrifuged at 2000rpm for 10 min in order to obtain plasma, then analyzed using CDC-real-time PCR protocols. A Gnasma-stained tick blood smear was formed for malaria microscopy. Odds ratios were
used to determine the level of association between socio-demographic factors, clinical features and the infection status. Overall, 301 patients were recruited: 198 in Yaoundé and 103 in Dizangue. For dengue, 110 patients were positive (94.3%) in Yaoundé and 109 (95.2%) in Dizangue and the disease’s prevalence was higher in urban compared to rural setting. Important prevalence (n= 50, 16.61%) of dengue-malaria co-infection was recorded. For chikungunya, one (0.3%) patient (Yaoundé) was tested positive after rRT-PCR. Abdominal and retro-orbital pains were significantly associated to acute dengue infection. All the four dengue serotypes were recorded with a predominance of DENV-3 (35.4%) and DENV-4 (25.5%), with DENV-4 reported for the first time in Cameroon as well as Central Africa Region. In conclusion, this study further confirms endemicity of both dengue and chikungunya in Yaoundé and Dizangue. These data stress the need for active surveillance of cases to prevent outbreaks occurrence across the country.

5278

SEROPREVALENCE OF SARS-COV-2 NEUTRALISING ANTIBODIES AMONG TRAVELERS ENTERING GHANA THROUGH THE MAJOR LAND BORDERS, 2022

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High mortality, morbidity, and transmission of circulating SARS-CoV-2 variants have been reported worldwide. The progression of the pandemic in Africa differs based on the pattern of death and the rate of contagion of the virus. SARS-CoV-2 was introduced into Ghana by travellers followed by subsequent community transmission. Lockdown of air and land borders were two key control strategies. Timely interventions to test and control air border users were implemented, however, land borders remained closed. This cross-sectional study aimed to determine the seroprevalence of SARS-CoV-2 amongst travellers entering Ghana through 10 approved land borders. Persons aged ≥ 18 years into this study. Truck drivers using the land borders and providing goods and services during the pandemic were the main focus in phase one of the study. Sampling later expanded to include all individuals aged ≥ 18 years using the land borders. A questionnaire was administered to each consenting participant and blood samples collected were processed to obtain serum for detection of neutralising antibodies using the WANTAI ELISA kit. Overall seroprevalence was 92.26% (4172/4522). This however varied across the country.

5279

ELIMINATION OF HEPATITIS B VIRUS USING ANTIVIRAL PROPHYLAXIS AND VACCINATION IN REMOTE SETTINGS THROUGH LOCALLY ADAPTED, INTEGRATED SERVICES: A MATHEMATICAL MODEL

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The Dolpa district of Nepal, has remote geographic landscape, low vaccination coverage, and high prevalence of hepatitis B (HBV) in pregnant women, which creates a condition for perpetuation of high mother to child transmission of the virus. This modeling study assessed the impact of vaccination and third-trimester tenofovir disoproxil fumarate (TDF) prophylaxis on HBV burden and elimination in Dolpa district. This deterministic compartmental model used four possible treatment scenarios: baseline 50% vaccination coverage (scenario I), 50% TDF and baseline vaccination coverage (scenario II), 90% TDF plus baseline vaccination coverage (scenario III), and 90% TDF and birth-dose plus 95% vaccination coverage (scenario IV). The main outcomes for the study were burden of HBV, incidence of HBV, and time to elimination. The study highlights that HBV elimination may not be achieved in Dolpa district by 2100 using the baseline interventions. The use of 90% TDF coverage with the baseline vaccination significantly reduces HBV prevalence and HBV-related mortality and elimination is possible in less than 60 years. Combined implementation and scale-up of 90% TDF and birth-dose and 95% infant HBV vaccination leads to HBV elimination by 2047. In the setting of geographical inaccessibility, a micro-elimination approach for HBV in the remote Dolpa district of Nepal using third-trimester TDF is an effective and equitable approach. This approach is likely to significantly reduce HBV burden and HBV-related mortality even before achieving elimination and partly avoids challenges from the need for cold chain and unaffordable cost of immunoprophylaxis.

5280

THE EPIDEMIOLOGY OF INFLUENZA B VIRUS IN GHANA, 2017 TO 2021

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Influenza B is characterized by two antigenic lineages; B/Victoria and B/Yamagata. These lineages circulate together with influenza A during seasonal flu epidemics with variation of the year-to-year incidence rates and geographic distribution. This study described the epidemiologic trends of influenza B in Ghana from 2017 to 2021 using surveillance data from the Ghana National Influenza Centre (NIC) sentinel sites. Demographic and laboratory-confirmed influenza data from influenza-like illness (ILI) cases in Ghana between 2017 and 2021 was obtained from the Ghana NIC. Data was analyzed as detection rates, trends, and regional distribution. The Ghana NIC confirmed 21,539 influenza cases. The detection rate of influenza B was 2.935 per 100,000 (632/21539) and was highest in age groups 0-5 years-old and 6-15 years-old. Over this five-year period, the detection rate of influenza B was highest in the years 2018 and 2019. However, the years 2017, 2020 and 2021 were below the average rate of 16,028 per 100,000 population (p-value <0.0001). The trend analysis showed that influenza B cases increased from epidemiological week 35...
(in the month September) for the years under review. In 2017 and 2018, both influenza B lineages were co-circulating, but from 2019 to 2020, the B/Victoria lineage dominated. Few influenza B infections (Victoria-1; Yamagata-1) were detected in 2021. Influenza B infections were sporadic with variable geographic (regional) distribution in Ghana. Secondary analysis of data from this five-year period helped better understand the frequency and distribution of influenza B in Ghana. Additionally, information from this five-year period was shared with the World Health Organization (WHO) Global Influenza Surveillance and Response System (GISRS) and contributed to recommendations for which influenza strains were incorporated into the southern hemisphere seasonal influenza vaccine. Findings from this surveillance activity highlight the importance for ongoing influenza surveillance and monitoring of the antigenic strains in circulation.

5281

A PROPOSAL FOR UTILIZATION OF PREGNANCY AS AN OPPORTUNITY FOR HCV ELIMINATION AND ERADICATION

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Hepatitis C Virus (HCV) is a major cause of morbidity and mortality globally. Since the development of highly effective direct acting antivirals, the WHO has set a goal of HCV elimination by 2030. Key to this strategy are increased screening and treatment for HCV. Pregnancy and the postpartum period represent an unique time when underserved populations globally have increased contact with the healthcare system. We propose a model in which antenatal care is used to maximize case identification, treatment, and prevention. Pregnant individuals are an ideal sentinel population for HCV surveillance. Contact with the healthcare system is driven by the pregnancy, rather than disease, and thus asymptomatic carriers can be identified. The population is well distributed geographically and socioeconomically, reducing potential sampling bias. Additionally, the infrastructure already exists, as antenatal centers routinely screen for other diseases like HIV. Universal screening in pregnancy can provide data on population level disease exposure and be used to identify geographic hot spots. Once cases are identified, we argue that pregnancy presents an opportunity for intervention. While treatment during pregnancy is not currently WHO approved, clinical trials are underway examining the safety of the direct acting antivirals antepartum. In the interim, identification of infection during pregnancy allows for optimization of the treatment cascade postpartum. It also ensures the exposed newborn and other close contacts can be identified and connected with HCV screening. Finally, we propose that pregnancy can be used as a time for prevention measures, connecting pregnant women to needle exchanges, counseling on risk reduction and providing education on the disease. As new technologies are developed, it may also represent an ideal time to perform vaccination. Taking advantage of patient engagement and existing infrastructure, pregnancy presents a unique opportunity to intervene in the fight for HCV eradication.

5282

ASSESSING JAPANESE ENCEPHALITIS VIRUS RISK IN ASIA USING HIGH-RESOLUTION REMOTELY SENSED DATA AND MACHINE LEARNING

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Japanese encephalitis virus (JEV) is a mosquito-borne virus endemic to large parts of Asia and the western Pacific and is the predominant cause of vaccine-preventable encephalitis in the region. JEV is estimated to have caused more than 20,000 deaths in 2019, but fewer than 1% of infections result in symptomatic disease. Occurrence data is sparse despite acute encephalitis syndrome (AES) surveillance in most countries, as cases are not always laboratory confirmed and spatially detailed data is typically not published. To enhance our understanding of the prevalence and distribution of JEV in Asia we conducted a literature review of available occurrence data and compiled all records which could be geocoded. We use this occurrence data with high-resolution remotely sensed covariates and a convolutional neural network (CNN)-based approach to model probability of occurrence at significantly improved resolution and scale over past research. The CNN method has been demonstrated to excel in similar spatial prediction tasks with sparse data and allows us to predict probability of occurrence at 100m x 100m resolution, a first for the region. Our 13 covariates include climatic and population data, as well as land use, vector habitat, and animal density rasters. Preliminary results are promising, with a 0.72 probability of occurrence predicted for the validation dataset, which contains 15% of total JEV observations withheld from model training and testing. These preliminary outputs focus on a subset of the region including the Indian subcontinent and surrounding area, successfully predicting higher probability in Uttar Pradesh, Bihar, and Bangladesh where we have high density of occurrence records, but also in Andhra Pradesh, Telangana, and parts of Myanmar where data is severely lacking. We expect further improvement in model accuracy as we incorporate more data and further develop the model. Our approach provides a more detailed view of the spatial distribution of JEV and has the potential to inform targeted intervention and control strategies for the disease, ultimately helping to reduce the burden of Japanese Encephalitis in these endemic regions.

5283

INVESTIGATION OF SEVERE DENGUE OUTBREAK IN MAUMERE, EAST NUSA TENGGARA, INDONESIA IN 2020: CLINICAL, SEROLOGY, AND VIROLOGICAL FEATURES

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Dengue, an acute febrile disease caused by dengue virus (DENV) infection, is endemic to Indonesia. The country continues to see cyclical outbreaks throughout the years. During early 2020, outbreak of severe dengue occurred in Maumere, Sikka district, East Nusa Tenggara province. Investigation was conducted to understand the cause and characteristics of the outbreak. Dengue is a major cause of morbidity and mortality globally. The high mortality rate and dengue hemorrhagic fever (DHF) are caused by more than 20,000 deaths in 2019. In 2020, Maumere is caused by DENV-3 which were introduced from nearby islands. The severe dengue outbreak in Maumere was identified as DENV-3 (87.3%), followed by DENV-4 (7.0%), DENV-2 (2.8%), and DENV-1 (2.8%). PRNT on DENV-3 infections. The dominant serotype was DENV-3 (87.3%), followed by DENV-4 (7.0%), DENV-1 (2.8%), and DENV-2 (2.8%). PRNT on DENV-3 secondary infections patients detected the presence of DENV-2 and DENV-4 neutralizing antibodies. Phylogenetic analysis revealed close evolutionary relationship of Maumere DENV to viruses from other Indonesian regions, especially Bali and Kupang. The presence of anti-dengue antibodies for multiple serotypes suggests a history of dengue transmission, with the serotype shift, likely from introduction through travel, as a possible contributor to this outbreak. The high proportion of anti-dengue IgG in young children also demonstrates a high infection rate in the area which may contribute to disease severity. The severe dengue outbreak in Maumere is caused by DENV-3 which were introduced from nearby islands. The secondary infection of this serotype most likely contributes to the severity of the disease during the outbreak.
PREVALENCE OF ANTI-VZV AMONG SAMPLE OF MEDICAL UNDERGRADUATES IN SRI LANKA: EXPLORING THE VALUE OF ‘RECALLED HISTORY OF CHICKENPOX’ AS AN INDICATOR OF IMMUNITY

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Immunity to varicella zoster virus (VZV) is indicated by the presence of antibody to VZV (anti-VZV). Chickenpox vaccine is not included in the National Immunization Program of Sri Lanka and immunoity to VZV usually develops following natural infection, unless an individual receives vaccination in the private sector. Recalled history of chickenpox is often used as an indicator of immunity when deciding post-exposure prophylaxis. The objective of this study was to determine the performance of ‘Recalled history of chickenpox’ as an indicator of immunity. A cross-sectional study was carried out with convenient sampling involving medical undergraduates at a state university in Sri Lanka from August to October 2020. Epidemiological data and blood sample for anti-VZV (by anti-VZV IgG ELISA, Euroimmun, Germany) was collected. Total of 142 undergraduates participated in the study. Median age was 22 years (IQR 22 -23). Almost half (45.1%, 64/142) gave history of chickenpox without history of vaccination. Another 7% (10/142) undergraduates who had no history of illness had taken chickenpox vaccination, with 60% (6/10) of them getting a single dose of vaccine. The balance 47.9% (68/142) had neither history of chickenpox nor vaccination. Of the 64 undergraduates who had chickenpox, 43.8% (28/64) had it at the age < 10 years, 18.8% (12/64) at 10 to 15 years and 34.4% (22/64) at > 15 years. Anti-VZV was detected in 48.6% (69/142) indicating immunity to chickenpox, including 91.5% (63/69) with history of chickenpox and 7.2% (5/69) with history of vaccination. Anti-VZV was detected in one (1.4%) who had no history of chickenpox or vaccination. Positive recalled history of chickenpox had a sensitivity 98.4%, specificity 98.5%, positive predictive value 98.4% and negative predictive value 98.5% for the presence of anti-VZV. Immunity to VZV is detected in less than 50% of the medical undergraduates at a state university in Sri Lanka, in majority following natural infection. Positive recalled history of chickenpox has a good predictive value of immunity and recalled history of chickenpox can be used in deciding the post exposure prophylaxis.

HIGH RISK OF DENGUE AND CHIKUNGUNYA VIRUS FOUND AMONGST CHILDREN LIVING IN INFORMAL URBAN SETTLEMENTS IN MAKASSAR, INDONESIA

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Increases in dengue (DENV) and chikungunya (CHIKV), viruses transmitted by Aedes aegypti and Aedes albopictus, have been highlighted as one of the most alarming health impacts of climate change. Increasing temperatures are ideal for Aedes life stages and extreme weather events, such as droughts and floods, increase water insecurity and promote Aedes breeding habitats. Additionally, the built environment and under-resourced water and trash management systems in informal settlements likely play critical roles in mediating the risk of Aedes-transmitted arboviruses. However, dengue and chikungunya risk in informal settlements with these dual risks from the built environment and climate vulnerability have not been well studied. In 12 informal settlements in Makassar, Indonesia, we conducted annual testing for past dengue and chikungunya infection by Abcam IgG ELISA in children under 5 years old. We then calculated annual incidence using the catalytic formula with age stratified seropositivity rates and using the seroconversion rate in children tested both years. Amongst 154 children tested during at least one of two testing campaigns in 2019 and 2020, seropositivity was 32% for dengue and 31% for chikungunya. We estimated the dengue annual incidence to be approximately 10-18%. We also found that children living in houses made of porous wall or floor materials were less likely to have evidence of a past dengue or chikungunya infection (OR=0.5; p<0.05). Additionally, children living in households with trash collection were less likely to be seropositive (OR=0.4; p<0.05) compared to those that disposed of their household trash locally in the settlement Changes in house construction and trash disposal practices offer opportunities for interventions to reduce dengue and chikungunya transmission in this highly vulnerable population.

GENOMIC CHARACTERIZATION OF SARS-COV-2 FROM AN INDIGENOUS RESERVE IN MATO GROSSO DO SUL, BRAZIL

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The COVID-19 pandemic had a major impact on indigenous populations. Understanding the viral dynamics within this population is essential to create targeted protection measures. A total of 204 SARS-CoV-2 positive samples collected between May 2020 and November 2021 from an indigenous area in Mato Grosso do Sul (MS), Midwestern Brazil, were screened. Samples were submitted to whole genome sequencing using the Nanopore sequencing platform. Clinical, demographic, and phylogenetic data were analyzed. We found the co-circulation of six main SARS-CoV-2 lineages in the indigenous population, with the Zeta lineage being the most prevalent, followed by B.1.1 (an ancestral strain), Gamma, and Delta. The estimated indigenous population mortality rate was 1.47%. Our results revealed that multiple independent SARS-CoV-2 introduction events had occurred over time, probably due to indigenous mobility since the villages studied here are close to urban areas in MS, and people are in constant movement between both areas. The mortality rate was slightly below the estimation for the state in the period studied, which we believe could be related to the low number of samples evaluated, the underreporting of cases and deaths among the indigenous population, and the inconsistency of secondary data available for this study. In this study, we showed the circulation of multiple SARS-CoV-2 variants in the indigenous population, which should be isolated and protected as they belong to the most fragile group due to their socioeconomic and cultural disparities. We reinforce the need for constant genomic surveillance to monitor and prevent the spread of new emerging viruses and to better understand the viral dynamics in these populations, making it possible to direct specific actions.
METAGENOMIC SEQUENCING REVEALS EXTENSIVE DIVERSITY OF RNA VIRUSES IN WESTERN AUSTRALIAN MOSQUITOES

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Mosquitoes harbour a wide diversity of microorganisms, including insect-specific viruses and viruses of public health importance. In recent years, metagenomic approaches have enhanced the study of these widely diverse and complex virus populations in field-collected mosquitoes. We used metagenomics to characterize the infectome in mosquitoes trapped as part of the Western Australia (WA) arbovirus surveillance program. Firstly, we performed high-resolution metagenomic sequencing on six mosquito species associated with medically important viruses: Aedes vigilax, Culex annulirostris, Cx. australicus, Cx. globocephalus, Cx. molestus and Cx. quinquefasciatus. We identified 41 RNA and one DNA viral species from 19 families, including 13 novel viruses. Culex mosquitoes exhibited a significantly higher diversity of viruses than Aedes; no virus was shared between the two genera. We observed heterogeneous distribution of viruses between geographical regions and between closely related species suggesting the possible role of geography and host species in shaping virome composition. Wolbachia bacteria were detected in three members of the C. pipiens complex, excluding C. globocephalus. Secondly, we characterized viruses from cytopathic effect (CPE)-positive tissue culture supernatants obtained by inoculation of mosquito homogenate, in which flaviviruses and alphaviruses were excluded via fixed-cell ELISA. Follow-up epidemiological investigations are needed to determine whether the other identified viruses infect humans or other animals. In summary, we have used an unbiased approach to expand and understand the diversity of RNA viruses and other microorganisms in WA mosquitoes.

A UNIQUE AMPLICON SEQUENCING TECHNOLOGY FOR INFECTIOUS DISEASE: LONG AND SHORT-READ SOLUTIONS

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The COVID-19 pandemic demonstrated that accurate, rapid, and sensitive viral genome sequencing is critical to directing the global response to disease and pandemics. IDT's xGen™ Amplicon technology allows over 3,500 target specific primers in a single multiplexed PCR reaction. This design can generate super-amplicons that maintain target coverage when a primer drop-out occurs due to novel viral mutations. Super-amplicons form when amplicon primers produce 2X or greater sized amplicons. A primer drop-out occurs due to novel viral mutations. Super-amplicons are produced but are too long for sequencing. Coverage was improved to greater than 96% for all variants; however a significant drop in viral mapping resulted. To circumvent the low viral mapping rate, an xGen Amplicon panel for HIV was designed using the Oxford Nanopore MinIon (ONT). For HIV we developed an xGen Amplicon panel using the reference genome HXB2. We tested five variants with 91-94.3% identity to HXB2. Coverage for the short read panel ranged from 77-90%. Gaps in coverage were mainly in the env region. We hypothesized that super-amplicons are produced but are too long for Illumina platforms. Here we show that the xGen Amplicon technology also provides sequencing solutions with long read sequencing (up to 4kb read length) using the Oxford Nanopore Minion (ONT). For HIV we developed an xGen Amplicon panel using the reference genome HXB2. We tested five variants with 91-94.3% identity to HXB2. Coverage for the short read panel ranged from 77-90%. Gaps in coverage were mainly in the env region. We hypothesized that super-amplicons are produced but are too long for Illumina sequencing. To confirm this, HIV amplicon products of the multiplex PCR were subjected to fragmentation and library prep followed by Illumina sequencing. Coverage was improved to greater than 96% for all variants; however a significant drop in viral mapping resulted. To circumvent the low mapping rate, an xGen Amplicon ONT panel for HIV was designed using the same panel design described above. Long read sequencing with the Minion generated coverage greater than 90% for the four HIV variants that were tested, while a high viral mapping rate was maintained. This demonstrates the ability of the panel to generate amplicons that can be used to sequence highly divergent regions of rapidly evolving viruses even when regions of the genome diverge significantly from the design genome. We are continuing with this approach for a number of other viruses, including Chikungunya, Zika, and Dengue.
**RE-EMERGENCE OF COSMOPOLITAN GENOTYPE OF DENGUE VIRUS SEROTYPE 2 IN SOUTHERN VIETNAM**

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Dengue is a mosquito-borne viral infection caused by one of the four dengue serotypes (DENV-1-4). Each serotype is subdivided into several genotypes. Asian 1 of dengue virus serotype-2 (DENV-2), has been the dominant genotype in southern Vietnam for the last decade. In this study, we have identified new DENV-2 lineages of cosmopolitan genotype in Ho Chi Minh City, not previously detected in Vietnam before (samples from 2017-2022). We infer the likely transmission routes of these new DENV-2 lineages into Vietnam from other South and Southeast Asian countries and when these events occurred. Of 45 DENV-2 samples, taken between 2017 and 2022, we identified 28 (62%) were Cosmopolitan genotype and 17 (37.7%) were Asian I genotype. The full DENV-2 phylogeny analysis will be presented, including the probable route of introduction of these viruses into Ho Chi Minh City and surrounding areas, the approximate timing of these events, and current outbreak dynamics. This re-emergence of the Cosmopolitan genotype, with new lineages, prompts an urgent need to update dengue serotyping techniques for surveillance in the region as well as set up studies on evolution, and transmission dynamics of this virus.

**DENGUESEQ: DEVELOPMENT AND VALIDATION OF A PAN-SEROTYPE WHOLE GENOME AMPICLON SEQUENCING APPROACH FOR DENGUE VIRUS**

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Amplicon-based sequencing (PrimaSeq) was developed in response to the Zika virus epidemic due to difficulties generating complete genomes using metagenomic approaches. Later this approach was adapted as the primary sequencing method for SARS-CoV-2 (i.e. the “ARTIC” protocol). Investments in global genomic infrastructure resulted in a significant increase in amplicon sequencing capacity that can be utilized beyond SARS-CoV-2, by swapping out virus-specific components such as primer schemes. Increased genomic surveillance of other viruses of public health concern, such as dengue virus, is needed to reduce the future burden of disease. Particularly, genomic surveillance of dengue virus can help to monitor the roll out of novel control strategies such as vaccines and release of mosquitoes carrying the virus-inhibiting Wolbachia bacterium. However, the majority of currently available sequences are partial, while complete genomes are needed to monitor and refine novel control tools. In this study, we developed and validated a pan-serotype whole genome amplicon sequencing approach for dengue virus. We sequenced a panel of virus stocks as well as clinical specimens from Florida to validate our approach with genetically diverse dengue viruses spanning the defined genotypes within each of the four serotypes. We show that the dengue primer schemes can be “plugged” into existing amplicon sequencing workflows, with high genome coverage across all four serotypes at a range of RNA titers (threshold of ~100 RNA copies/µL). The primer schemes can be either used as a serotype-specific assay (serotype known) or mixed into a unified pan-serotype assay (serotype unknown), with similar sensitivity. Our approach can help laboratories to quickly adapt their existing amplicon sequencing workflows to improve genomic surveillance of dengue virus.

**EVOLUTION AND CIRCULATION OF SARS-CO-V2 OMICRON SUBVARIANTS IN ODISHA STATE, INDIA, NOVEMBER 2021 TO NOVEMBER 2022**

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Omicron Variant of Concern (VOC) & its subvariants are characterized by immune evasion and reinfection. We present 1 year data (November 2021 to November 2022) of circulating SARS CoV2 variants in Odisha, India. We included 855 samples from SARS CoV2 positive patients by real-time RT-PCR referred to the Virus Research & Diagnostics Laboratory at the ICMR Regional Medical Research Centre, Bhubaneswar. Next-generation sequencing for SARS-CoV-2 was performed on Oxford Nanopore MinION Mk1C with Midnight protocol for library preparation. Bioinformatic pipeline included ARTIC field bioinformatics, lineage classification was done with PANGOLIN and Nextclade. Demographic data, history of vaccination & prior SARS CoV2 infection was included for analysis. Predominant circulating VOC in Odisha in November & December 2021 was B.1.617.2 in 87.3% and 58.8% respectively of sequenced samples while Omicron (B.1.1.159) VOC was detected in 3.23% of specimens in December 2021. BA.1 and BA.2 subvariants dominated between January-May 2022 and corresponded to a peak in COVID19 cases & high positivity between January-March 2022. A shorter peak in reported cases in July-August 2022 corresponded with predominance of BA.2,75 subvariant (40.3% and 60% of sequenced samples July & August 2022 respectively). BA.4, BA.5 were detected in limited samples (5% and 1% July 2022). XBB and further subvariants (XBB.1, XBB.2, XBB.3) were detected from September-November 2022 but did not correspond with increased cases or hospitalization. In cases of SARS CoV2 reinfection(N=166), the commonest subvariants were BA.2 (24.1%), BA.2.75 (24.7%) & XBB (10.8%) corresponding to the months in which these were the predominant subvariants. Reinfections were more common in ages 20-40 yrs and 40-60 yrs with few cases in >60 yrs. Primary 2-dose vaccination against SARS CoV2 was complete in all the reinfection cases, but in 39.2% of reinfections, booster 3rd dose had not been administered. In conclusion, Omicron and its evolving sub-variants are the current circulating VOC in our region in India and associated with considerable number of SARS CoV2 reinfections.

**DEVELOPING A DENGUE VIRUS LINEAGE CLASSIFICATION SYSTEM TO IMPROVE GENOMIC SURVEILLANCE**

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Last year, dengue virus caused nearly three million cases in the Americas. As climate change continues and Aedes species increase their geographical range, more populations are at risk of infection, leading to an increase in the disease burden. Interventions, including Wolbachia infection and vaccinations, are likely to be impacted by viral genetic diversity but dengue virus genetic diversity is currently not well described. Dengue virus genomes are categorized as one of four serotypes, and each of those have several
Identification of Genes Involved in the Type-I Interferon Response Elicited by the Live-Attenuated Japanese Encephalitis Virus SA14-14-2 Vaccine

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Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus that has both human and veterinary public health significance. The safe and highly effective SA14-14-2 live-attenuated vaccine (LAV) can elicit protective immunity with one single immunization and plays a major role in the control of human JEV. To date, little is known about the mechanisms contributing to the high immunogenicity of JEV SA14-14-2 LAV. Such knowledge can be translated to support the development of next-generation candidate JEV LAV and has important implications for the development of candidate LAVs for encephalitic flaviviruses. We undertook the genome-wide CRISPR-Cas9 knock-out screening of human A549 (GeCKO-A549) cells infected with the JEV SA14-14-2 strain, as a model system to investigate the host responses following immunization with JEV LAVs. Surviving cells were harvested, followed by the extraction of genomic DNA and next-generation sequencing analysis. Unique genes were selected based on the gene enrichment in the JEV resistant GeCKO-S49 cells as compared to the control cells. There was correlation between survival of GeCKO-A549 cells and deletion of tyrosine kinase 2, interferon alpha subunit 1, interferon beta receptor subunit 2, and signal transducer and activator of transcription 1 (STAT1). All four genes are associated with type-I interferon response against JEV. Although STAT1 knockdown did not result in the surviving phenotype, STAT1 gene was highly ranked. In comparison with wild-type JEV strains, the SA14-14-2 vaccine strain is capable of eliciting a broader spectrum of type-I IFN responses, providing a putative molecular basis for the high immunogenicity of the empirically developed human JEV live-attenuated vaccine (LAV). The knowledge can be translated to support the rational design of second-generation candidate LAVs to control human and swine JEV.

Molecular Phylogeny and Serotype Distribution of Dengue Virus in the Philippines, 2015-2022

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Dengue is the most prevalent arboviral disease in humans and it has caused tremendous burden to the tropical and sub-tropical countries. In the Philippines, dengue is considered as a major public health problem and is probably the most well-known arboviral infection and feared tropical disease. Despite being endemic in the Philippines with all four Dengue virus (DENV) serotypes, there is a limited information available about the circulating viral serotypes and genotypes. Serum samples (n = 30,594) from dengue suspected patients for the years 2015 to 2022 were sent to the Research Institute for Tropical Medicine (RITM) to detect DENV serotype by Real-time RT-PCR. The E gene was amplified using one-step RT-PCR and followed by direct Sanger sequencing. Phylogenetic analysis was done using Maximum Likelihood and General Time-Reversible +G model with 1,000 replicates for bootstrap (1,500 + n) by MEGA 7.0 for genotype identification of positive DENV samples. Dengue RNA was detected in 15,367 samples, 1,364 of these were successfully sequenced for the whole E gene. The predominant serotypes for 2015 were DENV 1 and 2. Subsequently, a shift to DENV 3 serotype from 2016 to 2019 was observed. However, in the year 2022, DENV 1 and 2 were detected again to be the dominant serotypes. It is also notable that in the same year, DENV 4 detection has increased compared to the previous years. Phylogenetic analysis showed that the Philippine DENV samples existing genotypes were: DENV 1 (GI = 13, GIV = 490), DENV 2 (Cosmopolitan = 292), DENV 3 (GI = 474), and DENV 4 (GI = 103). The eight-year phylogenetic data suggested that the existing genotypes for DENV 1 to DENV 4 in the Philippines originated mostly from Asian countries and French Polynesia. Constant monitoring of circulating strains is vital in understanding the phylogeographic patterns of disease outbreaks. This will aid in the development of future regional vaccines with antigenic and genetic compositions based on the surveillance.
PERSISTENCE OF SERUM IgM ANTIBODIES ANTI-CHIKUNGUNYA VIRUS FOR MORE THAN 24 MONTHS AFTER THE ONSET OF ACUTE SYMPTOMS

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(35 vaccinated, 44 unvaccinated) were recruited from the Sunyani West control design was employed for this study. Seventy-nine participants the transmission of the SARS-CoV-2 virus. In Ghana, data on the immune vaccine was highly effective in preventing not only the symptoms but also Preliminary data across the globe shows that the AZD1222 recombinant vaccine generated a higher antibody response compared to individuals who did not receive any dose of the vaccine. It remains to be seen how long the generated immune response will last in this population and whether a booster shot could be a useful strategy.

TO MODULATE OR NOT TO MODULATE: INCREASING IMMUNOGENICITY AND REDUCING IMMUNE EVASION OF SARS-COV-2 VIA NEXT GENERATION VACCINES

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Coronaviridae is a notable family of viruses that are responsible for multiple epidemics and pandemics throughout the 20th and 21st centuries. The SARS-CoV-2 pandemic and its generation of subsequent variants have magnified the importance of researching and understanding highly pathogenic emerging viruses and forging the advancement of vaccines to combat future pandemics that are likely to occur. The vaccines developed to combat the SARS-CoV-2 pandemic have significantly reduced the likelihood of hospitalizations and death in vaccinated individuals infected with the virus, however as the virus mutates to create emerging variants that evade prior immunity, the need for a robust and efficacious vaccine is the utmost importance. The type I interferon (IFN) response plays a dichotomous role in mRNA vaccines. Type I IFN has been shown to both inhibit and enhance the mRNA vaccine-driven response. Here we provide data showing our investigation of a type I IFN antagonist on mRNA vaccine immunogenicity and protection. As a model system, we developed an mRNA vaccine that expresses stabilized pre-fusion spike protein of SARS-CoV-2 and a type I interferon antagonist for immune modulation to confer robust immunity and protection against SARS-CoV-2 variants while reducing intra-host viral diversity relative to a vaccine encoding spike protein only. We have generated and validated our mRNA vaccine in vivo, leading to the demonstration of vaccine immunogenicity and protective capacity against SARS-CoV-2. Our results indicate that type I IFN antagonists improve vaccine immunogenicity and protective capacity against lethal challenge in comparison to parental mRNA vaccine (spike only). We intend to investigate the immune-modulated vaccine’s ability to alter viral population dynamics using SARS-CoV-2 barcode viruses in vaccinated mice post-infection to understand mechanisms of protection and control. Our findings on mRNA vaccine immunogenicity, vaccine efficacy through ancestral and variant challenges, and their impact on virus population dynamics will lead to advancements in the fields of virology, immunology, and evolutionary biology.

CROSS-SECTIONAL EVALUATION OF ANTI-SARS-COV-2 ANTIBODY RESPONSE TO AZD1222 RECOMBINANT VACCINE DEPLOYMENT IN THE BONO REGION, GHANA

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Preliminary data across the globe shows that the AZD1222 recombinant vaccine was highly effective in preventing not only the symptoms but also the transmission of the SARS-CoV-2 virus. In Ghana, data on the immune response generated by different vaccination doses is lacking. The present study aimed to compare the anti-SARS-CoV-2 antibody response among single and double-vaccinated versus unvaccinated individuals. A case-control design was employed for this study. Seventy-nine participants (35 vaccinated, 44 unvaccinated) were recruited from the Sunyani West Municipality and screened for the presence of SARS-CoV-2 specific IgG and IgM antibodies in plasma samples using a Standard COVID IgG and IgM Combo FIA test. Data analysis was carried out with STATA (Version 21). The current study showed that mean IgG levels among vaccine groups (Group 1: Not vaccinated, Group 2: 1 dose, Group 3: 2 doses) differed significantly (F2, 76=11.457, p<.001) between Group 1 and Group 3; and between Group 2 and Group 3. Participants in Group 2 and Group 3 were 4.1 and 12.5 times more likely to develop more antibody responses compared to their counterparts in Group 1 respectively. This baseline study demonstrated that in the short term, individuals who received either one or two doses of the AZD1222 recombinant vaccine generated a higher antibody response compared to individuals who did not receive any dose of the vaccine. It remains to be seen how long the generated immune response will last in this population and whether a booster shot could be a useful strategy.

SEROPREVALENCE OF HUMAN CORONAVIRUSES IN PEDIATRIC SAMPLES COLLECTED BEFORE COVID-19 PANDEMIC IN THE PHILIPPINES AND JAPAN

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Infections of four seasonal human coronaviruses (HCoVs); 229E, NL63, HKU1, and OC43, are common in children, and COVID-19 infections in
children are generally mild. This feature might be associated with protection conferred by recent seasonal HCoVs infection. This study aimed to determine the seroprevalence of HCoVs and SARS-CoV-2 neutralizing antibodies in children less than 5 years old. A total of 419 (Philippines: 315 and Japan: 104) serum samples collected before COVID-19 pandemic period (2015—2019) were tested for IgG antibodies against four seasonal HCoVs and SARS-CoV-2 using recombinant spike ectodomain proteins by enzyme-linked immunosorbent assay (ELISA). Neutralization antibodies against SARS-CoV-2 (wild-type) were also measured for samples collected in the Philippines that were positive for anti-SARS-CoV-2 IgG. As a result, about 90% of children less than 2 months old in the Philippines had IgG antibodies against four seasonal HCoVs. Then, the antibody prevalence was less than 20-47% between 6-11 months old and reached up to 80% by 2-3 years old. The seroprevalence of SARS-CoV-2 was low at about 3% between 6-11 months old and then reached about 50% at 4 years old. The seroprevalence of NL63, HKU1, and OC43 in samples collected in Japan showed a similar trend, although the seropositivity against 229E stayed low (63%) in those aged 4 years old. The median age of children in the Philippines who showed positive for anti-SARS-CoV-2 IgG antibodies were older and had significantly higher IgG antibody titer against four seasonal HCoVs, compared with those negative for SARS-CoV-2 IgG antibodies (age: 2.2 years vs. 0.9 years, p < 0.0001, antibody titer in each antigen: 7.7-13.5 vs. 1.3-6.13, p < 0.0001). These results suggest some cross-reactivity between SARS-CoV-2 and seasonal HCoVs. However, only one of the 69 reactive samples had a neutralization antibody against SARS-CoV-2. Although there is no neutralization capability in samples positive for anti-SARS-CoV-2 IgG antibodies, there might be some cross-reactive antibodies between SARS-CoV-2 and seasonal HCoVs, which might explain lower severity of COVID-19 infections among children.

5301

ASSESSING THE ROLE OF NON-NEUTRALIZING ANTIBODIES IN ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY OF DENGUE VIRUS INFECTED CELLS

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Dengue virus (DENV) is endemic in over 100 countries causing widespread morbidity and mortality. 400 million people are infected annually, resulting in 100 million symptomatic cases and at least 40,000 deaths. It has been previously described that antibodies against DENV E protein can cause antibody dependent enhancement during secondary DENV infection, increasing infection. However, there are other potential antigen targets created during DENV infection. Non-structural protein 1 (NS1) is a non-structural protein that is both secreted from and expressed on the surface of DENV infected cells. IgM, IgG, and IgA isotype antibodies against NS1 can be readily detected after DENV infection. Our study aims to determine: the capability of NK cells to clear NS1 expressing cells opsonized by antiNS1 antibodies via antibody-dependent cellular cytotoxicity (ADCC), confirm cytotoxic activation of NK cells with opsonized NS1-expressing cells, what receptors are used in both IgG and IgA isotype mediated killing of NS1 expressing cells, and if secreted NS1 functions to protect DENV-infected cells from ADCC. To this end, we will analyze ADCC using a flow cytometry based ADCC assay. We will assess the death of NS1-expressing cells in the presence of NS1-reactive antibodies and NK cells, and we will also assess the activation of NK cells using CD107a as a cytotoxic activation marker on NK cells. We will also assess receptor utilization of NK cells for IgG or IgA mediated ADCC. Using an αCD89 antibody known to block FcγR binding to IgA, we can assess potential reduced ADCC of opsonized NS1-expressing cells with an IgA monomolecular antibody. Similarly, using αCD16, 32, and 64 antibodies known to block FcγRs binding to IgG, we can assess reduced ADCC of opsonized NS1-expressing cells with an IgG monomolecular antibody. We have previously shown that secreted NS1 can block monocytic phagocytosis of NS1-expressing cells. We will also investigate the ability of secreted NS1 to block ADCC by NK cells and NK cells to decrease viral proliferation by killing infected cells.
INDIRECT IGG ELISA AND SEROTYPE-SPECIFIC NEUTRALIZING ANTIBODY TITERS ARE ASSOCIATED WITH DENGUE IN CHILDREN IN CEBU, PHILIPPINES
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Standard measures of dengue virus (DENV) antibodies have not been consistently associated with dengue disease. We assessed whether an indirect dengue IgG ELISA (PanBio; Brisbane, QLD, Australia), plaque reduction neutralization test (PRNT) to DENV1-4, and preparation of virus used in this assay (standard vs. mature) are associated with dengue risk and protection. Healthy children (n = 1,214) aged 9-14 years in Cebu, Philippines were enrolled in a prospective cohort study in May - June 2017, and febrile illness prompted dengue diagnostic testing through March 2020. At enrollment, all sera were tested for binding antibodies by ELISA, and random subsets were tested for neutralizing antibodies by PRNT to DENV1-4 using WHO reference (standard, n = 737) and mature clinical strains (n = 77). The probability of dengue was modeled as a function of baseline antibodies using logistic regression and generalized additive models adjusted for age, sex, and enrollment site. Inverse probability weighting was used to account for subset size. The probability of symptomatic dengue was 11.1% (95% CI: 5.6-20.9) in the naive group vs. 3.6% (2.0-6.3) for those with ELISA >= 3 (p = 0.005). By standard PRNT, geometric mean titers to DENV1-4 (GMTs) > 200 were protective (1.4% [0.6-3.1], p = 0.0001) with no enhancement at lower GMTs. Baseline standard PRNT titers to DENV2 and DENV3 were protective against a homotypic dengue case (DENV2: OR 0.28, 95% CI 0.12 to 0.65; DENV3: OR 0.27, 95% CI 0.13 to 0.57), but not heterotypic dengue. Among children who entered the cohort with multitypic immunity, none had GMTs < 40 by the standard PRNT, but 49% had GMTs < 40 by the mature PRNT. Using the mature PRNT, GMTs between 40-100 were enhancing compared to GMTs <40 (probability of dengue: 15.4% [8.2-27.1] vs. 7.1% [3.7-13.3], p = 0.024) while GMTs >100 were protective (1.4% [0.3-5.4], p = 0.024). In sum, a commercial ELISA and neutralizing antibodies are associated with dengue. We hypothesize that the mature PRNT has a higher threshold for antibody binding and thus more accurately identifies enhancing and neutralizing antibodies. These assays may be valuable for use in population and vaccine trials.

THE MAGNITUDE AND QUALITY OF NEUTRALIZING ANTIBODIES CORRELATE WITH PROTECTION AGAINST SYMPTOMATIC DENGUE VIRUS INFECTION AND DIFFER BY SEROTYPE, IMMUNE STATUS, AND ASSAY CONDITION
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Neutralizing antibodies (nAbs) are considered an essential component of the protective response against the four serotypes of dengue virus (DENV-1-4), yet measurement of their potency is primarily performed using a single cellular substrate and partially mature virions. This does not capture the full breadth of nAb activity and may lead to biased estimations of nAb potency and repertoire. Here, we evaluated the nAb response associated with protection against symptomatic DENV infection using samples collected after one or more DENV infections but prior to a subsequent symptomatic or inapparent DENV1, DENV2 or DENV3 infection in a long-standing pediatric cohort study in Nicaragua. We compared nAb titers in pre-inapparent and pre-symptomatic infection samples measured in Vero cells with or without DC-SIGN expression infected with mature or partially mature virions. This allowed us to measure the magnitude and quality of the nAb response and revealed that nAb correlates of protection are dependent on the individual’s prior DENV immune status (primary vs. secondary) and the infecting serotype. Higher cross-reactive nAb titers were associated with protection against DENV1 and DENV2 disease in participants with one prior infection (DENV1 and DENV2) or multiple prior infections (DENV2), while no difference was observed between the pre-DENV1 infection groups. The nAb potency and the protective NT50 correlate were greatly impacted by virion maturation state and cell substrate. For all serotypes combined, the median NT50 to partially mature virions in Vero cells was 202, compared with 42 to mature virions in Vero-DC-SIGN cells. nAbs to mature virions with cross-reactive neutralizing antibodies (Abs) like envelope-dimer epitope (EDE) Abs, which target quaternary epitopes on the E protein dimer and neutralize dengue viruses 1-4 (DENV1-4) without triggering ADE. Here, we investigated natural infection and vaccine-induced cross-reactive neutralizing Abs in children with prior DENV infection histories in a longitudinal vaccine cohort in Cebu, Philippines. In total, 1,214 children remained unvaccinated while 1,782 children received a single dose of Dengvaxia in June of 2017 during a mass vaccination campaign. Serum samples were collected one month before and one year (12-18 months) after the vaccine campaign. We selected a random subset of n = 223 polytypic DENV-immune children to measure baseline status and change in EDE-like Abs due to natural infection and vaccination. Paired samples were tested by Plaque Reduction Neutralization Test (PRNT) with mature DENV1-4 low passage clinical isolates and a Blockade-of-Binding (BOB) assay to detect Abs that prevent DENV Ab C8 from binding a DENV2 E protein dimer. An IgG ELISA against the DENV1-4 E protein monomer and DENV2 E protein dimer were also performed on the same participants for comparison to the mature PRNT and BOB. Both vaccinated and unvaccinated groups had high levels of EDE C8-like Abs at baseline. We also observed a significant increase in Ab level after one year in both groups across all assays. The level of C8-like Abs in a sample was correlated with how well sera were able to neutralize mature DENV1-4 in PRNT assays. This correlation was stronger in naturally infected sera than vaccinated sera, indicating differences in Ab quality. These results highlight the presence of EDE-like Abs that could possibly protect against the four DENV serotypes and provide insights for future vaccine candidates.

DETECTION OF ENVELOPE-DIMER EPITOPE-LIKE BROADLY PROTECTIVE ANTIBODIES IN DENGUE-IMMUNE CHILDREN IN THE PHILIPPINES FOLLOWING VACCINATION AND NATURAL INFECTION
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The dengue vaccine, Dengvaxia, has been described as inducing antibody-dependent enhancement (ADE) in dengue-naïve individuals. There is an urgent need to develop an effective dengue vaccine that elicits protective,
After primary dengue (DENV) infection or vaccination, individuals develop antibodies to different epitopes and domains on the DENV envelope (E) and pre-membrane (prM) glycoproteins. Of these, serotype-specific (TS) antibodies typically target E domains, while serotype cross-reactive (CR) antibodies typically target the pre-membrane (prM) protein and conserved regions of E. Age-dependent vaccine efficacy has been observed in Phase III trials of pediatric live-attenuated vaccination against DENV, where younger children have lower protection after vaccination compared to older children. A possible component of this difference may be variation in epitopes of neutralizing antibodies elicited by maturing immune systems.

To identify and quantify E-domain, neutralizing TS antibody responses in polyclonal sera, we developed a panel of chimeric DENV4/2 viruses that incorporate DENV2 envelope domain I, II, and III (DENV4/2-EDI, EDII, respectively) into the DENV4 E glycoprotein. The recoverable DENV4/2-EDI recombinants was dependent on the inclusion of chimeric DENV4/2 prM protein that maintained critical interactions with chimeric E. The ED-chimeric viruses preserved epitopes of TS and envelope dimer epitope (EDE) CR mAbs and had similar sensitivity to CR polyclonal responses as the parental strains. In natural infection and human challenge studies, the neutralizing activity of polyclonal sera predominantly targets EDIII. To evaluate age-based differences in antibody domain-level epitope targets controlling for prior exposure, we also present an analysis of primary DENV2 convalescent sera from the Pediatric Dengue Cohort Study located in Managua, Nicaragua. Additional studies in controlled experimental studies and well-described observational cohorts will examine if ED epitope targets correlate with protection against subsequent infection.

**5307**

**NEUTRALIZING IGM CONTRIBUTE SUBSTANTIALY TO BOTH PRIMARY AND SECONDARY DENGUE SEROTYPE 1 IMMUNITY**

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IgM antibodies can contribute to virus neutralization, but their role in immunity to dengue virus serotypes 1-4 (DENV1-4) remains a gap. DENV1-4 cause ~100 million infections per year, with 25-50% symptomatic cases. Using children’s plasma from our long-standing hospital-based study in Nicaragua, we compared DENV-neutralizing IgM antibodies in primary (1st; n=10) and secondary (2nd; n=19) DENV infections. Plasma from ~14-30 days post-symptom onset were IgM vs mock-depleted, and polyclonal IgM was eluted. We found that plasma IgM antibodies in both 1st (Mean NT50=0.23µg/mL) and 2nd (Mean NT50=2.6µg/mL) DENV1 cases demonstrated potent neutralizing activity against DENV1, the infecting serotype. Surprisingly, in 55% (10/18) of 2nd DENV1 cases, plasma IgM contributed an average of 30% (range 2-76%) of total plasma DENV1-neutralizing activity at early convalescence, which is the peak antibody response. In this subset, mean DENV1-neutralizing titer in plasma with IgM was higher than plasma without IgM (NT50 = 63,331 vs 49,458; p<0.05, Wilcoxon paired rank test). Even after controlling for IgG in each fraction, the DENV1-neutralizing titer of plasma with IgM was 21% higher than that without IgM. This demonstrates a substantial role of IgM in 2nd DENV1 cases. As a benchmark, plasma IgM from 1st DENV1 cases (n=8/9) contributed to a mean of 54% (24-76%) of total plasma DENV1-neutralizing activity. Additionally, we found that eluted plasma IgM from 2nd vs 1st DENV1 cases were phenotypically distinct, with more cross-neutralization of DENV2 and DENV3 in 2nd (Mean NT50 DENV2 =3.8; DENV3 = 2.4 µg/mL) compared to 1st (Mean NT50 DENV2 = 4.6; DENV3 = 12 µg/mL) DENV1 cases (p<0.05; Mann Whitney Test). Thus, plasma IgM from the early convalescence of both 1st and 2nd DENV1 cases demonstrate substantial antiviral activity, and breadth of IgM DENV-neutralization is greater in 2nd DENV1 immunity. Contribution of IgM to 2nd DENV1 immunity is particularly intriguing since this is a time at risk for enhanced dengue. Neutralizing IgM may have an underappreciated role in controlling DENV infection.

**5308**

**TYPE-SPECIFIC ENVELOPE-DOMAIN EPITOPES OF NEUTRALIZING ANTIBODIES AFTER PRIMARY DENV2: SUMMARY OF FINDINGS FROM NATURAL INFECTION, HUMAN CHALLENGE MODELS, AND YOUNGER AND OLDER CHILDREN FROM A PEDIATRIC OBSERVATIONAL COHORT**

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Dengue virus seropositivity is often measured by plaque reduction neutralization test (PRNT), which assesses the antibody response of a patient against one or more of the dengue virus serotypes 1-4 (DENV1-4). These tests consist of serum from a patient and an infectious virus, but the high antigenic diversity of dengue virus may mean that the neutralizing capacity of an individual’s serum is variable by strain. Additionally, viruses can emerge from cells at different stages of maturity (defined by the cleavage of the precursor membrane protein), with mature viruses possessing a more homogeneous structure and fewer antibody-accessible epitopes. To assess the effect of these variables on neutralization assays, we grew both standard and mature preparations of highly passaged DENV1-4 WHO reference strains, where lab adaptation may increase antigenic diversity. As anticipated, antibody titers were...
highest against the WHO-standard strains, but surprisingly, we also found that the WHO-mature strains had the lowest titers. When we scrutinized this trend by serotype, the WHO-standard strains consistently had the highest titers, while the titers of the other three virus preparations relative to one another were variable by serotype. Overall, the geometric mean titers of the SEA strains were significantly different from the titers of the WHO strains irrespective of maturation preparation (p < 0.001), but the SEA strains were less sensitive to changes in maturity (p = 0.09) than the WHO strains (p < 0.0001). Our results suggest that careful consideration should be given before selecting a viral strain for neutralization assays. A mature isolate may be more useful as a correlate of protection. However, for identifying any prior DENV infection, a standard lab-adapted virus may better detect weakly neutralizing antibodies.

**CIRCULATORY T FOLLICULAR HELPER CELL AND MEMORY B CELL FREQUENCIES IN A CONVALESCENT DENV IMMUNE COHORT**

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Dengue virus (DENV) is the cause of the most prevalent mosquito-borne viral disease worldwide, with an estimated 400 million cases and 21,000 deaths per year. Circulatory T follicular helper cells (cTfh) activation, a CD4+ T cell subset that helps B cells, requires antigen presentation through specific human leukocyte antigen (HLA). In some populations, HLA alleles are associated with DENV protection, with robust and multifunctional T-cell responses, supporting a role for T-cells during DENV infection mediated through individual HLA alleles. However, studies identifying conserved HLA alleles specific to DENV epitopes and HLA-restricted T cells (DENV-specific HLA-restricted T cells) across multiple DENV endemic populations, including Puerto Rico, still need to be included. In this context, we aim to characterize the breadth and magnitude of DENV-specific HLA-restricted cTfh cell responses in a convalescent DENV immune cohort to access specific cellular and host genetic factors mediating the adaptive immune response to DENV infection. Here we test the hypothesis that DENV-specific HLA-restricted cTfh cells persist over the convalescent period with heightened breadth and magnitude. HLA class II alleles from stored buccal samples will be genotyped by Next-Generation Sequencing using the MHC Core Library Prep and Capture Kit. We aim to describe the HLA alleles from a Puerto Rico cohort. The HLA alleles selected for the study will be the most frequent alleles worldwide for each locus and the unique alleles in the population. The investigators anticipate that DENV-specific HLA-restricted cTfh cells circulating in blood persist over convalescent infection with heightened breadth and magnitude.

**LINKING MULTIPLE SEROLOGICAL ASSAYS TO INFECT DENGUE INFECTION HISTORY ACROSS PAIRED SAMPLES USING MIXTURE MODELS**

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Dengue virus (DENV) is an increasingly important human pathogen, with half of the globe living in environments where DENV transmission may one day occur. Since only a minority of infections are captured by direct detection methods (PCR or antigen tests), serological assays play an important role in the diagnostic process. However, interpreting results from serological assays across different platforms remains challenging, particularly because interpretations from multiple assays may differ, creating uncertainty over how to generate finalized interpretations. Here we utilize mixture models, which provide a probabilistic framework to separate data into multiple distinct components, to infer infected vs. uninfected individuals from longitudinal serological samples. We develop a Bayesian mixture model that can jointly model data from multiple serological assays, and that can incorporate information from serum sampled at multiple time points. We fit to 3479 sampled pairs of acute and convalescent serum collected as a part of illness and household investigations across three longitudinal cohort studies in Kamphaeng Phet, Thailand which contained 298 from gold standard PCR confirmed infections. We compare the classification of the new model to prior standard interpretations that independently utilize information from either the hemagglutination inhibition assay (HAI) or enzyme-linked immunosorbent assay (ELISA). Our results provide a probabilistic framework through which multiple serological assays across different platforms can be combined across sequential serum samples to provide insight into whether individuals have recently experienced DENV. As the differences in results across these platforms reflect performance as well as biological differences, future work will explore the clinical and immunological differences that shape these heterogeneous immune responses to DENV and whether distinct phenotypes may shape subsequent clinical outcomes.
Additional experiments aiming at identifying soluble immune factors that could explain the development of TFH responses in in footpad-draining LN of co-infected mice are being carried out.

5313

EVALUATING THE CONTRIBUTION OF NS1 ANTIGENEMIA TO DENGUE-ELICITED NEUTROPENIA

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Neutrophils are an important part of the innate immune response and host protection from invading pathogens. One way pathogens try to impede this innate response is decreasing the number of neutrophils available, causing neutropenia. Neutropenia is a common clinical manifestation in viral infections, including dengue infection. Dengue virus (DENV) is responsible for dengue disease, a tropical disease responsible for an estimated 400 million infections per year and significant healthcare burden. The cause of dengue-elicited neutropenia is unknown but is an important question in the field due to culpability of activated neutrophils and their byproducts in the risk of hemorrhage in severe dengue. Neutropenia has a variety of causes, one prospect in the context of dengue infection is a cell death pathway known as NETosis. The cell releases its nuclear content complexed with antimicrobial proteins to immobilize invading pathogens; evidence of this process has been found during dengue infection. Using data collected from the Dengue Human Infection Model challenge study, our lab analyzed the early viral kinetics of dengue infection and identified the secreted form of DENV non-structural protein 1 (sNS1) in serum as a potential contributor to neutropenia. sNS1 plays an important role in dengue pathogenesis and is implicated in disrupting endothelial cell monolayer integrity and platelet activation, both important components of vascular destabilization during severe disease through interaction with TLR4. TLR4 engagement can induce NETosis, providing a potential link between sNS1 and neutrophils. To test sNS1 involvement in NETosis, we stimulated isolated neutrophils with varying concentrations of NS1 for three hours. Using fluorescent microscopy, we found that sNS1 appears to trigger cell death in neutrophils. We plan to preincubate neutrophils with a TLR4 blocking antibody to determine if TLR4 mediates sNS1 induction of neutrophil death. This begins to address the question of whether secreted NS1 interaction with neutrophils plays an important role in mediating dengue-elicited neutropenia through triggering of cell death pathways.

5314

ARBOVIRUS TRANSMISSION AND DISEASE PATHOGENESIS IN OBSESE AND TYPE II DIABETIC MICE

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In the last century, anthropogenic factors such as human movement to new ecotopes, agricultural expansion, and uncontrolled urbanization, have significantly contributed to zoonotic emergence and spillover at a global scale. Arthropod-borne viruses (arboviruses), maintained in nature through transmission cycles involving hematophagous arthropod vectors, are the most important contributors to disease and a public health concern worldwide. Concomitantly, another contemporary public health concern is the prevalence of chronic underlying conditions. According to the World Health Organization (WHO), chronic diseases kill approximately 41 million people each year, with cardiovascular disease, cancer, respiratory diseases, and diabetes accounting for the most deaths. Strikingly, clinical data indicate that patients with preexisting conditions such as diabetes infected with mosquito-borne viruses are prone to severe disease outcomes and mortality. It is reasonable to hypothesize that such conditions will impact the progression of arboviral replication and transmission in and from human hosts. In the present study, we aim to understand the arbovirus disease pathogenesis, viral kinetics, and mosquito acquisition of arboviruses in infected mice suffering from an obese state approximating a Type II diabetes mellitus (T2DM) phenotype. 10-week-old LEPRDB/DB, LEPRWT/DB, wild type C57BL/6J mice were pretreated with IFNAR blockading antibodies to render them permissive to MAYV infection through Aedes aegypti bite. No significant difference in viral load was observed during early infection (3 to 5 days post-infection) by MAYV among the three genotypes (medians ranging from 2.1 log10 to 3.1 log10 FFU/mL). This lack of significant difference in the viral load suggests that further histopathological and cytokine analysis must be conducted to analyze if there are differences in the MAYV infection outcome related to TSDM and the wild-type genotypes.

5315

EXPLORING MICRONA AS POTENTIAL DIAGNOSTIC BIOMARKER FOR ZIKA VIRUS INFECTION

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Zika virus (ZIKV) has emerged as a serious public health concern, which accurate diagnosis is of crucial importance, since it impacts pregnancy outcome and has lifelong consequences for newborns from infected mothers. However, current diagnostic tests are challenging for nucleic acid testing due to short-lived viremia and low viral loads, and for serological testing due to antibody cross-reactivity with other flaviviruses leading to misdiagnosis. This study aimed to identify and evaluate microRNAs (miRNAs) in plasma as biomarkers for accurate ZIKV diagnosis. In the discovery phase miRNA profiles were determined by Next-Generation sequencing using plasma samples from pre- and post-seroconversion phase of ZIKV infection, and from non-infected subjects as a control group. Analysis of miRNA results from the discovery phase led to the identification of 110 differentially expressed miRNAs (68 up- and 42 down-regulated) in ZIKV-infected subjects as compared to the control group. We selected 20 miRNAs with high levels of differential magnitude (up or down modulation) between ZIKV-infected and control groups for further evaluation and validation by qRT-PCR using sample from 72 subjects, including 48 ZIKV-infected (26 pre- and 22 post-seroconversion) and 24 from non-infected controls. A total of 5 miRNAs (miR-3929, miR-3615, miR-17-3p, miR-497-5p, and miR-1224-5p) exhibited the highest differential expression to discriminate ZIKV-infected from control, from which 2 (miR-3929 and miR-3615) were identified using the logit model as a signature-panel to distinguish infected from non-infected subjects. To verify and validate the identified signature-panel we performed blind-coded testing in a cohort of 36 plasma samples composed of ZIKV-infected pre- (n=12) and post-seroconversion (n=12) and non-infected control (n=12). Decoded analysis of results revealed that 19/24 infected samples and all uninfected were correctly identified, demonstrating feasibility of the miRNA signature-panel to be used as non-viral and non-immune biomarkers to diagnose ZIKV infection and potentially for monitoring pregnant women in endemic areas.

5316

CARDIAC ELECTROMECHEANICAL ALTERATIONS DURING CHIKUNGUNYA VIRUS INFECTION

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Chikungunya virus (CHIKV) is a re-emerging arbovirus that is endemic to several parts of the world, including Africa, Asia, South America, and Central America. CHIKV has been shown to infect several tissue systems, resulting in a spectrum of symptoms. While it is generally considered an abnormal presentation, there is significant clinical evidence of CHIKV infection of human cardiac tissues, including myocarditis, arrhythmia, and cardiac arrest. These reports span over five decades and include all lineages that were and are currently circulating in the world. As climate change increases the range of CHIKV and more people are at risk of infection, there is a need to understand how CHIKV can affect the heart. In this study, six lines of human pluripotent stem cell derived cardiomyocytes
(hiPSC-CMs) were infected with seven different strains of CHIKV, including field strains of East Central South African, Indian Ocean, Asian, and West African lineages. Microelectrode Array (MEA) was used to assess electrophysiological alterations of the cardiomyocytes, including beat rhythm, conducation, and contractility changes. All strains of CHIKV directly infected hiPSC-CMs within two days of infection. MEA analysis revealed differences between impact of CHIKV infection on female hiPSC-CMs and specific CHIKV strains. Multiple linear regression analysis showed that gender was a significant predictor for several electromechanical factors. Statistical analysis did not indicate that any CHIKV lineage caused significant electrophysiological changes. The data show significant changes in electrophysiology of CHIKV infected hiPSC-CMs, where specific CHIKV lineage does not appear to play a role, which is supported by previous clinical data. Further, the experiments conducted here support past mouse model work and reveal hiPSC-CMs as a relevant study model for CHIKV infection of the human heart, allowing for novel discoveries to be made.

5317
SOLUBLE UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR AS PROGNOSTIC BIOMARKER FOR SEVERE DENGUE IN ADULTS

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Dengue is the most common arboviral disease with an estimated 100 million symptomatic infections annually, and cases is expected to increase with warmer climate. Dengue associated illness requiring hospitalization is estimated at 19.0% in Asia, which may strain healthcare facilities during outbreaks. Current biomarkers are inconsistent in predicting SD in early disease, and a combination of biomarkers to predict SD are less feasible in resource limited settings. Together, poses a challenge in the management of dengue patients. In a longitudinal cohort (febrile, critical and recovery phases) of adult dengue patients recruited in Singapore (2016-2019), we evaluated the utility of soluble urokinase plasminogen activator receptor (suPAR) as a prognostic biomarker of SD. A total 129 patients: 40 dengue fever (DF), 46 dengue warning signs (DWS), 13 SD and 30 controls, were assayed for plasma suPAR levels by ELISA. In the febrile, critical and recovery phases, suPAR levels were significantly elevated in the dengue group compared with controls, and levels were significantly raised with increasing severity (all, P<0.001). By pairwise comparisons, suPAR concentrations were significantly raised in SD versus either DWS or DF in all disease phases, but no significant difference between the DWS and DF groups was observed. By logistic regression, a unit increase in suPAR level was associated with an increased risk of SD in the febrile phase [OR: 2.1, 95%CI (1.2-3.7), P = 0.009] and critical phase [OR: 1.70, 95%CI (1.27-2.28), P < 0.001]. In the febrile and critical phases, the AUROC for suPAR to predict SD was 0.82 (95%CI 0.63-0.99) and 0.86 (95%CI 0.75-0.97), respectively. Using a cut-off, suPAR levels at >4ng/ml, to predict SD, the sensitivity was 86.0% with a specificity of 69.0% in the febrile phase, and 91.0% sensitive and 68.0% specific in the critical phase. The PPV for SD in febrile and critical phases were 35.0% and 42.0%, respectively, and the corresponding NPV was 97.0% (febrile) and 98.0% (critical). In conclusion, plasma suPAR levels were elevated in adult dengue patients in proportion to disease severity and maybe a reliable predictor of SD.

5318
ATTENUATED CHIKUNGUNYA VIRUS STRAIN 181 CLONE 25 INFECTION IN IMMUNOSUPPRESSED Rhesus Macaques

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Chikungunya is a mosquito-borne infection caused by Chikungunya virus (CHIKV) which is transmitted to humans by the bite of infected mosquitos. CHIKV is a rapid-onset febrile disease characterized by fever, headache, lethargy, arthralgia, muscle pain, and rash. The attenuated CHIKV 181/clone 25 was developed for live vaccine production for human use in 1986 using CHIKV 15561 strain isolated from an infected patient from Thailand. CHIKV 181/clone 25 induced great protection against challenge and highest antibody titers in weanling mice and monkeys; however, it was withdrawn because 8% of volunteers in Phase II human clinical trials developed transient arthralgia. In this study, we studied the ability of attenuated CHIKV strain 181/25 to cause disease in immunosuppressed rhesus macaques (RM) using cyclophosphamide and dexamethasone. The immunosuppressed monkeys were inoculated with 107 PFU of CHIKV strain 181/25. After the infection, all animals exhibited asymptomatic infection as evidenced by lacking of core temperature change, joint swelling and/or lymph node enlargement. No critical changes in blood cell counts were observed following the infection. Viral RNA was detectable in the circulation from day 1 after intravenous infection, and longer bacteremia period was observed in an animal in the immunosuppressed group. Anti-CHIKV IgM and IgG were induced by day 4 post infection. CHIKV-specific cellular immune responses were determined by IFN-γ ELISPOT assay and showed the cellular responses during 7 to 14 days post infection. Cytokine profiling was different between the immunosuppressed and control groups. Viral RNA dissemination was found predominantly in lymph nodes and spleen with greater level in the immunosuppressed group. Infectious virus was detected after 1 to 2 days post infection in one animal in each group at relative low level. This study provided laboratory tools to evaluate CHIKV rhesus model for pre-clinical study or drug and vaccine testing. However, to establish symptomatic CHIKV illness, longer immunosuppression treatment may require.

5319
A MACHINE LEARNING AIDED COMPARISON OF LIVER PATHOLOGY AMONG FILOVIRUSES

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Ebola virus and Marburg virus are filoviruses known for causing hemorrhagic fever. Pathologic changes in the liver are abundant and develop early after infection. However, model specific differences in liver pathology are poorly understood. We performed a retrospective study of the liver pathology resulting from different filoviral models, using machine learning approaches, to investigate model specific differences. Liver slides and blocks were examined from rhesus monkeys infected with Ebola virus (EBOV Kikwit and EBOV Makona), and Marburg virus (MARV), and compared to clinical chemistries obtained during the experiment. All samples were from previous experiments on animals that did not receive treatment or vaccination and were infected intramuscularly with similar inoculant. A board-certified pathologist annotated slides for areas of parenchyma and necrosis in QuPath. Model training was done using a customized multi-class U-Net implemented in Tensorflow. Clinical chemistries showed higher levels of the liver enzyme ALT in animals infected with MARV (mean 1411) compared to EBOV Makona (mean 666, p= 0.001) or EBOV Kikwit (mean 387, p<0.001), but lowest levels of AST in animals infected with MARV compared to EBOV Makona or EBOV Kikwit. The percentage of parenchymal area with necrosis was highest in animals infected with MARV.
A CONSISTENT NONHUMAN PRIMATE MODEL FOR EARLY ZIKV-ASSOCIATED PREGNANCY LOSS

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The 2016 Zika virus (ZIKV) outbreak in the Americas revealed a previously unidentified risk of birth defects in babies born to mothers infected with African-lineage ZIKV during pregnancy. However, the impact of African-lineage ZIKV infection during pregnancy remains less understood, despite probable origination in Africa and its presumed endemic presence in many countries for decades. Nonhuman primates serve as a valuable model for studying ZIKV in pregnancy due to their gestational similarities to humans and susceptibility to ZIKV infection but limited animal numbers and inconsistent outcomes have hindered the study of ZIKV preventative and therapeutic strategies. Using a nonhuman primate model, we tested the effects of African ZIKV co-infection on pregnancy outcomes in SIV-positive rhesus macaques, we identified a specific combination of infection timing, dose, and ZIKV strain that consistently resulted in fetal demise. Regardless of SIV co-infection or antiretroviral therapy (ART) treatment, 11 out of 14 pregnancies (78%) ended in spontaneous loss within 3 weeks following infection at approximately 30 days gestation with an African-lineage ZIKV. ZIKV was detected in the placentas and fetal tissues in all cases of pregnancy loss. Due to the high rate of pregnancy loss among all ZIKV-infected dams, we could not assess the impact of SIV co-infection. However, we serendipitously developed a model with a consistent outcome necessary for testing medical countermeasures in pregnancy and potentially uncovered an under-appreciated risk of early pregnancy loss due to infection with African-lineage ZIKV.
alcohol heavily. The possible mechanisms of CKD causing HCC involve uremia itself, long-term dialysis status, and miscellaneous factors such as hormone alterations and dysbiosis.

**5323**

RECONSTITUTION OF HUMAN MICROGLIAL CELLS IN BRAIN CEREBRAL CORTEX AND CEREBELLUM OF HUMAN-IMMUNE-SYSTEM HUMANIZED DRAGA MICE

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Human microglial cells are a target and reservoir for human pathogens affecting the central nervous system such as HIV, SARS-CoV-2, and Zika virus leading to severe neuropathology in humans. The lack of animal models allows to reconstitute human microglial cells poses a challenge for investigating human neuropathology after infection and for testing efficacy of immunotherapeutics. The humanized DRAGA mouse (HLA-A2.HLA-DR4, Rag1KO.ILR2TyC.KO.NOD) constitutes a functional human-immune-system upon infusion with CD34+ hematopoietic stem cells (HSC) from HLA-matched human umbilical cord blood. Pluripotent HSCs infused into DRAGA mice migrate in various tissues where they can differentiate not only into hematopoietic-derived cells, but also into non-hematopoietic cells such as human epithelial/endothelial cells expressing the human angiotensin-converting enzyme 2 (hACE2), the primary receptor for the SARS-CoV-2 virus infection, as well as human hepatocytes. The DRAGA mouse, by virtue of reconstituting human cells and by eliciting specific human cellular and antibody responses following infection and vaccination, represents a surrogate “in vivo human model” able to sustain infection with human pathogens such as SARS-CoV-2, Influenza, P. falciparum, HIV, Zika virus, and dengue. Herein we show reconstitution of human microglial cells (hCD45+hCD18+) in the brain cerebral cortex and cerebellum of DRAGA mice by flow cytometry (FACS), with cell numbers averaging 6.3% of the total brain microglia. Immunofluorescence studies further indicated that the human microglial cells in the brain of DRAGA mice were organized in small patches in the cerebral cortex and cerebellum but absent in the control (non-HSC infused DRAGA) mice. The DRAGA mouse model thus represents a novel pre-clinical model to investigate infection and immunopathology of human pathogens targeting human microglia, and for testing the efficacy of novel vaccines and immunotherapeutics.

**5324**

EFFICACY OF HUMAN SERA FROM SUBJECTS VACCINATED WITH A CHIKUNGUNYA VIRUS VIRUS-LIKE PARTICLE VACCINE IN CYMONOGULUS 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. 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. The vaccine has demonstrated protection against viremia after a virulent challenge in non-human primates. To determine the protective efficacy of antibodies induced in humans by the CHIKV VLP vaccine, sera from vaccinated volunteers was used to passively immunize cynomolgus macaques (NH-PrS). Four dose levels of CHIKV immune sera pooled from subjects who had been vaccinated once with 40 µg of CHIKV VLP vaccine were administered intravenously to six NH-PrS per group, and an additional six NH-PrS received negative control sera. All NH-PrS were challenged subcutaneously at 24 hours with a rescued clone of CHIKV outbreak strain LR2006-OPY1, a strain heterologous to the Senegal strain used to derive the CHIKV VLP. Animals were monitored for ten days following challenge. Analysis of serum immediately before challenge demonstrated that CHIKV serum neutralizing antibody (SNA) levels in NH-PrS increased in a dose-dependent manner. No animals that were administered CHIKV sera developed viremia at any time during the course of the study, while all animals that were administered control sera developed viremia that peaked two days post-challenge and resolved by day 4 post-challenge. Viral RNA was detected by quantitative reverse-transcriptase PCR in all control animals and in some animals in the two groups that were administered the two lowest dose levels (0.3 and 0.6 mL/kg) of CHIKV sera. No CHIKV RNA was detected in any animals that were administered the two higher CHIKV sera dose levels (1.2 and 2.4 mL/kg) or that had a pre-challenge SNA titer ≥ 25.7. This study demonstrated that sera from vaccinated individuals was sufficient to protect NH-PrS from viremia and the presence of viral RNA following heterologous CHIKV challenge and that an SNA titer of 25.7 was associated with that protection.

**5325**

INVESTIGATION OF A SUSPECTED CASE OF MONKEY POX, IBOKE, HEALTH DISTRICT OF TABOU, CÔTE D’IVOIRE, JULY 2022

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Monkeypox is a global public health problem. In West Africa more than 35 cases have been reported to WHO. On July 30, 2022, the health district of Tabou was informed by the detection of a suspected case of monkeypox in Iboke, a health area bordering Liberia, a country to which a confirmed case of monkeypox was reported. An investigation was carried out with the aim of describing the case, searching for other cases and proposing prevention and control measures. A cross-sectional descriptive study was conducted. A case was defined as any person presenting with an acute rash & one or more of the following signs or symptoms headache, fever, lymphadenopathy, myalgia, body aches, asthenia. The data were taken from consultation registers, interviews with identified contacts. Sociodemographic and clinical characteristics were collected. The data were analyzed in Excel & frequencies measures were calculated. 35-year-old young man with disseminated rashes on the face & the rest of the body accompanied by fever, with no notion of travel outside Iboke but having been in contact with a confirmed case. The result of his sample was negative. Ten (10) contacts were identified without signs & symptoms, of which 9(90%) linked to the suspected case and 2(20%) linked to the confirmed case in Liberia. 80% of the contacts of the suspected case live in Iboke versus 50% (1/2) for the confirmed case. The median age of contacts is 28.5 (range: 5 - 43). The sex ratio is 1.7 male to 1 female. Young man had contact with the confirmed case from Liberia. The majority of contacts lives in Cote d’Ivoire. No case of monkeypox has been detected in Cote d’Ivoire so far. Case management has been recalled and measures have been taken to strengthen cross-border surveillance between the two countries.

**5326**

SYLTVIC STRAINS OF DENGUE VIRUS HAVE DISTINCT REPLICA KINETICS IN HUMAN CELLS

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Dengue disease is caused by each of the 4 types of dengue virus, infecting an estimated 390 million people each year and causing significant morbidity and economic burden. These viruses are endemic in tropical areas of the planet where the competent mosquito vector is available. Dengue viruses are thought to have emerged as a spillover from the dengue sylvatic cycle, where the virus is transmitted among non-human primates in Southeast Asia and Africa. Based on phylogenetic analyses, 4 independent spillover events resulted in the successful generation of the dengue virus types that
circulate in humans today. Other contemporary infections of humans with sylvatic strains have been reported, however, these have resulted in dead-end transmission chains without the generation of a new clade of viruses circulating in humans. What restricts most sylvatic strains of dengue virus to gain efficient transmission in humans remains unknown. Other scientific groups have generated conflicting data on the possible differences among the virus clades. To approach this question, we decided to use a set of complimentary methods to identify differences in replication of lab adapted, clinical isolates, and sylvatic strains of dengue viruses, using human cells as a model. Our data shows that clinical strains of dengue can initiate a complete replication cycle in primary human macrophages, although reaching lower infectious titers compared to lab adapted strains. Among the tested sylvatic strains, all presented lower infectious titers compared to the clinical isolates. As expected, all strains replicated to similar levels in mosquito cells. Currently, we are increasing the number of isolates from clinical and sylvatic origin to better understand inter-strain variability. This research will help understand what regulates the success of spillover events in flavivirus and help to anticipate if risk remains of new clades of dengue viruses entering the human transmission cycle.

5327
SEVERE DENGUE RISK: SPECIAL POPULATIONS WITH REPEATED HIGH-RISK EXPOSURES: CHARACTERISTICS AND A FRAMEWORK FOR RECOMMENDATIONS
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As a re-emerging tropical disease, a growing body of literature has been dedicated to the emergence of Dengue serotypes throughout the world, an increase in the vector range and seasonality, the discovery of dengue in previously unaffected populations and locations, and characteristics that place individuals at greater risk for severe dengue—the most complex and life-threatening form. However, no cohesive synthesis of the pathogenesis of the disease and its impact on specific non-traditional populations who are at a heightened risk of frequent and/or prolonged exposures. A literature review identified the comprehensive factors that determine the severity and determinates of the disease, the characteristics of non-traditional high-risk populations, and mitigation and control measures. Humanitarian aid workers, Peace Corps volunteers, missionaries, international business travelers, travelers visiting friends and relatives, and military service members are at a heightened risk for dengue infection due to recurrent and long-term travel to dengue-endemic regions, where they may have continuous or repeated exposures. A comprehensive, One Health approach should be employed to fight the spread of dengue infections. Synthesizing the clinco-epidemiology of dengue with available prevention and mitigation measures, including the use of vaccines, allows for the development of a framework for recommendations for travelers with repeated high-risk exposures. One of the best prevention methods is a tailored pre-travel health assessment covering various topics, including illness risk, primary prevention of vector contact, and health history. After establishing previous infection through a serum study, administering vaccines for travelers to endemic and hyperendemic areas should be considered. Travel health providers must be aware of barriers to prevention, such as non-compliance, inaccurate risk perception, and vaccine hesitancy, and devise strategies to mitigate these barriers. Dengue is a real and present threat whose reach will expand with travel, trade, and the expansion of the habitable range of the vector.
Yellow fever virus (YFV) is a mosquito-borne flavivirus endemic in Sub-Saharan Africa and tropical South America. The disease yellow fever causes approximately 51,000 annual deaths worldwide. A live-attenuated vaccine developed in 1937 is essential to control the spread of YFV, but the shelf life and manufacturing constraints of egg-based vaccine production, the vaccine’s rare severe adverse events, and the lack of effective therapeutic options for yellow fever disease create an urgency for the development of new YFV vaccines and therapeutic tools. YFV-neutralizing antibodies could be a promising passive immunization and treatment that may also guide development of effective non-replicating YFV vaccines. In this study, we captured natively paired heavy and light chain antibody libraries from two donors that were immunized with the YFV 17D vaccine and generated yeast surface display libraries for functional antibody analysis. By screening yeast libraries with YF virus-like particles purified by chromatographic techniques, we identified three virus-neutralizing antibodies with potent neutralizing activity against circulating strains from Western Africa and South America, including one extremely potent antibody with a neutralizing IC50 < 5 ng/mL against the 17D vaccine strain. Passive transfer of these monoclonal antibodies protected mice in the YFV neurotropic disease mouse model via intracerebral challenge with the 17D strain. The new YFV antibodies we describe here have the potential to support development of novel YFV vaccines and may also serve as YFV outbreak countermeasures for treatment or prevention.

EXPLORING POTENTIAL INDICATIONS FOR REMDESVIR BEYOND COVID-19

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Remdesivir (RDV; GS-5734; VEKLRF®), a monophosphoramidate prodrug of an adenosine analog, is the first FDA-approved antiviral therapy for COVID-19. Rapid approval and distribution of RDV for SARS-CoV-2 was enabled by preexisting safety, efficacy, and pharmacokinetic data generated during its initial development for RSV and Ebola. Beyond SARS-CoV-2, RDV and its parent nucleoside (GS-441524) have antiviral activity against multiple RNA viruses through potent inhibition of their viral RNA-dependent RNA polymerases (RdRps). Cell-based assays have shown that RDV is potent against viruses within the Paramyxoviridae, Pneumoviridae, Filoviridae, Coronaviridae, Flaviviridae, and Picornaviridae families. In vivo, the pharmacologically active nucleoside triphosphate is efficiently produced in lung and peripheral blood mononuclear cells, and antiviral efficacy has been demonstrated in animal models of RSV, Ebola, Marburg, Nipah, MERS-CoV, SARS-CoV, and SARS-CoV-2 infection. Because RDV requires intravenous administration, orally bioavailable prodrugs of the parent nucleoside are being evaluated for expanded use in outpatient populations. GS-621763, an orally bioavailable prototype prodrug of GS-441524, reduced SARS-CoV-2 replication in human primary lung cell cultures as well as in mouse, ferret, and nonhuman primate challenge models. GS-5245, another oral prodrug of GS-441524, has completed Phase I pharmacokinetic and safety evaluations and is currently in Phase III clinical trials for the treatment of high-risk COVID-19 patients. Expanded in vivo efficacy against other emerging viruses is an area of active research. Collectively, these data support continued exploration of the antiviral prophylactic and therapeutic indications of RDV and oral nucleoside analogs. Proactive characterization of preclinical efficacy across other priority pathogens can enable rapid deployment of an effective treatment in response to emerging viral threats.

EVOLUTION OF A FUNCTIONALLY INTACT BUT ANTIGENICALLY DISTINCT DENGUE VIRUS (DENV) FUSION LOOP

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A hallmark of Dengue virus (DENV) pathogenesis is the potential for antibody-dependent enhancement (ADE), which is associated with deadly DENV secondary infection, complicates the identification of correlates of protection, and negatively impacts the safety and efficacy of DENV vaccines. ADE is linked to antibodies targeting the fusion loop (FL) motif of the envelope (E) protein, which is completely conserved in mosquito-borne flaviviruses and required for viral entry and fusion. In the current study, we utilized saturation mutagenesis and directed evolution to engineer a functional variant with a mutated fusion loop (D2-FL) which is not neutralized by FL-targeting monoclonal antibodies. The FL mutations were combined with our previously evolved pre-membrane (prM) cleavage site to create a mature version of D2-FL (D2-FLM), which evades both prM- and FL-antibodies but retains sensitivity to other type-specific (TS) and quaternary cross-reactive (CR) antibodies. CR serum from heterotopic (DENV4) infected non-human primates (NHPs) showed lower neutralization titers against D2-FL and D2-FLM than isogenic wildtype DENV2 while similar neutralization titers were observed in serum from homotypic (DENV2) infected NHPs. We propose D2-FL and D2-FLM as valuable tools to delineate CR antibody subtypes in serum as well as an exciting platform for safer live attenuated DENV vaccines suitable for naive individuals and children.

PHARMACODYNAMIC MODELS TO INFORM THE DESIGN OF PHASE 2 ANTIVIRAL THERAPEUTIC TRIALS FOR DENGUE

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There are currently no effective antiviral therapeutics for the treatment of early symptomatic dengue infection. A consensus methodology for the pharmacometric assessment of candidate dengue antiviral drugs would be important for comparing trial results and improving phase 2 trial design. The time to viral clearance and the area under log-viremia curve (AUC), assessed from serial qRT-PCR measurements in plasma samples, are the most widely reported measures of virological response in clinical trials. These endpoints have not been compared formally with other metrics, notably model-based estimates of the rate of viral clearance. We analyzed prospectively gathered viral clearance profiles from >600 patients recruited in clinical trials of repurposed drugs for dengue conducted by the Oxford University Clinical Research Unit in Viet Nam over the last 15 years. We fit different phenomenological pharmacodynamic models and summary measures (single exponential decay, bi-exponential, penalized splines, AUC) and show that the rate of viral clearance, estimated from a mixed effects single exponential decay model, is a robust pharmacodynamic summary of viral clearance. The rate of viral clearance, estimated from viral densities during the first 5 days following enrollment, provides increased statistical power (reduced type 2 error) compared with time to clearance and AUC. Using these data, we take a simulation approach to derive sample size requirements for hypothetical effective antiviral drugs with varying effects on acceleration of viral clearance. We recommend that pharmacometric antiviral assessments should be conducted in patients with early dengue illness (less than 72 hours from fever onset) with twice daily serial qRT-PCR plasma samples taken over 5 days.
Studies conducted in China early in the pandemic, demonstrated that antiviral activity of antimalarial compounds. Even though at present several antiviral drugs against this disease has renewed interest in the possible Syndrome virus (SARS-CoV-2) that causes COVID-19, the search for new public health intervention strategies but may have global impacts as well. Sustainable and unvaried analysis of samples can not only improve local results. This validated technology identifies previous exposure to filovirus assay performance across countries, laboratories, and standardized cutoff criteria. The standardized cutoff criteria involve readouts from positive and negative control samples, population limits of detection, and validation using gaussian mixture modeling. These data suggest that our multiplex filovirus bead-based assay, used in three distinct detection, and validation using gaussian mixture modeling. These data show the robustness of the assay, it’s hypothesized that filovirus multiplex bead-based technology in resource settings in Low Middle-Income Countries (LMICs) and High-risk settings in Low Middle-Income Countries (LMICs) and High-Income Countries (HICs). On the example of “real-life” detection of filovirus-specific IgG responses using Magpix multiplex bead-based technology in laboratories in the Democratic Republic of the Congo (DRC), Liberia, and Hawaii, United States, assay performance across countries, laboratories, and technologists has been assessed. Sources of potential variation were identified; however, there was limited variability across assays performed in each locale after assessments were conducted using correlation analyses and standardized cutoff criteria. The standardized cutoff criteria involve readouts from positive and negative control samples, population limits of detection, and validation using gaussian mixture modeling. These data suggest that our multiplex filovirus bead-based assay, used in three distinct geographical and resource settings, performs well with reproducible results. This validated technology identifies previous exposure to filovirus antigens, including vaccine and natural infection responses, globally, with limited biases due to geographical or resource setting changes. Sustainable and unvaried analysis of samples can not only improve local public health intervention strategies but may have global impacts as well.

IN VITRO EFFICACY OF SELECTED ANTIMALARIALS AGAINST VARIANTS OF SARS COV 2 VIRUS CIRCULATING IN PANAMA DURING 2020-2022

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With the appearance of new variants of the Severe Acute Respiratory Syndrome virus (SARS-CoV-2) that causes COVID-19, the search for new antiviral drugs against this disease has renewed interest in the possible antiviral activity of antimalarial compounds. Even though at present several chemotherapeutic agents had been screened for activity against the SARS-CoV2 virus, only a few drugs had been approved for use in humans. Studies conducted in China early in the pandemic, demonstrated that chloroquine (CQ) and hydroxychloroquine (HCQ), 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 cells (Calu-3), but the use of CQ and HCQ in humans remains controversial. In this study, we aim to compare the in vitro efficacy of a series of selected antimalarials compounds against SARS-CoV-2 variants circulating in Panama with the hypothesis that the efficacy of the compounds is variant dependent. For this purpose, we first screened twenty-six compounds for cytotoxicity in both cell lines using the methyl thiazolyl tetrazolium (MTT) assay to determine their minimal cytotoxic concentration. Preliminary results indicated that 17/26 (65%) of the compounds had a viability ≥ 80% in Vero-E6 cells and 26/26 (100%) in Calu-3 cells. Of these, 13/17 (76%) showed antiviral activity in Vero-E6 cells against the Delta variant, and 15/17 (88%) against the A.2.5 variant. In summary, 15 compounds including 4 and 8 aminoquinolines, quinolinomethanols, sesquiterpene lactones, among others were down selected for further analysis. The inhibitory concentration 50% (IC50), cytotoxic concentration 50% (CC50), selectivity index (SI) and pre and post infection activity will be determined for these compounds. In conclusion, in this study we demonstrate that selected antimalarial, endectocidal, antiviral and antineoplastic-immunosuppressive compounds from various classes had in vitro antiviral activity in Vero-E6 and Calu-3 cells against the Delta and A.2.5 variants of SARS-CoV-2 circulating in Panama.
A UNIVERSAL PURIFICATION METHOD FOR SARS-COV-2 VARIANT SPIKE ANTIGENS

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Many vaccine platforms, including recombinant subunit protein vaccines, have been used to prevent severe disease subsequent to SARS-COV-2 infection. Previously we developed an advanted, lyophilized SARS-CoV-2 protein vaccine capable of preventing lung pathology in non-human primates. Immunoaffinity purification with the monoclonal antibody CR3022 was employed for antigen purification. Due to the evolution of the SARS-CoV-2 receptor binding domain in new variants, purification of current variant spike antigens with this efficient method is impossible. We demonstrate here a universal, tagless purification method applicable to all SARS-CoV-2 like spike proteins with minimal impact on the remaining antigen receptor binding sites by restoring the CR3022-binding epitope in our engineered BA.1 and BA.5 spike variant constructs. Reverted spike protein genes of BA.1 and BA.5 sub-variants were transfected into Drosophila S2 cells to produce stably expressing cell lines. Supernatants containing native BA.1 or the engineered versions of BA.1 and BA.5 were used for purification on CR3022- and ACE2-coupled affinity columns. Initial purification of native BA.1 spike construct using CR3022-immunoaffinity failed to extract measurable quantities of purified protein but purification with ACE2 resulted in low yields of purified protein. Reversion of the mAb epitope in BA.1 and BA.5 constructs allowed for efficient purification using CR3022. Binding affinity to ACE2 receptors remained the same between naïve and reverted forms of BA.1 and BA.5 spike proteins. Restoring the CR3022 epitope on the Omicron variant spike therefore allows for effective immunoaffinity purification of a tagless, minimally modified antigen. This may therefore constitute a universal method for purifying SARS-CoV-2 spike proteins with minimal impact on the remaining epitopes and may streamline production of antigens for serosurveillance and rapid subunit protein vaccine production with a uniform, pre-preparation process to combat new and evolving variants. It could potentially also be used for the generation of other purpose-engineered vaccine antigens.

DESIGNING THERAPEUTICS BIOSIMILAR OF COMMERCIALIZED MABS TO MINIMIZE LETAL EFFECTS OF DENGUE HEMORRHAGIC FEVER: IN-SILICO APPROACH

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Dengue virus (DENV) with its 4 flavivirus serotypes, is responsible for dengue hemorrhagic fever (DHF) and dengue shock syndrome and contributes to 390 M infections yearly, worldwide. Due to antibody-dependent enhancement (ADE), vaccines are worsening DHF. Unavailability of therapeutics, drive this study to design biosimilars of existing FDA-approved therapeutics mAbs to neutralize Dengue Ag-Ab immune complex and reduce the pathogenesis of DHF:Envelope protein sequence of 4 serotypes of DENV, their conserved domain, epitope regions, antigenicity were identified, predicted and aligned respectively. The 7 selected FDA-approved therapeutic mAbs (PDB ID: 6VJA, 6TCS, 7DHA, 5GGU, 5VL3, SUDE and Nirsevimab) and Fcy of Anti-Dengue specific IgG sequence and structure, were retrieved. Molecular docking was conducted between therapeutics mAbs with Ag, Ab separately. Later, amino acids modification were performed on existing mAbs to design biosimilar model with a wide coverage range for blocking all serotypes of DENV, and validated them. Finally, DENV Ag-Ab immune complex with modeled biosimilar mAbs were docked for final screening. Out of the 7, both existing and modified Nirsevimab, Tremlimomab, and Omalizumab showed better binding affinity (ΔG values range -10 to -20.4 kcal/mole, where ΔG < -9.3 kcal/mole preferable for therapeutic drugs), dissociation score and smallest eigenvale with dengue Ag-Ab immune complex. The 306-318 amino acid sequence of the E protein of DENV is the conserved epitopic domain with an antigenic score of 0.8 (where >0.7 is considered an immune response generating threshold). Among 13 amino acids, FVKETOH amino acids are common among all serotypes, and others are unique. The modified mAbs cover the entire epitopic C terminal region of all serotypes, along with the Fc gamma region of anti-dengue IgG3. The proposed 3 candidate biosimilars of commercialized mAbs fulfilled the criteria for the therapeutic potential to neutralize ADE in DHF. Further in vitro studies are required for biopotency and toxicity determination to investigate their effectiveness in immediate patient management.

NIPAH VIRUS THERAPEUTICS: A SYSTEMATIC REVIEW FOR CLINICAL PRIORITISATION

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First identified in 1998 in Malaysia and Singapore, Nipah virus infection is a bat-borne zoonotic disease spread through contaminated body fluids of infected mammals. The case fatality rate (CFR) is 40-75% and debilitating long-term neurological complications are common in survivors. The ongoing Bangladesh outbreak is the largest since 2015 with 14 cases and 10 deaths to date. There are no specific therapeutics. In recognition of this and the high CFR, Nipah is a priority pathogen on the WHO R&D Blueprint. We conducted a systematic review to identify existing therapeutic monoclonal antibodies (mAbs) and small molecules for Nipah virus and other Henipaviridae to assess the evidence available for the safety and efficacy of each to support candidate prioritisation for potential compassionate use and clinical trials. We searched (last on 30 May 2022) 7 bibliographic databases, 3 trial registries, and the Trip database and WHO website. Studies were included if they contained minimum primary data on the safety and/or efficacy of mAbs (in vivo) or small molecules (in vitro) for the treatment of Nipah, Hendra, and related Henipaviridae. From 1469 records screened, we identified 56 eligible studies: 12 on 6 sets of mAbs and 25 on 10 groups of small molecules with in vivo data, and 19 on 18 sets of small molecules with in vitro data only. Limited data were available in humans with only one clinical trial (a phase 1 study of the anti-Hendra G glycoprotein mAb m102.4 in healthy volunteers), and 8 outbreak reports, 7 of which were case series of <10 patients and 6 of which used ribavirin. There were 23 animal studies all except one of which were challenge studies with Nipah or Hendra virus. Only m102.4, remdesivir, ribavirin, and fusion inhibitory lipopeptide have been tested in non-human primates, m102.4 and remdesivir protected all and lipopeptide 2 of 6 treated primates, while ribavirin delayed but did not prevent death. Risk of bias was critical in all clinical studies other than the one trial and high or unclear in all except one of the animal studies. A rationale is presented for clinical prioritisation.

DENGGUE ALLIANCE: ADVANCING DENGUE ANTIVIRALS FROM IN VITRO TO CLINICAL EFFICACY STUDIES OF CONCEPT

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In 2019 dengue was listed by World Health Organization (WHO) as one of the top ten global health threats, with a 30-fold increase in incidence over the last 50 years reaching 400 million infected cases per year globally with majority of the disease burden in Asia and Latin America. Climate change is predicted to contribute to expansion of the mosquito vectors to new geographical areas thus leading to further increase in the number of infections. Despite significant progress on vaccine and vector control, until now, no specific dengue therapeutics are available. The Dengue Alliance includes leading research institutions from dengue-endemic countries namely India, Thailand, Malaysia, and Brazil, and was established by the Drugs for Neglected Diseases initiative (DNDi) to provide a framework for the rapid identification and progression of antivirals for dengue to clinical proof-of-concept studies by drug repurposing strategies. Here we describe the drug repurposing approach taken by the Dengue Alliance to validate antivirals as well as the initial results and overall strategy for prioritization of compounds. The in vitro efficacy for 23 compounds with reported dengue or flavivirus antiviral data were determined using cell-culture based DENV assays available at the Dengue Alliance partner labs. These assays represent a broad coverage of the DENV-1-4 serotypes as well as clinical isolates and lab strains using different infection systems. The results aligned well between the labs, with most compounds ranked similarly, independent of host cell system or DENV serotype. Compounds with EC50 in low micromolar range were further evaluated by pharmacokinetics studies in mice. Two of the compounds were finally tested in A129/AG129 model for inhibition of dengue infection.

5340 DEVELOPMENT OF A PSEUDOTYPED LENTIVIRAL REPORTER VIRUS SYSTEM FOR NIPAH AND HENDRA VIRUSES

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The Nipah and Hendra henipaviruses are highly virulent zoonotic parvoviruses, a family of negative-sense single-stranded RNA viruses. Henipaviruses have been isolated from bats in Central and South America, Asia, Oceania, and East Africa and can cause disease in humans, with fatality rates of up to 75%, and across a range of mammals. Nipah virus (NiV) infection in humans can cause respiratory illness and encephalitis and outbreaks have been reported in Malaysia and Singapore and are seen almost annually in eastern India and Bangladesh and. Hendra virus (HeV) are reported nearly annually in the eastern states of Australia, primarily infecting horses but also humans. There are no specific treatments or vaccines for NiV and HeV in humans, and they are classified as Biosafety Level-4 (BSL-4) pathogens. Their infection uses the interaction of the virus envelope G protein (for cell attachment) and F protein which performs infectious membrane fusion. To provide critical reagents for analyses of NiV and HeV infections, we have developed a pseudotyped lentiviral reporter virus system for Nipah (strain Malaysia 2008) and Hendra (strain Hendra horse virus/Australia/Hendra/1994) viruses, with these reporter virus particles (RVPs) displaying both G and F proteins. These replication-incompetent RVPs perform one round of infectivity and enable safe (BSL-2) and reproducible virus neutralization assays with luminescent or fluorescent readout. We are testing the ability to neutralize the NiV and HeV RVPs using anti-G and anti-F antibodies. The use of henipavirus RVPs will overcome biosafety level restrictions on research into vaccines and therapies for these viruses.

5341 MAPPING ANTIBODY EPITOPES USING A COMPREHENSIVE MUTAGENESIS LIBRARY OF SARS-COV-2 S PROTEIN

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To characterize the immune response to SARS-CoV-2 infection, we created a comprehensive Ala-scan mutagenesis library of the SARS-CoV-2 S protein. We use this library to epitope map 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 quantified by high-throughput flow cytometry, allowing us to identify the S protein epitope residues with the highest energetic contributions to MAb binding. We have mapped over 150 MAbs targeting 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 MAbs or serum immune responses to SARS-CoV-2 infection, we developed a pseudotyped lentiviral reporter virus system for SARS-CoV-2, with reporter virus particles (RVPs) displaying S protein. These 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 70 SARS-CoV-2 RVP types incorporating variant S proteins. We also used our MPS antibody isolation platform to obtain MAbs against S protein, some of which neutralize the major Omicron variants.

5342 DEVELOPING NOVEL INHIBITORS AGAINST VENEZUELAN EQUINE ENCEPHALITIS VIRUS BY TARGETING VIRUS-HOST INTERACTIONS

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Venezuelan equine encephalitis virus (VEEV) remains one the most important zoonotic pathogen in the family Togaviridae. VEEV is endemic to the Americas and has been responsible for periodic outbreaks of febrile and neurological disease in both equines and humans, with an associated case fatality rate of up to 10% in humans. Moreover, it is classified as a select agent by both the CDC and USDA due to its low infectious dose and ease of aerosolization and manipulation. Concerningly, there are currently no FDA-approved therapeutics or licensed vaccines against VEEV infection in humans. The VEEV capsid protein is an essential virulence factor of VEEV. The capsid protein can simultaneously bind to the host’s nuclear import receptors, importin α/β1, and the host export receptor, CRM1 to form a tetrameric complex. This complex accumulates at the nuclear pore channel, halting nucleocytoplasmic trafficking, downregulating host transcription and inhibiting cellular antiviral response. We hypothesized that chemical inhibitors capable of disrupting the interaction of capsid with importin α/β1 should increase cellular antiviral response, resulting in reduced viral titers and rescue of cells from VEEV-induced cell death. Two small molecule inhibitors, I2 and 1564, were designed to disrupt the interaction between capsid and importin α. These inhibitors were well tolerated by HMC3 microglial cells with CC50 of >250 µM and >500 µM for I2 and 1564, respectively. These compounds impacted VEEV TCID50 titer with >1 log10 decrease at 9 hpi. Furthermore, I2 displayed an EC50 of 2.96 µM and 1564 an EC50 of 5.38 µM against VEEV. Both compounds also rescued infected cells from VEEV-induced cell death. In order to evaluate the impact of these compounds on the capsid-importin diteraction, we cloned two viruses that contain a V5 tag at the N-terminus of the capsid. The replication kinetics of these new viruses were similar to that of parental viruses. Moreover, they
Evaluating the BinaxNov® Malaria Rapid Diagnostic Test, which received FDA approval in 2007. A total of 808 samples were tested, and 696 samples were confirmed malaria species positive. P. falciparum was the dominant species detected, followed by mixed infections of P. falciparum/P. vivax, P. falciparum/P. malariae, P. falciparum/P. vivax/P. malariae, and P. falciparum/P. vivax/P. ovale. Mutations in kelch13, Pfort, pfhrf, pfhrp, cytochrome b, and Pfmdr1 genes were the major targets of detection. The first-line of treatment for uncomplicated P. falciparum infection during the time of the collection of these samples was mefloquine alone or in combination with sulfadoxine/pyrimethamine (SP) and with artesunate in more recent years. No PICTYM mutations associated with atovaquone resistance were detected. We detected mutations in Pfdmcr 1 at positions N86Y, Y184F, and N1042D that confer mefloquine resistance. Mutations in genes that are associated with S/P resistance, pfhrf and pfhrps, are high in these samples. A double mutation of Pfdhrf at positions 59R and 108N were presented at >99% while the majority of Pfdhps had multiple mutations, 540E and 581G. The only K13 mutations detected were a mutation at position P574L (3.33%, 1/30) in a 1998 sample, position N86Y (4.9%, 2/42) in 1998 samples, and position R539T (0.22%, 1/460) in 2001 samples. Both Y493H and R539T are WHO confirmed K13 mutations conferring resistance to artesinin.

**5346**

**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 to monitor the emergence and spread of drug-resistant pathogens. These markers are comprised of either single nucleotide polymorphisms (SNPs) or concatenated SNPs in microbial genes that interact with drugs. Although various technical improvements in sequencing methods have been introduced to identify SNPs, current tools (conventional approach) used to measure molecular markers of anti-malarial resistance do not allow discrimination of mixed infections, and must be improved for more sensitive surveillance of anti-malarial resistance to better inform control strategies. We developed a new method to quantify molecular markers of anti-malarial resistance discrimination of mixed infections, and must be improved for more sensitive surveillance of anti-malarial resistance to better inform control strategies.
Malaria is one of the most devastating diseases in the world, causing around 65,000 deaths in 2021, and almost 50% of the world population is at risk of being infected. Reports of resistance to Artemisinin in different regions, especially in South East Asia and recently also in Africa, is a major concern. There is an urgent need of new antimalarial treatments to replace those compromised due to resistance. Phenotypic screening has been the cornerstone in the search for new antimalarials in the last decades. However, the chemical diversity in the screening collections is currently exhausted, and little progress has been made in the identification of new chemical starting points for hit to lead programs.

Drugs resistance genes [Plasmodium falciparum dihydrofolate synthase (PfDHPS) and P. falciparum dihydrofolate reductase (PfDHFR)] by standard sequencing of amplicons and bioinformatic estimation of proportions of different genotypes in individual samples. Using parasite mixtures with known alleles, we observed a highly significant correlation between the predicted proportion of each allele with the proportion measured by sequencing and deconvolution. This was observed for PfDHPS at codons 436F, 437G and 612S/T and for PfDHFR at codons 51I, 59R and 164L (p < 0.001). In studies of field samples, the mean fraction of PfDHPS was greater than 20% at codons 436F/A (95.9%), and 437G (49.9%), but not at 431V, 540E, 581G, and 612S/T (4.7%, 0.0%, 1.2% and 1.5%, respectively); corresponding prevalences of PfDHFR were 100%, 72.5%, 50.0, 0.0%, 25.0%, and 12.5%, respectively. The mean fraction of PfDHFR was greater than 20% at codons 51I, 59R and 108T/N (89.0%, 98.3% and 74.7%), respectively, but not at 16V(0.6%), 50R(11.1%), 140L(8.6%) and 164L(8.7%); corresponding prevalences of PfDHFR were 100%, 100%, 100%, 12.5%, 75.0%, 50.0%, and 28.6%, respectively. Our results demonstrate qualitative discrimination of varying proportions of sensitive versus resistant alleles using a cost-effective approach that incorporates Sanger sequencing of PCR amplicons with novel informatics tools for deconvolution.

IDENTIFICATION OF NEW ANTIMALARIALS TARGETING THE PLASMODIUM FALCIPARUM PROLINE TRNA SYNTHETASE

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Identification of new antimalarias targeting the P. falciparum proline tRNA synthetase Benigno Crespo, Carlos Alemparte, Pilar Almenara, Emilia D’oria, Andrew Clifton, Tony Choudhry, Gang Yao, Apirat Chaikuad, Audrey Tolbert, Chloe Oxford and Nathan Gittens

Malaria is one of the most devastating diseases in the world, causing around 65,000 deaths in 2021, and almost 50% of the world population is at risk of being infected. Reports of resistance to Artemisinin in different regions, especially in South East Asia and recently also in Africa, is a major concern. There is an urgent need of new antimalarial treatments to replace those compromised due to resistance. Phenotypic screening has been the cornerstone in the search for new antimalarials in the last decades. However, the chemical diversity in the screening collections is currently exhausted, and little progress has been made in the identification of new modes of action. Thus, more target-based approaches are required to find new chemical starting points for hit to lead programs. Cytoplasmic prolyl-tRNA synthetase (ProRS) is one of the most chemically and genetically validated targets in Plasmodium falciparum (Pf). ProRS is the main target of Febrifugine, a Chinese traditional medicine used for centuries in the treatment of Malaria. However, Febrifugine and synthetic analogues like Halofuginone cannot be developed as antimalarials due to an unacceptable safety profile associated to their low selectivity against the human homologue. In this work, we present the strategy followed for the identification of new chemical inhibitors of Pf ProRS. GSK collection has been screened by affinity selection mass spectrometry (ASMS) and by encoded library technology (ELT) in different conditions, including ProRS alone as well as combinations of the protein with its different substrates (proline, ATP and tRNA). Identified binders were progressed to a biochemical assay to assess their activity against Pf ProRS and its human counterpart. Mode of action of selective compounds will be elucidated using resistant strains, thermal shift, biochemical assays and cryo-crystallography.

SIMPLE, INEXPENSIVE IN VITRO DRUG SURVIVAL ASSAY FOR MONITORING ANTIMALARIAL DRUG SENSITIVITY IN MALARIA ENDEMIC REGIONS

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Resistance to cheap antimalarial drugs and the current artemisinin combination therapies has contributed to the sustained burden of malaria. For malaria elimination, the efficacy of current drugs needs to be monitored especially in Africa, which bears over 96% of the burden of malaria. Drug efficacy monitoring in laboratories use in vitro inhibition concentration (IC50) tests, which has been highly inconsistent and cumbersome to implement across endemic settings. Here, we present a simple and inexpensive in vitro malaria drug survival assay (mDSA) for monitoring antimalarial failure in low resource settings. In vitro drug survival assay determines the % survival and the reinvasion rate of parasite isolates exposed to a 48-hour pulse of lethal concentrations of antimalarial drugs. In this study, 32 field isolates of malaria parasites in the Gambia and the control isolates (DD2 and 3D7) were exposed to (10X IC50 cut-off) of dihydroartemisinin (DHA): 24nM, lumefantrine (LUM): 200nM, chloroquine (CQ): 200nM and piperaquine (PPQ):200nM respectively for 48h, followed by another cycle of culture in drug-free medium. The % survival and the reinvasion rate were estimated after 24h and 48h drug-free growth. Our preliminary findings show that this simple assay can consistently differentiate resistant from sensitive parasite strains for each of the antimalarial drugs. With CQ for example, the % survival was 72, 118 and 6% for the field isolates, CQ-resistant strain (DD2) and CQ-sensitive strains (3D7) respectively (p <0.05). The high % survival of the field isolates, evidence of CQ resistance, was supported by the significantly higher reinvasion in the field isolate than 3D7 (0.4 vs 0, p <0.05) and the no significant difference between the field isolates and DD2 (0.4 vs 1.4, p >0.05). mDSA confirmed persistent CQ resistance in the Gambia, mDSA can therefore be used for surveillance of antimalarials and early detection of failure. It could facilitate drug policy to improve malaria control.

ANALYSIS OF THE SUITABILITY OF USE OF MUTATIONS IN THE PVCRT-O AND PVMDR1 GENES AS MARKERS OF RESISTANCE OF PLASMODIUM VIVAX TO CHLOROQUINE IN AMAZONIC BASIN

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In Brazil, approximately 139,000 malaria cases were recorded in 2021, 83% of them caused by P. vivax. In the absence of an antimalarial vaccine, the control policy is based on case management through prompt diagnosis and treatment. With the rapid emergence and dispersion of parasite drug resistance, it is necessary to monitor the effectiveness of drugs used in the treatment. In Brazil, a percentage of up to 10% of vivax malaria cases resistant to chloroquine (CQ) have already been registered. Unlike P. falciparum, there are no established molecular markers of P. vivax chemoresistance (QR) to CQ. Therefore, we investigate whether the polymorphisms in the genes pvct-o and pvmdr1 can be markers of QR to OC. Additionally, the pvmdr1 and pvmdr2 genes associated with QR to the sulfadoxine-pyrimethamine (SP) were analyzed to find out the potential of using this drug in cases of QR parasites to CQ. For this, 130 samples from the Amazon Basin were studied through the polymerase chain reaction followed by target DNA sequencing. In the pvct-o exons, the K10 insert was present in 14% of the samples. Regarding pvmdr1, the SNPs T958M and F1076L had a frequency of 95% and 3%, respectively, while the SNP Y976F was not detected in the samples. In pvmdr1, we observed the presence of double FRTHNL (76%), triple FRTHNL (21%), and quadruple FRTHNL (2%)
FRTRNL (8%) and wild-type haplotype was not found. As for pvdhps, we detected the wild type (38%) and single SGKAEV (50%), and double mutants CGKAEV (12%). Allelic combination of the pvdrfr/ pvdrps genes showed that the triple FRTRNL + SGKAEV (42%) and the double FRTRNL + SAKAEV (31%) mutants were the most prevalent. Thus, it is concluded that: i) mutations in the pvdr-o gene seem to have a low potential for association with the phenotype of QR to CQ because K10-pvdr-o and F1076L/Y976F/T986M-pvmdr1 polymorphisms were detected in samples from patients who responded well to CQ treatment and; ii) the frequencies of double and triple pvdrfr and pvdrps mutants associated with the low percentage of non-mutated parasites seems to indicate that SP cannot be introduced as an alternative drug in cases of QR to CQ in P. vivax.

5350

IDENTIFICATION OF B-CARBOLINE DERIVATIVES ACTIVE AGAINST QUIESCENT ARTEMISININ-RESISTANT PLASMODIUM FALCIPARUM

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Malaria is a devastating disease that caused 619,000 deaths in 2021 worldwide. Cases of malaria have increased from previous years in part by the quick development of resistance to current antimalarials. Due to the rising resistance to current antimalarial drugs such as chloroquine and artemisinin, there is an urgent need to discover and develop new chemotherapeutic agents that engage new targets in the malaria parasite. This research focuses on a novel antimalarial with a unique β-carboline scaffold known as PRC1584. It is known that exposure to dihydroartemisinin (DHA) induces a quiescent state in Plasmodium falciparum ring stage. Quiescence is a mechanism of Plasmodium survival especially from drug treatment. This phenomenon increases the risk of clinical failures following artemisinin-based combination therapies by slowing parasite clearance and allowing the selection of parasites resistant to partner drugs. We investigated if short exposure of PRC1584 also induces quiescent and/or kills the proliferating ring stage and if PRC1584 has activity against DHA-induced quiescent ring stage in the presence or absence of DHA-resistance. We used the ring survival assay (RSA) and the quiescent-stage survival assay (QSA) to assess the antiplasmodial activity of PRC1584 and its analogs in the presence and absence of DHA-resistance. Our studies revealed that only 8 hours of exposure to PRC1584 kills both the proliferating ring stage and the DHA-induced quiescent rings of P. falciparum independently of the presence of DHA-resistance. In addition, we are using these assays to guide optimization of this series as preclinical leads. Altogether, these results revealed that PRC1584 displays a fast-killing profile and that it may act through a novel mechanism of action. Identifying if new antimalarials may also facilitate the development of quiescent-ring stage is extremely important, as this could result in recrudescence and treatment failure.

5351

PLASMODIUM FALCIPARUM KELCH13 R561H SPREAD AND EMERGENCE OF OTHER ARTEMISININ PARTIAL RESISTANT MUTATIONS ACROSS RWANDA USING A SITE AND TEMPORAL RAPID POOLING STRATEGY

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Recent monitoring has detected multiple emerging Plasmodium falciparum kelch13 (K13) propeller gene mutations across East Africa. Mutations, such as R561H in Rwanda and C469Y/F, and A675V in Uganda, have been increasing and are associated with artemisinin partial resistance mainly manifesting as delayed clearance after treatment with ACTs and recrudescence. Coordinated surveillance efforts are necessary to track these K13 mutations and inform control efforts. Here we apply pooled sequencing to provide a rapid initial assessment of population allele frequency at collection sites. Whole blood samples (n=2,703) from malaria-positive patients were collected from 20 Rwandan health centers from May to December 2022. Samples were pooled at equal volume, stratified by site and month to generate 104 pools. DNA was extracted using magnetic beads and genotyped using molecular inversion probe targeted sequencing of K13 and other resistance genes. Site allele frequencies were calculated weighted by the number of samples in a given pool. From a single round of sequencing, R561H was detected at 13 of 17 sites with an average frequency of 23.5% (0 to 70.3%). The highest site frequency clustered in the center of the country. At sites bordering the DRC (n=2), no R561H was found, suggesting R561H may not have spread west. In Bugaragara, in northeast Rwanda, we found 49.7% average frequency that progressively increased monthly from October to December (0.54% to 94.1%). Notably, A675V, previously seen in Uganda, was found at 6.81% across 11 sites. Other mutations observed to be spreading in Uganda, C469F and C469Y, were not observed at any site. Concerningly, G449A was at 7.11%, a WHO candidate resistance mutation never before reported in Africa but associated with a two-fold prolonged parasite clearance half-life in Asia. It was detected at 2 sites including Tanda (20.8%) where a large outbreak has been occurring. Overall, it appears multiple K13 mutations are rapidly expanding in Rwanda, further endangering control efforts and treatment efficacy with the potential of engendering partner drug resistance.

5352

PLASMODIUM FALCIPARUM DRUG RESISTANCE MARKERS AND GENETIC STRUCTURE IN MOZAMBIQUE, 2015-2022

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Antimalarial drug resistance is a global threat to malaria control and elimination, and is of particular concern in Mozambique, one of the four African countries which account for over half of all malaria deaths worldwide. Here we aimed to describe antimalarial resistance markers in Mozambique and interrogate parasite population structure using genome-wide microhaplotypes. To achieve this, we performed Plasmodium falciparum amplicon and whole genome sequencing using Next Generation Sequencing in 2251 malaria-infected blood samples collected in 2015 and 2018 in seven provinces of Mozambique. Another batch of samples (1303) from 2021 and 2022 are being tested at the laboratory and the results will be presented at the conference. The preliminary results revealed
a total of 32 non-synonymous mutations in the kelch13 gene, although none of them are currently associated with artesinin resistance. No mutations or frequencies above 1% were observed for the rest of the markers, except for 184F in pfmdr1 (59%), 511V/590R/108N in pfdhfr (>95%) and 437G/540E in pfdhps (>8%). The frequency of pfmdr1/pfdhps quintuple mutants increased from 80% in 2015 to 90% in 2018 (p<0.001), with a lower expected heterozygosity and higher relatedness of microhaplotypes surrounding dhps mutants than wild-type parasites suggestive of recent selection. pfdhfr/pfdhps quintuple mutants also increased from 72% in the north to 95% in the south (2018; p<0.001). This resistance gradient was overlaid with a concentration of mutations in position 436 of pfdhps (17%) in the north, a south-to-north increase in the genetic complexity of P. falciparum infections (p<0.001) and a microhaplotype signature of regional differentiation. The results of this study revealed several public health implications such as, appropriate efficacy of artesinin-based combination therapy for P. falciparum treatment, chemoprevention with sulfadoxine-pyrimethamine is still recommended in areas with high rates of pfmdr1/ pfdhps quintuple mutations, the evidence of a return of chloroquine therapeutic efficacy in Mozambique.

5353

INADEQUATE ARTEMETHER-LUMEFANTRINE TREATMENT RESPONSE IN A 15-MONTH OLD PATIENT WITH UNCOMPLICATED FALCIPARUM MALARIA IN WESTERN KENYA: A CASE REPORT

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Despite global efforts against malaria, inadequate treatment response and resistance to artesinin combination therapies (ACTs) are becoming increasingly common, posing a threat to malaria elimination efforts. In endemic zones, ACTs are recommended as first-line treatment of uncomplicated malaria. This case report describes a 15-month old male patient with uncomplicated falciparum malaria who did not respond to artemether-lumefantrine treatment. The patient weighed 9.0 kilograms and presented at Kisumu sub county referral hospital with classical symptoms of uncomplicated falciparum malaria. The first dose of Artemether Lumefantrine (AL) was administered by a health worker who monitored the patient for an additional 30 minutes to confirm drug retention. Subsequent treatment doses were given to the parent for continuation at home. The patient returned on day 7 without symptoms, but was diagnosed with malaria by RDT and microscopy, revealing a Plasmodium falciparum single species infection at 0.2% parasitemia. Molecular diagnosis was done using PCR followed by genotyping for merozoite surface proteins 1 (MSPI), MSP2 and GLURP, which confirmed the presence of P. falciparum and recrudescence from identical alleles between the initial and subsequent samples. No non-synonymous mutations were observed in the Kelch 13 propeller gene of the parasite. Pharmacokinetic analyses, in vitro drug sensitivity tests, and further genotyping of putative drug resistance markers are underway. This case highlights the challenge of inadequate treatment response to artemether-lumefantrine in a young child with uncomplicated falciparum malaria in Western Kenya. The molecular diagnosis and genotyping results confirmed recrudescence of P. falciparum from identical alleles between the initial and subsequent samples. This case report emphasizes the need for close surveillance of antimalarial efficacy and parasite response to current antimalarial drugs in endemic zones. Further research is required to identify the underlying mechanisms of artemether- lumefantrine treatment failure and to develop effective strategies to combat resistance.
**Sulfadoxine-pyrimethamine (SP)** is the recommended treatment for perennial malaria chemoprevention (PMC). The triple mutation in the Plasmodium falciparum dihydrofolate reductase (dhfr) gene (N51/51/S59/S108N) affects tolerance to pyrimethamine and it is largely fixed and saturated across sub-Saharan Africa. Additional mutations in the dihydropteroate synthase (dhp5) gene may threaten the protective efficacy of SP, but this relationship has not yet been quantified. Here, we retrospectively analyse PCR-corrected re-infection data from therapeutic efficacy trials to quantify protective efficacy and duration of protection offered by SP for SP-artesunate (AS) against parasites with different genetic levels of SP resistance. We use a mathematical model that accounts for site-specific genotype frequencies and incidence rates and fit Weibull survival curves to the duration of drug protection using Bayesian methods. In a study in Benin (2003-2005), where 87.1% of patients had the quadruple mutation (dhfr triple + dhp5K104E), we estimated the mean duration of protection to be 29.9 days (95% Credible Interval [Crit]: 10.4-61.0) for SP and 27.0 days (95% Crit: 11.1-44.9) for SP-AS. The 30-day protective efficacy was 89.7% and 84.9%, respectively. In Malawi in 2005, where 91.6% of participants had the quintuple mutation (quadruple + dhp5K104E), SP-AS provided 14.8 (95% Crit: 10.3-30.3) days protection (30-day protective efficacy: 41.7%). In Tanzania in 2006, SP was estimated to provide 14.8 days (95% Crit: 3.6-39.9) protection against quintuple mutant parasites and 10.7 days (95% Crit: 6.1-30.1) against sextuple mutant parasites (quintuple + dhp5A81C). These findings suggest that accumulation of dhp5 mutations is associated with a reduced ability of SP to protect against infection. Further data will be added to refine these estimates and to quantify protection against different parasite strains. These findings, along with recent molecular survey data, can help parameterize models quantifying the impact of seasonal or perennial chemoprevention in areas with different genotype frequencies to inform scale-up of these interventions.

**IMPACT OF SEASONAL MALARIA CHEMOPREVENTION (SMC) ON MOLECULAR MARKERS OF RESISTANCE TO PLASMODIUM FALCIPARUM ANTIMALARIAL DRUG RESISTANCE IN KOULIKORO HEALTH DISTRICT, MALI**

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With the scale-up of Seasonal Malaria Chemoprevention (SMC), emergence of antimalarial drug resistance may impair the efficacy of the control strategy. This study examined potential changes in Plasmodium falciparum genes related sulfadoxine-pyrimethamine plus amodiaquine (SP+AQ) and dihydroartemisinin-piperaquine (DHA-PQ) resistance. The study was carried out during effectiveness trials using DHA+PQ as an alternative drug treatment to SP+AQ among children 3-59 months old in Koulkoro region, Mali. DNA was extracted from dried blood spots and P. falciparum PCR positive samples underwent targeted amplicon sequencing (Miseq Illumina) for drug resistance profiling, including SNPs in Pfmdr1, Pfmdr1, Pfcr1, Pfcrt, PfKelch13 and Pfexo. Furthermore, copy number variations (CNVs) in Pfmdr1 and Plasmsesp12 were analyzed by qPCR. In total, 215 and 341 P. falciparum positive samples were successfully genotyped for 2019 and 2020, respectively. High prevalence of the triple mutated Pfdrh1 haplotype at 51I-59R/S108N was observed for both 2019 (95%) and 2020 (93.3%), with no significant difference between the years and the drug arms (p=0.03). For Pfdrhs, overall the most prevalent haplotypes observed were the single mutant haplotypes at 431-436-437G-540-581-613 (IAGKA/ ISGKA), at approx 60% while more prevalent haplotypes were observed in low numbers: for 2020 e.g. VAGKAA (0.6%), VAGKGG (1.2%) and ISGKAA (2.4%). No significant differences between the years and between the drug arms were observed (p=1.57). For Pfdhfr and Pfcrt haplotypes, the most prevalent Pfdhfr haplotype, NFD (86-184-1246) at >50% did not differ between the years and drug arms while the Pfcrt wildtype CVMNK haplotype increased from 39% to 52% from 2019 to 2020 (p=0.01), no difference between the drug arms were observed (p=0.44). For PfK13 gene, few non-synonymous SNPs were observed, none seemed important. Finally, few CNVs (<2%) in Pfplasmepsin2 and Pfdhfr1 were observed for 2019 and 2020. Despite some minor variations from year to year, deploying SMC using either SP+AQ or DHA-PQ does not seem to select for molecular markers of drug resistance in the tested population in Mali.

**SYSTEMATIC REVIEW & GEOSPATIAL MODELLING OF MOLECULAR MARKERS OF RESISTANCE TO ARTEMISININS & SULFADOXINE-PYRIMETHAMINE IN PLASMODIUM FALCIPARUM IN INDIA**

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Surveillance for genetic markers of resistance can rapidly provide valuable information on the likely efficacy of antimalarial drugs but needs to be targeted to ensure optimal use of resources. We conducted a systematic review of publications in 7 databases and 5 languages to compile resistance marker prevalence data from studies in malaria endemic states of India published between Jan 2014 and Mar 2020. The most identified study was conducted in 1994 and the most recent in 2019 with a median time lag from sample collection to publication of 4 years. Fewer studies were conducted in states with moderate or low malaria endemicity, with no studies conducted in Uttarakhand. In all, pfk13, pfdrh, and pfdrhs genotype data from 2953, 4148, and 4222 samples, respectively, were extracted from these publications. They were combined with data from the WorldWide Antimalarial Resistance Network (WWARN) Molecular Surveyor database and fed into a hierarchical geostatistical model to produce maps of the predicted prevalence of the pfk13 and pfdrhs markers and of the associated uncertainty. Zones with a high predicted pfdrhs S406E prevalence of >15% were identified in Central, Eastern and Northeastern India. The predicted prevalence of pfk13 mutants was non-zero at only few locations outside the Northeastern states but these were within or adjacent to the zones with high prevalence of pfdrhs S406E. The highest uncertainty in the predicted prevalence was from zones where fewer or no studies were conducted but also from locations where conflicting data were collected, possibly reflecting evolving prevalence over time. This study identified regions with high predicted pfdrhs S406E prevalence where there may be a higher probability of artesunate-sulfadoxine-pyrimethamine failures due to the predicted co-prevalence of pfk13 mutants. However, the accuracy of these predictions remains to be confirmed as they are based on very sparse data. This work can be applied to conduct systematic, targeted,
The emergence of drug-resistant clones of Plasmodium falciparum is a major public health concern, and the ability to detect and track the spread of these clones is crucial for effective control and treatment. However, in endemic settings, patients are often infected with more than one clone at the same time making it likely to miss drug resistant clones using traditional molecular screening methods. The PHARE pipeline is a novel method for detecting these minor haplotypes in multilocus samples sequenced using the Oxford Nanopore MinION platform. The pipeline was validated on three control datasets containing P. falciparum DNA of four laboratory strains at varying mixing ratios, achieving high recall and accuracy rates in all control datasets. Additionally, the pipeline was successfully tested on clinical samples from children in a paediatric hospital in the Central African Republic infected with P. falciparum. The pipeline can be used on any raw and was tested with amplicons of pfdhps, pfdhfr, and pfk13. The PHARE pipeline helps to provide a complete picture of the population structure of the malaria parasite and can be easily adapted to other pathogens and genes.

Malaria is a major public health problem in Burkina Faso and is the leading cause of hospital visits, hospitalization and death. Malaria transmission is seasonal, with peaks observed during the rainy season. Seasonal malaria chemoprevention (SMC) is a major prevention intervention to reduce malaria burden in children under five, the most vulnerable group. It is recommended that SMC be implemented in areas where Sulfadoxine-Pyrimethamine (SP) and Amodiaquine (AQ) remains effective. Resistance to SP or AQ may reduce the chemopreventive efficacy of SPAQ from clinical malaria in children. To date, there is little data on the prevalence of SP and AQ resistance in Burkina Faso. After more than 7 years of SMC implementation, there are no up to date data on the level of molecular markers of SP and AQ resistance. This study aims to investigate the levels of parasite resistance to SP and AQ in the health district of Boussé in the Central Plateau region. A cross-sectional survey in health facilities was conducted. A total of 150 RDT positives samples were collected prior to the start of the 2022 SMC campaign from 08th to 10th July 2022. After the 4 SMC cycles in the area, 150 other RDT positive samples were collected between 7th to 11th November 2022. Parasite DNA was extracted using Quiagen Kit at Molecular Biology laboratory. Parasite genotyping is ongoing and the main outcome measure is the prevalence of molecular markers associated with SP (codons 108, 51 and 59 in dhfr and 437, 540 and 581 in dhps) and amodiaquine (codons 72-76 Pfcrrt and 86, 184, 1034 and 1246 pfmdr1) in collected blood samples. This study will provide evidence of the resistance level of AQ and SP in Burkina Faso. Furthermore, this study will inform policy makers about the selective pressure of the SMC intervention on the circulating parasite population in Burkina Faso.
births. New diagnostics with the potential to improve access to safe radical cure treatment options for Plasmodium vivax (Pv) malaria, such as point-of-care (POC) glucose-6-phosphate dehydrogenase (G6PD) tests and novel malaria rapid diagnostic tests (RDTs), are being deployed. Of particular interest are plasmodium lactate dehydrogenase (pLDH)-based RDTs with improved limits of detection (LODs) that translate into robust clinical performance for Plasmodium falciparum (Pf) infections with histidine-rich protein 2 (HRP2) and HRP3 gene deletions and for Pv clinical infections. In the Brazilian Amazon, where malaria is prevalent, such tools have the potential to optimize malaria case management. Venous blood specimens in K2EDTA were collected in Porto Velho, Rondônia, Brazil, as part of a cross-sectional diagnostic accuracy study to evaluate the performance of the POC STANDARD G6PD Test (SD Biosensor, South Korea) against a reference spectrophotometric G6PD assay. At the time of specimen collection, all participants were tested for malaria by microscopy per the standard of care. Stored frozen specimens from this study were used to evaluate the proportion of three novel RDTs with improved LODs for LDH: the BIOCREATIT Malaria Ag Pf/Pv (pLDH/pLDH), Pf (pLDH), and Pf (HRP2/pLDH) (Rapigen Inc., South Korea). A commercially available comparator RDT (Malaria Pf/Pv Pan ECO Teste - TR.003, ECO Diagnóstica, Brazil) was also run. RDT performance was evaluated against a reference assay for Pf and Pv malaria. The study evaluated 981 specimens, including 70 Pf-positives, 262 Pv-positives, 15 positives for both Pf and Pv, and 631 negative specimens by PCR. Diagnostic performance of the RDTs in terms of sensitivity and specificity will be reported. The implications of test performance for malaria case management will also be considered. The proportion of PCR-confirmed Pv malaria cases that would be eligible for radical cure treatment under different testing algorithm scenarios will be presented, considering the BIOCREATIT Ag Pf/Pv (pLDH/pLDH) RDT results, the microscopy results, and the individual's G6PD status.

5364

LOW PREVALENCE OF PFHRP2 AND PFHRP3 DELETIONS AND NON-FALCIPARUM MALARIA INFECTIONS IN OUTPATIENTS SAMPLED DURING THE 2021 BENIN HEALTH FACILITY SURVEY

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Benin’s entire population is at high risk for malaria infection and Benin relies on rapid diagnostic tests (RDTs) detecting the Plasmodium falciparum histidine-rich protein 2 (HRP2) to diagnose malaria. However, no such research has been conducted in Benin to determine the prevalence of non-falciparum malaria infections, which are undetectable by HRP2-based RDTs, are also lacking in Benin. In this study, a total of 1413 outpatients at a national representative sample of 115 health facilities in Benin in 2021 were enrolled. Participants were tested using an HRP2-based RDT (Bioline Malaria Ag Pf Device TSF60, Abbott) and provided dried blood spot samples for multiplex screening of six Plasmodium antigens and PCR genotyping for pfhrp2 and pfhrp3 deletions. In total, 729 participants (51.6%) tested positive for any Plasmodium antigen, and 673 (47.6%) had high HRP2 antigen levels. 56 Plasmodium antigen-positive isolates with low HRP2 antigen signals had DNA extracted for genotyping. Six non-falciparum single-species infections were identified by PET-PCR among study participants: two were P. ovale, and seven were P. malariae (0.5%, 4/729). No P. falciparum isolates lacking the pfhrp2 gene were found. Two infections (0.3%, 2/729) carried
P. falciparum parasites with pfhrp3 gene deletions, but these persons still tested positive by HRP2-based RDT and had received appropriate antimalarial treatment. Using laboratory HRP2-antigen positivity as the comparator assay, the sensitivity of the field HRP2-based RDT was found to be 89.5% (614/686), and the specificity was 86.4% (616/713). Overall, this study identified no pfhrp2 gene deletions in P. falciparum parasites and few non-falciparum Plasmodium infections. In conclusion, these data provide evidence for the continued use of HRP2-based RDTs for malaria case management in Benin.

5365

PLASMODIUM FALCIPARUM KELCH13 MUTATIONS IN ERIETEA AND ASSOCIATIONS WITH PFHRP2 AND PFHRP3 DELETIONS

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Eritrea was the first African country to report a high prevalence of pfhrp2/3-deleted P. falciparum parasites causing high rates of false-negative RDT results and to switch away from exclusive use of HRP2-based RDTs in 2016. While country’s high reliance on malaria RDTs was likely the major driving force behind the rapid expansion of pfhrp2/3-deleted parasites, antimalarial (artesunate-amodiaquine) use and host immunity could also exert selection pressures contributing to the spread. We retrospectively examined SNPs in the P. falciparum kelch13 gene from samples collected from the Northern Red Sea (Semenawi Keih Bahri) Zone in 2016 before the RDT switch and from the Gash Barka, Anseba and Debub Zones in 2019 where pfhrp2/3 status had been determined. No non-synonymous changes were identified from the 2016 sample set. However, five different single non-synonymous SNPs were detected in samples collected after 2019. The most prevalent non-synonymous SNP was R622I as it was detected in samples collected from all locations with an overall prevalence of 11.7% (ranging from 2.5% to 28%). Preliminary analysis suggests that the pfk13 R622I variant is twice as likely to occur in single pfhrp3-deleted than dual pfhrp2/3-deleted parasites, and three-times as likely compared to parasites without pfhrp2/3 deletions. Parasites carrying the R622I mutation have diverse microsatellite marker haplotypes, suggesting that they have evolved from different genetic backgrounds. The results suggest that there is an association between pfhrp3 deletion and pfk13 R622I mutation, and indicate an interaction between RDT and artemisinin selection pressure on the parasite population. Continued monitoring of the trend of pfhrp2/3 deletion and pfk13 R622I mutation will help to decipher this interaction. Full analysis will be presented at the meeting.

5366

SURVEILLANCE OF PLASMODIUM FALCIPARUM HRP23 GENE DELETIONS IN MOZAMBIQUE: A PROSPECTIVE STUDY

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The integration of genomic data into routine surveillance activities has the potential to increase actionable intelligence for making programmatic decisions on the optimal mix of control and elimination measures. With the aim of informing Mozambique’s national malaria control and elimination strategies, we are conducting a prospective genomic surveillance study to monitor molecular markers of drug and diagnostic resistance, characterize transmission sources in low transmission settings, and assess the value of genomic data to infer levels of transmission. For the use case of assessing Plasmodium falciparum histidine rich protein 2/3 (pfhrp2/3) deletions, associated with parasite escape from rapid diagnostic test (RDT) detection, sampling took place in 2022 at 40 health facilities from five districts across four provinces of medium/high transmission during both rainy and dry seasons from 2-10 year-old children seeking care for malaria symptoms were tested by both routine HRP2-based rapid diagnostic test (RDT) and P. falciparum lactate dehydrogenase (LDH)-RDT, to identify potential false negative results due to pfhrp2/3 deletions among malaria clinical cases. Dried blood spots (DBS) were collected from those testing positive by one or both RDTs (n=200). Samples were identified according to discrepant results between LDH-RDT and HRP2- RDT, and all DBS from children with a negative HRP2-RDT but positive LDH-RDT will be tested for the presence of deletions by multiplex real time quantitative polymerase chain reaction qPCR. This approach not only detects the pfhrp2 and pfhrp3 genes but also the presence of the human reference genes humtubb (human beta-tubulin) and the pfldh gene. Samples with the following results (humtubb+, pfldh+ and pfhrp2/3-) and (humtubb+, pfldh+ and pfhrp2/3+) will be considered with deletion and no deletion respectively. The results will be incorporated into the Integrated Malaria Information Storage System (IMISS), so that the NMCP has full access to them and can recommend a change in RDTs if the prevalence of pfhrp2/3 gene deletions causing false-negative RDTs among symptomatic patients at national level is above 5%.

5367

AVAILABILITY OF FREE MALARIA RAPID DIAGNOSTIC TESTS AT THE LEVEL OF PRIVATE PHARMACIES FOR THE CONFIRMATION OF THE DIAGNOSIS OF MALARIA PRIOR TO ANTIMALARIAL TREATMENT: RESULTS OF A PILOT PROJECT IN BENIN: MARCH TO DECEMBER 2022

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World Health Organisation recommends that all suspected cases of malaria undergo parasitological testing to confirm the diagnosis. Private pharmacies are a reality of the supply of care corresponding to the demand of an increasingly growing urban population. They hold 1/3 of the volume of antimalarial sales in the private sector, which represents 70% of the total volume of antimalarial sales in Benin. Most antimalarials are sold in these pharmacies without confirmation of the parasitological diagnosis. In order to improve the diagnosis of uncomplicated malaria in these private pharmacies in Benin, the National Malaria Control Program initiated the training of pharmacists for the free implementation of rapid diagnostic tests for malaria and artemisinin-based therapeutic combinations subsidized in 44 out of the total of 340 private pharmacies currently operating in Benin. These 44 pharmacies were selected according to well-defined criteria, in particular possession of an authorization to open and operate, adherence to malaria control guidelines, agreement to comply with the rules for the transfer of subsidized ILPs, monthly reporting of consumption data on DHIS2 and the availability of a space to carry out malaria rapid diagnostic tests within the pharmacy. This training of pharmacists, followed by the implementation of the inputs, extended over the period from March to December 2022. Two supervision visits were carried out in July and December 2022 to assess the use rapid diagnostic tests in pharmacies in accordance with relevant directives. Of the 44 pharmacies trained, 35 (80%) started the diagnosis of malaria in accordance with the standards. A total of 2,030 patients suspected of uncomplicated malaria agreed to be tested as a preliminary to treatment for uncomplicated malaria in the 35 pilot pharmacies. Of the 2,030 patients who were tested, 560 tested positive and received antimalarial treatment, representing a positivity rate of 27.58% of rapid
diagnostic tests. Clients who had a negative RDT test result were referred for further assessment. A follow-up will be done for compliance with malaria diagnostic guidelines in the pharmacies.

MALARIA PARASITEMIA ESTIMATES BASED ON HRP2 AND PLDH ANTIGEN CONCENTRATIONS FROM A LARGE HOUSEHOLD SURVEY IN NIGERIA: HOW MUCH DIFFERENCE DOES RAPID DIAGNOSTIC TESTS (RDTs) PERFORMANCE MAKE?

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Countries increasingly rely on rapid diagnostic tests (RDTs) to estimate malaria prevalence in large surveys. Estimates depend on RDT ability to detect malaria antigens, especially when asymptomatic low-density infections predominate. We assessed how malaria prevalence varies by RDT sensitivity, using antigen data from a large national household survey in Nigeria. Dried blood spots (DBS) prepared during the 2018 Nigeria HIV/AIDS Indicator and Impact Survey were tested for Plasmodium falciparum-specific HRP2 and pan-species pLDH antigens on a multiplex bead assay. Standard antigen concentration curves were also created by testing known concentrations of HRP2 and pLDH on the bead assay. For HRP2, we calculated prevalence that would result from a conventional RDT (cRDT, assumed limit of detection (LoD)=6.7 nanograms HRP2/mL, the median concentration in pre-qualification testing samples) and hypothetical 10-fold more sensitive RDTs (μRDT) and 2-fold less sensitive RDTs (pRDT). For pLDH, we used thresholds for a “good” RDT (LDHRDT, LoD = 13.6 nanograms/mL) and a 2-fold more sensitive one (sLDHRDT). Estimates account for sampling probability and complex survey design. Our sample comprised all children <15 years(n=31,468), a sample of women of reproductive age (n=9,634), and all respondents 15+ years in 11 of Nigeria’s 36 states (n=47,811) with a DBS. Estimated prevalence varied significantly by HRP2 RDT, at 24.3% (95% CI: 23.0, 24.8) by μRDT, 45.1% (95% CI: 44.7, 45.6) by pRDT, and 19.5% (95% CI: 19.1, 19.9) by pRDT. Estimates significantly differed by RDT for all age groups, including children <5 years (C5), the target for malaria household surveys (μRDT: 28.7% (95% CI: 27.3, 30.1); pRDT: 47.0% (95% CI: 45.3, 48.7); pLDH RDT differences were smaller but significant for all age groups, including C5 (LDHRDT: 31.9% (95% CI: 30.3, 33.4); sLDHRDT: 35.8% (95% CI: 34.2, 37.3)). Malaria prevalence estimates can differ significantly depending on RDT sensitivity. RDT selection is especially important in settings with asymptomatic, low-density infections.

MALARIA MISDIAGNOSIS IN THE ROUTINE HEALTH SYSTEM IN ARBA MINCH AREA DISTRICT IN SOUTHWEST ETHIOPIA: AN IMPLICATION FOR MALARIA CONTROL AND ELIMINATION

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Accurate diagnosis of malaria is vital for the appropriate treatment of cases and to interrupt its transmission. The objective of this study was to compare microscopy with nested polymerase chain reaction (PCR) to diagnose malaria infections. A cross-sectional survey on health facilities was conducted from November 2019 to January 2020. Two hundred fifty-four microscopically negative and 193 microscopically positive malaria cases were assessed. Of the 193 malaria-positive patients, 46.1% (95% confidence interval (CI): 38.9-53.4) (89/193) were Plasmodium falciparum infections, 52.3% (95% CI: 45.0-59.5) (101/193) were P. vivax infection, and 1.6% (3/193) had mixed infection of P. falciparum and P. vivax. Of the microscopically positive cases of P. falciparum, 84.3% (75/89) were confirmed as P. falciparum, 3.4% (3/89) were P. vivax and 11.2% (10/89) were mixed infections with P. falciparum and P. vivax, and only one case was negative molecularly. Similarly, of the microscopically positive cases of P. vivax cases, 92.1% (93/101) were confirmed as P. vivax, 5.9% (6/101) were P. falciparum and 1% (1/101) was a mixed infection. A single case was negative by molecular technique. Of the 254 microscopically negative cases, 0.8% of patients tested positive for P. falciparum and 2% for P. vivax. Using PCR a reference, the sensitivity of microscopy for detecting P. falciparum was 89%, and for P. vivax, it was 91%. The specificity of microscopy for detecting P. falciparum was 96%, and for P. vivax, it was 98%. However, the sensitivity in detecting mixed infection of P. falciparum and P. vivax was low (8%). Thus, many P. falciparum and P. vivax mixed infections were microscopically overlooked and underreported. In addition, there were cases left untreated or inappropriately treated. Therefore, the gaps in the microscopically-based malaria diagnosis should be identified and regularly monitored to ensure the accuracy of the diagnosis.

USER PERCEPTIONS OF A SMARTPHONE-BASED MALARIA RAPID DIAGNOSTIC TEST (RDT) AID FOR COMMUNITY AND PRIVATE CLINIC-BASED HEALTH WORKERS IN WESTERN KENYA

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The increased use of malaria rapid diagnostic tests (mRDTS) has helped bridge a quality case management gap since their adoption. This is especially true in remote areas with limited access to malaria microscopy. However, health workers face challenges with proper administration and interpretation of mRDT results. Audere, PS Kenya, and PSI partnered on a study in Busia County, Western Kenya to assess the acceptability and feasibility of using HealthPulse, a smartphone RDT reader app developed by Audere, to aid health workers in mRDT administration, interpretation, and tracking with the aim of improving the mRDT testing process. 233 community health volunteers (CHVs) and 23 private clinic health workers (PCHWs) used their personal mobile devices during the study. Using a pre-and post-quantitative design, the study compared the accuracy of health worker mRDT interpretations before and after HealthPulse was introduced. During the intervention phase, process data, including participant interactions with the app, stock, and medication distribution, were recorded in addition to qualitative baseline and end line surveys. Results indicated that HealthPulse holds potential as a mobile tool that can be scaled up for adoption in low resource settings with possible utility as a supportive supervision, diagnostic, and surveillance tool. Among the 5,278 uploaded encounters, 879 (16.7%) included an image of a previously used mRDT. Process control data showed that most encounters (94.9%) were uploaded within the recommended 30-minute time frame and that 73.4% of uploaded photos passed the app’s artificial intelligence (AI) quality check on the first submission. The majority of participants felt that the app was helpful to the diagnostic process and end line survey results showed that 99.6% of participants found the app useful and 90.1% found the app easy to use. Follow-on qualitative work with 25 study participants indicated that HealthPulse provided an opportunity for health workers to submit evidence of their RDT result reporting activities, which enabled them to build credibility and trust with their peers, supervisors, and community members.
LACK OF MUTANT PLASMODIUM FALCIPARUM PARASITES WITH PFHRP2 AND PFHRP3 GENE DELETIONS IN ALONG VENG AND KRATIE, CAMBODIA

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Malaria transmission remains low in Cambodia after intensive intervention measures aimed towards malaria elimination over a decade ago. HRP2-based RDTs have been widely used as a diagnostic tool to guide antimalarial treatment, in particular to contain artemisinin resistance. Despite these, no RDT failures caused by mutant Plasmodium falciparum parasites with pfhrp2 and pfhrp3 gene deletions have been reported in the country. In this study, we investigated the genes’ status in 116 P. falciparum infected blood samples collected between Oct 2018 and Oct 2019 in Anlong Veng and Kratie towns, Cambodia during an antimalarial therapeutic efficacy trial. Plasmodium spp. was confirmed by microscopy, RDT and 18S rRNA multiplex PCR, pfhrp2 and pfhrp3 gene status was determined by conventional PCR and probe-based multiplex qPCR. HRP2 expression was also verified by ELISA and samples with negligible levels of HRP2 were tested for pLDH. Microsatellite genotyping was also performed to determine polyclonality, MOI and genetic relatedness. Despite low transmission levels (polyclonality=3.3%, MOI=1.03) and widespread use of HRP2-based RDTs that provide a fertile ground for the emergence of mutant parasites lacking HRP2, no pfhrp2 and/or pfhrp3 deletion was detected in this cohort of samples from northern and western region of Cambodia by both PCR methods. This was confirmed by parasite expression of HRP2. The result suggests a very low population frequency of pfhrp2/3 deletions in the survey location at the time of survey. The continued use of HRP based RDTs is not likely a public health issue at this time. However, continuous surveillance in Cambodia is still required to monitor possible emergence of mutant parasites causing malaria RDT failures.

PERFORMANCE AND USABILITY EVALUATION OF NOVEL MALARIA RDTs FOR IMPROVED CASE MANAGEMENT IN KÉDOUGOU, SENEGAL

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The emergence of pfhrp2/3-deleted parasites threatens histidine-rich protein 2 (HRP2)-based rapid diagnostic test (RDT) performance. High-performing, heat-stable RDTs that include Plasmodium falciparum (Pf)-lactate dehydrogenase (LDH) targets are needed to address limitations of current products and improve management of malaria patients. From November 2021 to February 2022, a cross-sectional diagnostic accuracy study was conducted in Kédougou, Senegal, to evaluate the clinical performance and usability of three novel RDTs with improved limits of detection for pLDH. Febrile patients aged >6 months were recruited at health facilities. Capillary blood was tested using a standard-of-care RDT (SD Bioline Ag Pf [H200F]), and three novel RDTs: the BIOCREDIT Malaria Ag Pf (pLDH), Pf (HRPII/pLDH), and Pf/Pv (pLDH/pLDH) (Rapigene Inc., South Korea). Venous blood was collected to repeat the novel RDTs, and microscopy slides were prepared. Venous samples were frozen and tested with a reference polymerase chain reaction (PCR) assay. Antigen concentration was determined using the Q-Plex™ Human Malaria array (5-Plex) (Quansys Biosciences, USA). A questionnaire was used to evaluate the ability of health workers to comprehend labels and interpret results of the Ag Pf (pLDH) and Pf (HRPII/pLDH) tests. Among 220 febrile patients enrolled in the diagnostic accuracy study, 154 (70%) were Pf-positive by reference PCR. No Pf. vivax cases were detected on any assay, and one suspected HRP2/3 deletion case was identified by antigen concentration results. Of all RDTs evaluated, the BIOCREDIT Ag Pf (HRPII/pLDH) had the highest performance at 78% sensitivity (70.9%–84.5%) and 89% specificity (79.4%–95.6%). RDTs performed better in terms of sensitivity and specificity when compared to the Quansys antigen than when compared to the PCR reference. All RDTs performed significantly better than microscopy (with a sensitivity of 53%) in this setting. Among 20 health workers, both evaluated tests yielded acceptable usability scores. The BIOCREDIT RDTs show promising usability and performance to address current diagnostic gaps.

DEVELOPMENT OF A FIELD-DEPLOYABLE RT-PCR DIAGNOSTIC SYSTEM FOR PLASMODIUM DETECTION IN ANOPHELES SPECIES.

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Surveillance of infectious mosquitoes is important for the implementation of appropriate vector control strategies. Diagnostic assays are essential for monitoring the infection prevalence and geographical range of pathogens in mosquito vector populations. We have evaluated a field-deployable Real-Time PCR platform as a molecular diagnostic tool for the detection of Plasmodium species. This surveillance system includes field deployable preparation of mosquito DNA samples and RT-PCR using MGB probe targeting 18S rRNA gene and other stage-specific genes using the portable bQUBE RT-PCR machine connected to an automatic data interpretation system. Parasite genomic DNA was detected in individually infected Anopheles mosquitoes and in pools of 5 to 50 mosquitoes. Additionally, emerging drug resistant Plasmodium infection using markers containing K13 SNP mutations, were detected in the field Anopheles population. Conclusively, this surveillance system provides an efficient molecular approach for the detection of Plasmodium in anopheline vectors in the field and therefore has a potential as a practical field-deployable diagnostic test for vector-borne disease surveillance programs.

EVALUATION OF MALARIA RAPID DIAGNOSTIC TEST SERVICES PERFORMANCE AT HEALTH POSTS IN ETHIOPIA

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The use of RDT by health extension workers at community level in Ethiopia has been a core element of diagnosis since 2005. However, there is limited information regarding malaria RDT use, performance and factors influencing utilization among health extension workers in Ethiopia. Evaluating performance of testing malaria using RDT at health post and addressing the bottlenecks is a vital step to improve community-based malaria treatment and inform decision at local levels is worth addressing. The aim of this study was to evaluate the performance of malaria rapid diagnostic test services at health posts as well as factors influencing malaria rapid diagnostic tests utilization in health posts in Ethiopia. A cross-section survey was conducted in 221 health posts and their 330 staffs found in 72 districts between March and October 2020. The districts were randomly sampled from nine regions and one City Administration. Interview of health extension worker using a structured questionnaire and standardized checklist and panel test was employed to collect data from selected health posts. Open Data Kit software was used to collect and share data with EPhI server. A total of 330 health extension workers from 221 health posts took part in the survey, which implies 1.49 on average. Nine out of ten health posts practice malaria diagnosis using RDT following the SOP, while discrepancies are observed.
in the rest particularly in Somal and Gambella Regional States. A moderate agreement, 89.5% (k: 0.46), was observed between the malaria RDT result and the blood film reading using expert microscope reading. The sensitivity and specificity of the RDTs were 70.2% and 91.1%, respectively, referring to expert microscopy. Conclusions, this study demonstrated that most of the health posts practice the standard national guideline and moderate agreement between RDT and expert microscope reading is obtained. Future capacity building scheme must prioritize on how to improve the use of standard guideline and avoid discrepancy in malaria diagnosis.

5375

A NOVEL COMPETITIVE ELISA ASSAY TO MEASURE AMODIAQUINE CONCENTRATION IN CHILDREN RECEIVING SULFADOXINE-PYRIMETHAMINE PLUS AMODIAQUINE FOR SEASONAL MALARIA CHEMOPREVENTION IN KOULIKORO, MALI.

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Seasonal malaria chemoprevention (SMC) was recommended by the Word Health Organization in 2012. The strategy involves monthly administration of the antimalarials sulfadoxine-pyrimethamine (SP) and amodiaquine (AQ) to all children aged under six years during the malaria season. Monitoring SMC efficacy to protect against clinical malaria requires a detectable concentration of AQ in the blood at least four weeks after treatment. However, rigorous and affordable laboratory methods to quantify AQ are lacking in malaria-endemic countries. This study aimed to validate a new ELISA-based method to reliably measure AQ and its metabolites. We also assessed the kinetic of AQ concentration from day 0 to day 28 after treatment with respect to infection status in Koulikoro, Mali. AQ concentration was measured among 37 children under 10 years who received SP+AQ for SMC and 15 children not treated with SP+AQ as a control group. At enrollment within the treated group, 15 children were parasite-negative by microscopy, 17 parasite-positive with a parasite density of <5000/μL, and 5 parasite-positive with a parasite density of ≥5000. A blood sample (2 mL) was collected from all participants on days 0, 4, 7, 14, 21, and 28 of treatment and used for AQ ELISA. Results show that the AQ concentration peaked on day 4 regardless of the infection status in the treated group. In contrast, AQ was barely detectable in the control group. The median AQ concentration varied between subgroups with 158.75 (9.2-734.16), 138.26 (21.45-224.14), 41.85 (30.71-106.22) ng/mL, respectively, in non-infected children, infected ones with parasite density of <5000, and those with parasite density of ≥5000. Using a mixed linear model, we found that the mean AQ concentration significantly increased by 121.43 ng/mL on day 4 and 64.57 ng/mL on day 7, compared to the AQ level at enrollment (p-values <0.05). A weak but significant negative correlation was found between parasitemia and AQ concentration (r=-0.26, p=0.01). We demonstrated that a validated ELISA technique could be used to accurately assess compliance with SMC and treatment outcomes in children from malaria-endemic countries.
recognize and amplify different Plasmodium/Pf/Pv in one-pot. Species-specific probes labeled with FAM, Hex, Texas Red, and Cy3 were designed to differentiate between Pan, Pf, Pv, and controls respectively. Following our newly designed “DipQuick” protocol for simple extraction, isothermal amplification occurs inside our engineered “White Lotus” device. Amplification was detected through the excitation of specific fluorescent probes. MLAMP products are then applied to the NALFIA device where the amplicons carrying different fluorophores are captured by specific antibodies coated on the strip. To avoid amplicon contamination, the whole assay occurs in a closed cartridge system, in which multiple bands are formed to visually differentiate among different Plasmodium species. Preliminary data demonstrate that different Plasmodium species were detected ~20 mins. MLAMP-NALFIA is a rapid and ultrasensitive portable low-cost assay for the species-specific detection of malaria to further progress elimination.

5378

MALARIA RAPID DIAGNOSTIC TESTS (RDTs) INTERPRETATION ACCURACY OF HEALTH WORKERS COMPARED TO ARTIFICIAL INTELLIGENCE (AI) AND PANEL READ IN KANO STATE, NIGERIA

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One strategy found to decrease malaria prevalence and mortality rates is early diagnosis using rapid diagnostic tests (RDTs). Although RDTs are known for their cost effectiveness, speed of result, and ease of use - misadministration and misinterpretation errors remain a concern amongst healthcare workers (HWs). This study investigated whether RDT use could be paired with a mobile application to improve the accuracy of mRDT interpretations amongst Health Care Workers in Kano State, Nigeria. We also investigated future applicability of result digitization and automated interpretation using artificial intelligence (AI) algorithms. The analysis assessed the accuracy of RDT interpretations against a trained group of RDT readers (Panel Read) and artificial intelligence algorithms (AI). Assessments were completed for: (1) AI interpretation compared to a Panel Read interpretation, (2) HW interpretation compared to a Panel Read interpretation, (3) HW interpretation compared to an AI interpretation, and (4) the AI performance for faint positives. The analysis first determined the AI interpretation accuracy by comparing the AI interpretation to the ground truth of the Panel Read for 2,479 RDTs. The AI performed well, correctly interpreting positives 95.54% of the time and negatives 96.90% of the time. The Panel Read was then compared to the HW interpretation, finding agreement 97.54% of the time on positives and 92.28% on negatives. Interpretation accuracy of the HWs was then determined by comparing their interpretations to the AI, providing a real-world use case for AI. With 96.76% agreement on interpretation of positives and 94.50% agreement on negatives. Overall accuracy was determined using a weighted F1 score, yielding 96.4 for the AI compared to 95.3 for experienced, well-trained health workers. The AI performed even better than HWs on faint lines, identifying 14.1% more faint positives than HWs, indicating AI’s strengths in elevating performance of humans when evaluating RDT results. Together the HW and AI are a good team to support HW decision making, and ensure accurate reporting.
generation was measured by electron paramagnetic resonance (EPR) using a SO-specific spin probe. EPR detected only low levels of SO from 5,6-POQ incubated with common electron donors, NADH, NADPH, or N-ribosylhydronicotinamide (NRH). However, robust SO generation was observed when either of the two most abundant quinone reductases from RBCs were added in the presence of their cofactors. Biliverdin reductase B (BLVRB) plus NADPH or N-ribosylhydronicotinamide quinone reductase 2 (NOQ2) plus NRH accelerated SO generation by 6.3- and 13-fold, respectively. Specific inhibitors prevented SO generation for BLVRB (Phloxine B; p < 0.0001) or NOQ2 (S29434; p < 0.0001). These findings identify two RBC reductases that are alone sufficient, in vitro, to reduce 5,6-POQ and promote redox cycling. These findings also have two translational implications. First, adding inhibitors of BLVRB and/or NOQ2 to primaquine may mitigate hemolysis in G6PDd patients requiring treatment for relapsing malaria. Because primaquine is redox cycled in hepatocytes by cytochrome P450s, which is not expressed in RBCs, BLVRB and/or NOQ2 inhibitors are not predicted to interfere with radical cure. Second, nutritional status may affect primaquine induced hemolysis in G6PDd patients, as NR, the precursor of NRH, is diet derived.

5381

POTENT ACYL-COA SYNTHETASE TEN INHIBITORS KILL PLASMODIUM FALCIPARUM BY DISRUPTING TRIGLYCERIDE FORMATION

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Conditional knockdown data demonstrate that ACS11 is not essential for triacylglycerols and a buildup of its lipid precursors. On the other hand, while inhibition of ACS10 by MMV665924 or MMV897615 leads to a reduction in lipid synthesis, confirming ACS10 as the likely target of these compounds. ACSs are enzymes that activate fatty acids scavenged from the host. Inhibition of ACS10 validating ACS10 as the likely target of these compounds. ACS genes ACS10 (PF3D7_0525100) and ACS11 (PF3D7_1238800).

Mutations in ACS10 showed either resistance to all compounds (M300I and F427L) or collateral sensitivity to another compound (A268D 10-times more susceptible to MMV665924). Introduction of M300I mutation into wildtype parasites phenocopied the resistance phenotype of the selected line. ACS genes ACS10 (PF3D7_0525100) and ACS11 (PF3D7_1238800).

Identifying how small molecules act to kill malaria parasites can lead to new "chemically validated" targets. By pressuring Plasmodium falciparum asexual stage parasites with three novel structurally unrelated antimalarial compounds (MMV665924, MMV019719 and MMV897615), and performing whole-genome sequence analysis on resistant parasite lines, we identify multiple-generation mutations in the P. falciparum acyl-CoA synthetase (ACS) genes ACS10 (PF3D7_0251100) and ACS11 (PF3D7_1238800). Mutations in ACS10 showed either resistance to all compounds (M300I and F427L) or collateral sensitivity to another compound (A268D 10-times more susceptible to MMV019719, MMV897615, and performing whole-genome sequence analysis on resistant parasite lines, we identify multiple-generation mutations in the P. falciparum acyl-CoA synthetase (ACS) genes ACS10 (PF3D7_0251100) and ACS11 (PF3D7_1238800).

5382

FIGHTING MALARIA WITH “IRRESISTIBLE” DRUGS

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Malaria is a major global disease which is transmitted to humans through the bites of infected female mosquitoes. According to the last WHO Malaria report there were an estimated of 247 million cases with 619,000 deaths worldwide. Plasmodium falciparum is the species accountable for nearly 90% of these deaths. Children under 5 are particularly susceptible to malaria illness, infection and death. In addition, malaria has severe socioeconomic impact in endemic countries. In fact, approximately 25% of the endemic countries incomes are devoted to treating and minimizing the impact of this disease. With the actual emergence of resistance against all antimalarial treatments including the standard of care artemisinin-based combination therapies (ACTs), first in the Cambodia and Thai-Burmese border region but now spread to certain African regions, there is an urgency to develop novel combination treatments. New compounds with novel modes of action and potent activity against sensitive and resistant Plasmodium parasites, are urgently needed to withstand resistance issues. Among the new opportunities identified as result of GSK whole cell phenotypic screening, a novel pyrazine class of antimalarial with promising potential has been identified. As one of the most outstanding properties, this series has demonstrated an extremely low propensity to select resistant parasites in vitro. The capability of preventing the emergence of malarial resistance renders this molecule an “irresistible” profile and offers a unique opportunity as an additional tool for future novel antimalarial combination.

Furymazine the pyrazine molecule selected as preclinical clinical molecule displays an appropriate pharmacokinetic profile along with a rapid mechanism of action that support potential for single oral dose cure. This profile could increase adherence and improve treatment efficacy whereas contributing to hampering parasite resistance selection.

5383

LEVERAGING RWANDA’S COMMUNITY HEALTH WORKERS TO CONDUCT A THERAPEUTIC EFFICACY STUDY IN AREAS OF DECLINING MALARIA TRANSMISSION

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Regular monitoring of artemisinin-based combination therapies (ACTs) is important to promptly detect and respond to emerging antimalarial resistance. The last two therapeutic efficacy studies (TESs) in Rwanda conducted in 2013-2015 and 2018, showed an artemether-lumefantrine (AL) efficacy over the 90% WHO benchmark for treatment policy change. However, day 3 parasitemia levels exceeded 10% in two of the three study sites, a criterion for suspected artemisinin resistance. In June 2021, Rwanda began a follow-up TES evaluating AL, the first line treatment, and dihydroartemisinin-piperaquine (DP), the second line treatment, in three health centers (HCS): Masaka, Rukara and Bugarama. Six months into the study, only 9 (1.7%) and 39 (7.4%) participants were enrolled in Masaka and Bugarama, respectively, and none in Rukara. This was due to malaria illness, infection and death. In addition, malaria has severe socioeconomic impact in endemic countries. In fact, approximately 25% of the endemic countries incomes are devoted to treating and minimizing the impact of this disease. With the actual emergence of resistance against all antimalarial treatments including the standard of care artemisinin-based combination therapies (ACTs), first in the Cambodia and Thai-Burmese border region but now spread to certain African regions, there is an urgency to develop novel combination treatments. New compounds with novel modes of action and potent activity against sensitive and resistant Plasmodium parasites, are urgently needed to withstand resistance issues. Among the new opportunities identified as result of GSK whole cell phenotypic screening, a novel pyrazine class of antimalarial with promising potential has been identified. As one of the most outstanding properties, this series has demonstrated an extremely low propensity to select resistant parasites in vitro. The capability of preventing the emergence of malarial resistance renders this molecule an “irresistible” profile and offers a unique opportunity as an additional tool for future novel antimalarial combination.

Furymazine the pyrazine molecule selected as preclinical clinical molecule displays an appropriate pharmacokinetic profile along with a rapid mechanism of action that support potential for single oral dose cure. This profile could increase adherence and improve treatment efficacy whereas contributing to hampering parasite resistance selection.

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to a decline in malaria cases (~41% in 2021 compared to 2020), likely a result of the successful malaria control interventions in place. In addition, 55% of all malaria cases in Rwanda are currently diagnosed and treated at the community level with only a small percentage managed in the HCs. To encourage malaria patients to attend the study HCs, Rwanda developed a strategy to engage community health workers (CHWs) to enhance study recruitment. In addition, routine malaria data from 2020 to 2021 were analyzed to replace Rukara HC with Ngoma HC, in a higher burden area. Through meetings with local leaders, partners and the malaria program, and trainings, CHWs were encouraged to refer all malaria RDT positive cases to the study HCs. In total, 92 CHWs from 46 villages in Masaka, 194 from 97 villages in Bugarama, and 64 from 32 villages in Ngoma were trained on the study protocol. From October 2021 to January 2023, 358 of the 528 (68%) required participants were enrolled and 78% of these were referred by CHWs. Innovative enrollment strategies are key to conducting TES in areas of declining malaria transmission. In a country like Rwanda where over 50% of malaria management is at the community level, active involvement and collaboration with CHWs and local leaders is essential.

5384

NOVEL MULTIPLE-STAGE ANTIMALARIAL PRODIGININES

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Prodiginines are a family of intriguing pyrrolopipermethene alkaloid natural products, produced by actinomycetes and other bacteria. Recently, there has been an increase of interest in natural and synthetic prodiginines because they have shown a broad range of therapeutic applications. While a few natural prodiginines have been evaluated for antimalarial activities, synthetic prodiginines have not been explored until our recent investigations. The modes of action are unknown for these prodiginines and the unique structure along with their pan-sensitivity against a large panel of MDR malaria parasites suggest potential to discover a new drug target to combat malaria parasites. Over the past few years, our research has focused on the discovery and development of novel antimalarials from the natural sources and we have developed prodiginine natural products as novel antimalarials that are effective against multiple life-cycle stages of the malaria parasite. Our prodiginine scaffold is a unique chemotype as compared to existing antimalarials, and potentially operates by a novel mode of action. In this context, we present the detailed optimization and structure-activity relationships of the novel prodiginine chemotype.

5385

ACRIDONES AS NOVEL LIVER STAGE ACTIVE ANTIMALARIAL

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The global impact of malaria remains staggering despite extensive efforts to eradicate the disease. The challenges for a sustainable elimination include the failing effectiveness of front-line artemisinin-based combination therapy (ACT) due to emerging resistance and safety concerns associated with limited radical cure options for relapsing Plasmodium vivax. There is an urgent need for novel, effective, affordable and safe antimalarial drugs to overcome drug resistance, and ideally, such agents would be efficacious against both blood stage and liver stage malaria infections. We have developed a novel antimalarial acridone chemotype with dual stage efficacy against both liver stage and blood stage malaria, as well as single-dose cure ability and potential to prevent relapsing infection. Our novel acridone chemotype represents a broad-spectrum approach with potential to vanquish many challenges.

5386

SAFETY AND EFFICACY OF PRIMAQUINE IN PATIENTS WITH PLASMODIUM VIVAX MALARIA FROM SOUTH ASIA: A SYSTEMATIC REVIEW AND INDIVIDUAL PATIENT DATA META-ANALYSIS

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Relapsing malaria caused by Plasmodium vivax causes substantial morbidity. Currently, primaquine is the only available antimalarial to prevent relapses in South Asia, however its optimal regimen remains unclear. A systematic review identified P. vivax clinical efficacy studies published between January 1, 2000, and August 23, 2021. Available individual patient data were pooled using standardised methods. The cumulative risks of recurrence between days 7 and 42, and days 7 and 180 were assessed.
by Kaplan-Meier methods with the effect of primaquine total mg/kg dose and treatment duration on rate of P. vivax recurrence investigated by Cox regression with random effects for study site. The number of patients with a >25% drop in haemoglobin to <7 g/dL, or an absolute drop of >5 g/dL between day 1-14 were categorised by primaquine daily mg/kg dose. The presence of vomiting, diarrhoea or anorexia following primaquine were assessed as a composite endpoint. Of 17 eligible studies, data were available from 7 studies including 791 patients from 4 countries. The cumulative risk of recurrence at day 180 was 61.1% (95% CI 42.2-80.4; 201 patients followed; 25 recurrences) after treatment without primaquine, 28.8% (95% CI 8.2-74.1; 398 patients; 4 recurrences) following low total dose primaquine (2-5 mg/kg) and 0% (96 patients; 0 recurrences) following high total dose primaquine (≥5 mg/kg). After controlling for confounders, the rate of first recurrence between days 7 and 42 was reduced following treatment with low total dose primaquine compared to treatment without primaquine (adjusted hazard ratio: 0.4 (95% CI 0.1-2.8)); no recurrences were observed following high total dose primaquine. No patients had a >25% drop in haemoglobin to<7g/dL. No data were available to assess gastrointestinal tolerability. Treatment with primaquine led to a marked decrease in the risk of P. vivax recurrences following low (−3.5 mg/kg) and high (−7 mg/kg) total dose primaquine regimens, with no reported severe haemolytic events. However, few data were available to compare efficacy of different primaquine regimens with prolonged follow up.

5387

EFFICACY OF THREE ARTEMISININ-BASED COMBINATIONS FOR THE TREATMENT OF UNCOMPPLICATED MALARIA IN CHILDREN IN BURKINA FASO

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The World Health Organization recommends regularly monitoring the efficacy of artemisinin-based combination therapy (ACT), a critical tool in the fight against malaria. This study evaluated the efficacy of three ACTs recommended to treat uncomplicated P. falciparum malaria in Burkina Faso in three sites across different epidemiological zones of malaria: Niangoloko, Nanoro, and Gourcy. This was a three-arm randomized controlled trial to assess the efficacy of artesunate-lumefantrine (AL), dihydroartemisinin-piperaquine (DP), and artesunate-pyronaridine (As-Pyr) in children aged 6 months to 12 years old followed up during supervised treatment. The primary outcomes of the study were uncorrected and PCR-corrected efficacies at day 28 for AL and day 42 for DP and As-Pyr. Day-7 lumefantrine concentrations for the AL arm were measured. We enrolled 1080 children: 190 in the AL arm, 30 in the DP arm, and 30 in the As-Pyr arm per site. PCR-uncorrected 28-day efficacy in the AL arm was 64.7% [95% confidence interval (CI) 57.1-71.1], 64.0% [95% CI 56.4-70.0] and 56.7% [95% CI 49.1-63.6] in Gourcy, Niangoloko, and Nanoro. PCR-uncorrected 42-day efficacy in the AL arm was 86.7% [95% CI 77.3-92.4], 88.0% [95% CI 78.8-93.3] and 89.9% [95% CI 81.5-94.6] in Gourcy, Niangoloko and Nanoro. PCR-uncorrected 42-day efficacy in the As-Pyr arm was 64.6% [95% CI 53.0-74.0], 70.9% [95% CI 60.1-79.3] and 54.8% [95% CI 43.5-64.7] in Gourcy, Niangoloko and Nanoro. Median day-7 lumefantrine concentrations were similar across sites and end-point classification. The day-3 positivity rate was null in all arms in Nanoro, in the DP and As-Pyr arms in Gourcy, and in the DP arm in Niangoloko. The day-3 positivity rate was 1.1% in the AL arm in Niangoloko and Gourcy and 1.2% in the As-Pyr arm in Niangoloko. The study results show evidence of high rates of PCR-uncorrected treatment failures in the AL and ASPY arms. The low uncorrected efficacy in the AL arm is consistent with previous studies. PCR correction is under way. These findings raise concerns about the most appropriate drug policy in Burkina Faso.

5388

EXPLORING DIMETHYL FUMARATE AS AN ADJUNCTIVE THERAPY FOR CEREBRAL MALARIA IN EXPERIMENTAL CEREBRAL MALARIA MODEL

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Approximately 1% of Plasmodium falciparum infected-children develop cerebral malaria (CM), which presents as a coma and is characterized by cerebral microvascular parasite sequestration. High rates of mortality and long-term neurocognitive impairment can occur in survivors despite potent antimalarial therapy. Adjunctive therapy is needed to improve CM outcomes. Our previous studies demonstrated the association of upregulation of transcription factor, Nr2f2 in children with CM who had good clinical outcomes. Nr2f2 modulates antioxidant and anti-inflammatory pathways, which may impact the pathophysiology of CM. Dimethyl fumarate (DMF) upregulates the Nr2f2 pathway and is used to prevent relapses of multiple sclerosis. We assessed the potential of DMF in the experimental cerebral malaria model (ECM). We examined the effects of DMF on survival, neuro-cognition and blood brain barrier permeability in C57BL6 female mice infected with P. berghei ANKA using state-of-the-art techniques. DMF significantly increased survival in mice during ECM (p = 0.0002). No difference was observed in total body or brain parasite sequestration, using in vivo imaging in mice infected with P. berghei ANKA GFP-Luciferase strain, suggesting that the protective property of DMF is not due to an antiparasitic effect. In ECM mice treated with DMF, we also observed improvement of neurocognitive function during the course of infection using the rapid murine coma and behavior scale. Furthermore, DMF reduced the blood-brain barrier disruption in ECM using the Evans blue extravasation assay on day 6 post infection (p = 0.01). We also examined the effect of DMF on brain volume in ECM using magnetic resonance imaging. DMF did not impact the increased brain volume which occurs during ECM. Taken together, this data supports DMF, an FDA approved drug, as a potential adjunctive therapy in CM as it increases survival and reduces the neurological decline in ECM.

5389

METABOLISM OF TAFENOQUINE AND TAFENOQUINE DRUG COMBINATIONS IN LIVER CELL CULTURES

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Antimalarial drugs of the 8-aminoquinoline class (pamaquine, primaquine (PQ), and tafenoquine (TQ)) have been the basis of malaria chemotherapy to prevent relapses and block transmission for nearly a century. Despite their wide-spread use, their metabolism and mechanism of action are still poorly understood. The importance of metabolism was suggested by an extensive 8-aminoquinoline testing program conducted by the US Army which found that 8-aminoquinolines without redox activity were unable to inactivate the latent residual parasites in the liver (hypnozoites) responsible for relapsing malaria. The precise mechanism of action of 8-aminoquinolines has been a subject of interest and conjecture for many years. It is known that monoamine oxidase (MAO) and cytochrome P450 2D6 (CYP2D6) convert PQ to the inactive carboxyprimaquine. Hydroxylated-PQ metabolites (OH-PQm) are responsible for efficacy against liver and sexual transmission stages of Plasmodium falciparum. The major CYP2D6 metabolite, formed by degradation of the unstable 5-hydroxy-PQ was found to be 5,6-OQ (5,6-orthoquinone). We have observed, quantified and partly characterised the hydroxylated metabolite of TQ (5,6-OQTQ) that is a marker of oxidative metabolism. Using high resolution accurate mass spectrometry and sophisticated compound structure elucidation software we have identified
the production of 5,6-OQTQ and other stable TQ metabolites in response to TQ alone and drug combinations of TQ over time. In addition, we have used western blotting to demonstrate CYP1A1 enzyme level modulation by TQ and drug combinations of TQ. The CYP1A1 transcript has previously been shown to be modulated by TQ. Our results may help explain the reduced therapeutic efficacy of TQ when combined with artemisinin combination therapies as well as to identify suitable TQ partner drugs in the treatment and prevention (i.e., post exposure prophylaxis) of P. vivax infections.

5390

PREDICTION OF ADENYLYLUCOCCINATE LYASE 3D STRUCTURE A PROMISING THERAPEUTIC TARGET IN PLASMODIUM FALCIPARUM AND ITS POTENTIAL INHIBITORS FROM AFRICAN NATURAL COMPOUND DATABASES

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The emergence and spread of resistant strains of Plasmodium falciparum constitute the main obstacles for the control of malaria. So, the discovery of new drugs is still urgent and requires continuous effort to identify new therapeutic targets. In this study we present P. falciparum Adenyllyuccinate lyase (PF ADSL) as a highly promising therapeutic target. This important enzyme is involved in de novo purine biosynthetic pathway, unique in P. falciparum, which results to the formation of adenosine monophosphate (AMP). Because of its role in purine metabolism, this enzyme appears as a promising drug target in P. falciparum. We aimed to construct the 3D structure of PF ADSL and predict its potential inhibitors from three databases of natural products. The 3D structure of PF ADSL was modeled using Alphafold server, and natural compounds were extracted from AFRODB, SANCDB, ANPDB. ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) predictions were performed using SwissADME, and virtual screening was performed with AutoDock-vina. After virtual screening, the ten (10) compounds with the best binding energies, good ADMET properties and also demonstrated the best results in protein-ligand interactions were selected. The binding energies were ranged between -9.6 and -8. kcal/mol and the selected compounds can provide a basis for in vivo studies. The obtained results therefore need to be validated experimentally to confirm their inhibitory activities.

5391

ARTEMETHER-LUMEFANTRINE VERSUS PYRONARIDINE-ARTESunate FOR THE TREATMENT OF MALARIA IN SARS-COV-2 INFECTED PATIENTS IN KENYA AND BURKINA FASO: A RANDOMIZED OPEN-LABEL TRIAL (MALCOV)

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ARTESUNATE FOR THE TREATMENT OF MALARIA IN ARTEMETHER-LUMEFANTRINE VERSUS PYRONARIDINE-ARTESunate is a promising drug candidate for treatment of malaria which is suggested as potential therapeutic candidates for COVID-19, particularly pyronaridine-artesunate (PA). A previous trial in COVID-19 patients without malaria showed that PA enhanced viral clearance compared to placebo. We conducted an open-label randomised trial comparing standard 3-day treatment with PA and artemether-lumefantrine (AL) in newly diagnosed SARS-CoV-2 infected patients aged ≥6 months with rapid diagnostic test or microscopy-confirmed non-severe malaria in Kenya and Burkina Faso. SARS-CoV-2 was assessed by RT-PCR on days 3, 7 (primary endpoint), 14 & 28 and symptom resolution daily for 14 days by FLUPRO+. Complete case analysis was conducted using log-binomial regression for binary outcomes, cox-regression for time-to-event outcomes and Poisson regression for count outcomes, adjusted for age, disease severity and viral load at enrolment. From February 2021 to January 2022, 143 participants were enrolled and 133 (AL:68, PA:65) with confirmed SARS-CoV-2 RT-PCR or positive serology/seroconversion contributed to the mITT analysis; median age 20 yrs (IQR 13-38). The baseline characteristics were comparable. Viral clearance by day 7 was slower with PA (adjusted hazard ratio [aHR]:0.62, 95% confidence interval 0.43-0.89, p<0.010) and median (IQR) SARS-CoV-2 viral load on day-7 was higher (AL:1337 [204-7519] vs PA:12,881 [272-46,624], p<0.000). The proportion SARS-CoV-2 by day 7 was AL:58% (39/66) vs PA:48% (30/63) (RR:0.81, 0.68-1.01, p=0.20), and by day 14 AL:34% (22/65) vs PA:54% (34/61) (RR:0.67, 0.51-0.88, p=0.028). The time to symptoms clearance was similar (aHR=1.01, 0.69-1.48, p>0.90). There were 8 serious adverse events (AL:3, PA:5), including 3 hospitalisations (AL:2, PA:1) and 2 deaths (AL:1, PA:1). PA in COVID-19 patients co-infected with malaria was associated with slower viral clearance than standard treatment with AL and with similar symptom resolution.

5389

LEVERAGING COMMUNITY OWNED RESOURCE PERSONS (CORPS) TO REACH THE UNDERSERVED POPULATION THROUGH INTEGRATED COMMUNITY CASE MANAGEMENT (ICCM) TO FIGHT MALARIA IN TANZANIA

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Malaria modeling in Tanzania demonstrates that case management (CM) should target ≥85% malaria cases appropriately managed in high malaria transmission areas to increase CM effectiveness and decrease transmission. Tanzania’s policy against the use of community volunteers for malaria transmission areas to increase CM effectiveness and decrease transmission. Tanzania’s policy against the use of community volunteers for malaria case management services. The mapped villages had high malaria risk, were hard to reach as well as overstretched health services. The service operates within the routine delivery system by using current logistic and M&E frameworks. Sensitization was done with regional and council health management teams, and CORPs were trained. 104 (33%) CORPs were trained in 311 villages that also have 434 Community Health Workers (CHWs). Between June and December 2022, CORPs attended to 35,409 patients and tested 33,030 (93.3 %) for malaria. Among them, 10,631 (32%) had confirmed malaria. Community level diagnosis accounts for 5% of national malaria cases and 16% in those Councils during the reporting
period. Community malaria CM promotes the early recognition, prompt testing and appropriate treatment of malaria among all age groups in areas with limited access to facility-based health care providers. Following the experience and lessons learned, the Ministry of Health through the NMCP plans to: 1) review protocol for iCCM implementation to address the challenges (eg. low numbers of CORPS per village); 2) advocate for using CHWs in place of CORPS for the sustainability of the intervention; and 3) review data collection and support supervision tools to address challenges.

5393

MALARIA AND THE INTERMITTENT PREVENTIVE TREATMENT FOR FOREST-GOERS IN CAMBODIA: PRELIMINARY RESULTS AND LESSONS LEARNED

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Malaria risk in Cambodia is highest in forest and forest fringe areas and linked to forest-goers. To address this challenge, the National Center for Parasitology, Entomology and Malaria Control (CNM), alongside the World Health Organization (WHO), began implementing “Last Mile Elimination” (LME) in 2021 - an innovative collection of interventions which includes providing intermittent preventative treatment for forest goers (IPTf) to prevent malaria infection. In each of the 117 LME villages, a census was conducted to identify male forest-goers between the ages of 15 and 49 who would be eligible for IPTf. Once identified, village malaria workers (VMWs) went door-to-door weekly to distribute IPTf to those planning to go to the forest in the following 30 days. Based on the preliminary findings of the initial implementation, the rate of this population taking IPTf has not surpassed 50% since the start of the program. Several contributing factors have been identified, including concerns about side effects and their impact on an individual’s ability to work and the fact that some forest-goers were engaged in illegal forest activities and were reluctant to disclose their forest-going habits. CNM has responded to these challenges by adapting its approach to IPTf, yielding important lessons learned the application of prophylactic approaches. For example, due to reports of persistent side effects, CNM shifted from arteunate-mefloquine to arteunate-pyronaridine starting in August 2022. CNM has also incentivized treatment completion to ensure forest-goes are fully protected from getting malaria. Despite the low uptake of IPTf, LME villages in Kampong Speu and Kratie implementing the full package of interventions have seen P. falciparum and mixed infections decrease by 82% over the last two years and there are ongoing analyses to ascertain whether this decrease was due, in part, to the distribution of IPTf. As Cambodia approaches elimination, documenting and sharing lessons learned and identifying which programs are truly accelerating elimination will be instrumental in aiding both Cambodia and the region in reaching their elimination targets.

5394

PLANT-DERIVED ADJUVANTS PROVIDE A PATH TO THwartING EMERGING DRUG-RESISTANT MALARIA

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Plasmodium falciparum’s drug resistance has become a significant issue in recent decades. Humans developed drug resistance to most antimalarial drugs, which caused severe side effects and failed malaria elimination programs. Hence, there is a need to investigate chemotherapeutic agents with low cost and causing minimal toxicity for the treatment of malaria. Combination therapy is a more emerging trend to control drug-resistant malaria. Plant-derived products are highly accessible and available and can be used as partner drugs with recommended ACTs and other clinically used drugs. Considering the above problems, a systematic study was conducted to search for reported plant-based adjuvants and combination therapy. Identifying practical, synergistic/additive drug combinations could improve drug-resistant malaria control. This review critically appraises the available evidence regarding plant-derived adjuvants to combat the problem of drug resistance in malaria. My presentation will highlight my previous work with current drug scenario available in past years for plant-derived adjuvants for combination therapy to combat drug resistance in malaria.

5395

SPILLOVER EFFECTS OF REACTIVE, FOCAL MALARIA ELIMINATION INTERVENTIONS IN NAMIBIA

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A recent factorial cluster-randomized trialed in Zambezi region, Namibia found that interventions targeted within 500m of index cases reduced malaria incidence (NCT02610400). To investigate whether interventions also reduced incidence in nearby areas, we measured spillover effects among untreated individuals within 500m and up to 1km from index cases. The trial randomized 56 clusters to: 1) reactive case detection (RACD) with rapid diagnostic testing and treatment with artelmerether-lumefantrine (AL) and single-dose primaquine, 2) reactive focal mass drug administration (rFMDA) with presumptive treatment with AL, 3) rFMDA + reactive focal indoor residual spraying (rIRS) with indoor residual spraying using pirimiphos-methyl, and 4) rFMDA + rIRS. Our primary outcome was the cumulative incidence of locally acquired Pf malaria in untreated individuals up to 1km from index cases. We defined spillover effects as cumulative incidence ratios (CIRs) for treatment contrasts based on the parasite reservoir targeted: 1) human reservoir (rFMDA vs. RACD); 2) mosquito reservoir (rFMDA vs. no rFMDA); and 3) human & mosquito reservoir (rFMDA + rIRS vs. RACD). We estimated unadjusted CIRs and adjusted CIRs accounting for statistical interference between outcomes of nearby untreated individuals in different study clusters using hierarchical targeted maximum likelihood estimation. For all treatment contrasts, cumulative incidence in untreated individuals was lower in the treatment vs. comparison arms. Unadjusted CIRs were 0.85 (95% CI 0.75, 0.95) for any rFMDA vs. RACD, was 0.67 (0.60, 0.75) for rFMDA vs. no rFMDA, and 0.57 (0.48, 0.67) for rFMDA + rIRS vs. RACD. Using TMLE and accounting for interference, CIRs were 0.78 (0.26, 2.37) for rFMDA vs. RACD, 0.93 (0.45, 1.90) for rFMDA vs. no rFMDA, and 0.60 (0.34, 1.05) for rFMDA + rIRS vs. RACD. Overall, our findings suggest that targeted interventions produced spillover effects and may hold promise for malaria elimination. Measuring spillover effects can inform a precision public health approach using focal interventions to reduce malaria in and near hot spots in low transmission settings.

5396

EFFECTS OF METEOROLOGICAL FACTORS AND ELEVATION ON MALARIA TRANSMISSION IN ELIMINATION TARGETED DISTRICT OF ETHIOPIA

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Malaria remains a significant public health issue in Ethiopia despite impressive progress made toward the 2030 targets of eliminating the disease. Several factors, most notably climate change, and related environmental factors have been challenging the progress. This study aimed to ascertain the transmission dynamics of malaria and associated factors in the elimination-targeted districts in the country. Malaria morbidity and meteorological data recorded from 2010 to 2017 were obtained from the
PRESENT STATUS OF THE PRIVATE SECTOR ENGAGEMENT IN MALARIA CASE MANAGEMENT IN BANGLADESH

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Bangladesh has achieved remarkable progress in malaria control and sets a target to become malaria-free by 2030. Currently, private sector involvement is limited to, partner NGOs in the NMEP platform. We conducted this study to understand the role of the for-profit private sector in malaria case management not partnering with NMEP in Bangladesh. Based on the endemicity and strategic priorities, we purposively selected 15 Upazilas for this study. Among them, eight were from high transmission districts (control area), six were from low transmission districts (endemic area) and one from non-endemic districts that have borders with high transmission districts. We have listed and enrolled all the for-profit private sectors in those selected upazilas that have some capacity for malaria case management.

A structured questionnaire was developed and used with the advice of the expert panel members. Between August to September 2022, a total of 104 health facilities were enrolled from 15 upazilas. More than half (54.8%) of health facilities were consulted & diagnostic centers followed by private clinics (18.3%) and drug stores with malaria diagnostic facilities (13.5%). Overall, 80.8% of facilities provided malaria testing while only 18.3% offered treatment services. Most of the health facilities in the control areas had facilities for malaria testing by RDT (77.7%), however, it was quite higher (95.0%) in endemic areas than control area. The price of RDT test varied widely from 50-1000 Bangladeshi Taka in different areas. Overall, 80.8% of facilities provided malaria testing while only 18.3% offered treatment services. Most of the health facilities in the control areas had facilities for malaria testing by RDT (77.7%), however, it was quite higher (95.0%) in endemic areas than control area.

Among the providers, 62.9% indicated willingness to work with the NMEP platform. Our study concludes that the for-profit private sector is somehow engaged in malaria case management but their contribution is not recognized. There is interest among the providers to work under the NMEP platform. However, there is an urgent need for a strategy to incorporate them into the rest of the NMEP surveillance systems.

FORMULATION OF G6PD HEMOGLOBIN CONTROL FOR POINT-OF-CARE G6PD DIAGNOSTICS

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Radical cure of Plasmodium vivax malaria requires glucose-6-phosphate dehydrogenase (G6PD) testing. A significant gap for the implementation of point-of-care (POC) G6PD testing is the availability of reagents to support quality control (QC) of G6PD products along the supply chain from the manufacturer to the end user. Key attributes to the utility of these reagents are stability and unitized packaging. Although reagents and systems exist to support QC of laboratory screening tests for G6PD, they are not formulated or packaged adequately to support programmatic quality assurance (QA) programs for POC G6PD tests. PATH has developed a G6PD-hemoglobin combined control reagent that can be used in POC settings. To represent high or normal G6PD concentration, human recombinant G6PD was created with contrived whole blood in K2EDTA, whereas low or deficient G6PD was used for spiking whole blood in K2EDTA, whereas low or deficient G6PD was created with contrived whole blood sample. Prior to lyophilization, a protective formulation was mixed in the blood sample to protect the G6PD enzyme activity against degradation from denaturation. Formulated blood samples were aliquoted into individual single-use tubes for lyophilization. Post lyophilization, the freeze-dried reagents were placed in individual packs with desiccants and stored at different temperatures for a one-year stability.
INVESTIGATING THE IMPACT OF LARVICIDING AS A SUPPLEMENTARY MALARIA VECTOR CONTROL TOOL IN RURAL SOUTH EASTERN TANZANIA: A SIMULATION STUDY

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Despite tremendous gains in reducing the malaria burden due to the massive use of insecticide-treated nets (ITNs) and indoor residual spraying (IRS), transmission continues in most of sub-Saharan Africa. Rufiji district located in rural southeastern Tanzania, still has malaria transmission occurring despite high ITN coverage (80 - 85%), which may be explained by the evolution of insecticide resistance and behavioral changes in malaria vectors. Therefore, there is an urgent need for additional interventions to complement ITN use. To investigate the potential impact of larviciding in different scenarios and coverage levels, a mathematical model, Vector Control Optimization Model was adapted and simulated with 80% ITN coverage as a baseline. To evaluate the effect of the application of larviciding on the mortality rate of An. gambiae, matured and immature mosquitoes were collected in two phases, before (2016-2017) and after (2019-2021) larviciding application. The entomological inoculation rates (EIR), reproduction number (R0) and biting rate were used as the primary outcome measures during the simulation. For the period of 1 year, larviciding was predicted to reduce EIR by 76.43% more than when only ITN was used, took over from 42 down to 9.9. Additionally, deploying larviciding together with ITNs was predicted to have a large impact, as it was estimated to reduce mosquito biting rate (approximately 60%) relative to the scenario without larviciding. Sensitivity analysis over a range of likely values for the biting rate, mosquito lifespan, and mosquito carrying capacity shows comparable the estimated impact between scenarios. This indicates that the predicted impact of larviciding is robust to uncertainty in model parameters and assumptions. The application of larviciding has practical challenges such as hardship in attaining high coverage but gives an assurance to vector control especially targeting the spreading An. Stephensi. This study supports larviciding as a successful strategy that policymakers and public health professionals, like the NMCP may use to control malaria vectors based on WHO application recommendations.

IMPACT OF MASS DRUG ADMINISTRATION AND INDOOR RESIDUAL SPRAYING ON MALARIA BURDEN IN A HIGH TRANSMISSION SETTING: A QUASI-EXPERIMENTAL STUDY DESIGN

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Mass drug administration (MDA) is likely to accelerate vector control impact. Yet exists little evidence on its impact on the malaria burden. We test the hypothesis that MDA plus indoor residual spraying (IRS) accelerates malaria burden reduction as compared to IRS. The study used a quasi-experimental design in three sub-counties of high malaria transmission endemicity where all received the standard of care. Toroma and Kapujan sub-counties received four rounds of IRS using permiphos-methyl (Actellic SC300) six to eight months apart from December 2016 till December 2018. Kapujan sub-county received simultaneously with IRS, MDA using dihydroartemisinin-piperaquine (DHA-PQ). Patient data were collected routinely from health facilities and aggregated in the health management information system (HMIS), at the facility level, and into the District Health Information Software (DHIS2). Data were analyzed using interrupted time series (ITS) and difference-in-difference (DID) to estimate the differences in changes in malaria case incidence rate and test positivity rate and the value of MDA on IRS. Safety was recorded through passive surveillance and patients managed following the standard of care. This study was registered with the Pan African Clinical Trial Registry under PACTR 20180716695568. Malaria cases dropped by 83% (IRR: 0.17 (0.16- 0.18); p=0.001) while in the IRS arm, these drops were 47% (IRR: 0.53 (0.51, 0.56); p=0.001) in children under 5 years. A total of 6.85 (CI 95: 2.53, 11.19) cases per 1000 persons per month were prevented in the IRS+MDA arm compared to the IRS arm in under 5 years. TPR dropped at a rate of 21 positives per 1000 persons (p=0.003) and a month-to-month decline of 0.09 positives per 1000 persons (p=0.40) in children under 5 years. An additional decrease of 60 (p-value, 0.040) mean malaria cases among children under five years and a mean decrease in TPR of 16·16 (p-value, 0.001) was observed. MDA reduces malaria burden among children under 5 years suggesting it’s a potential key strategy for malaria control and elimination in high transmission settings.

ASSESSMENT ON THE RATIONAL USE OF ANTIMALARIAL DRUGS IN HEALTH FACILITIES OF ETHIOPIA, CROSS SECTIONAL STUDY

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Malaria is one of the major causes of morbidity and mortality in in Ethiopia. Appropriate use of antimalarial drugs is vital in the effective management of malaria. This study helps to assess antimalarial drug use and level of adherence to national malaria treatment guideline and identify improvement areas at health facilities. To assess the rational use of anti-malarial drugs on the management of malaria cases in selected six health facilities in Ethiopia. Indicator based cross-sectional study was conducted by reviewing medical records of malaria patients who were treated from August 1, 2021, to June 30, 2022. Medical records of 540 patients were selected using systematic random sampling for data collection by standard questionnaire, exported to SPSS and descriptive analysis was done. 540 malaria patient medical records were reviewed. Most of the patients were male (55.7%), and a mean decrease in TPR of 16·16 (p-value, 0.001) was observed. MDA reduces malaria burden among children under 5 years suggesting it’s a potential key strategy for malaria control and elimination in high transmission settings.

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ASymptomatic malaria and its treatment effectiveness in gia lai and phu yen provinces of Vietnam for the malaria elimination roadmap

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A significant challenge for malaria elimination in Vietnam is the detection and elimination of asymptomatic malaria infections, which presents a hidden reservoir for malaria transmission contributing to the spread of drug-resistant malaria parasites. Gaining an understanding of the prevalence, distribution and persistence of asymptomatic malaria will help design intervention strategies to accelerate malaria elimination. Between 11 June 2022 and 17 February 2023, we screened 1,200 asymptomatic people (adults and children ≥ 5 years old) residing in Krong Pa district, Gia Lai province (n=1,045 people) and Song Hinh district, Phu Yen province (n=155) in Central Vietnam. Of these, 400 people (cohort 1) were living in close proximity to the previous year’s symptomatic malaria cases and 800 people (cohort 2) were close contacts of the “index” symptomatic malaria cases associated with a concurrently run therapeutic efficacy study of pyronaridine-artesunate (Pyramax®). Finger prick blood samples were collected. All subjects were malaria negative by rapid diagnostic tests and blood film microscopy. RNA was extracted from 150 µL of blood and malaria parasites were detected by one-step RT-qPCR targeting 18S ribosomal RNA transcripts of Plasmodium falciparum, P. vivax, P. ovale and P. malariae. Of the 1,200 subjects, 77 (6.4%, CI 95%: 5.2-7.9%) were positive for malaria parasites by RT-qPCR, with 73 (94.8%) cases infected with P. falciparum and four cases of unknown speciation. The prevalence of asymptomatic malaria in cohort 1 of 1.8% (CI 95% 0.9%-3.6%) was significantly lower (P<0.0001), than that in cohort 2 amongst close contacts of asymptomatic malaria in cohort 1 of 1.8% (CI 95% 0.9%-3.6%) was significantly lower (P<0.0001), than that in cohort 2 amongst close contacts of the current symptomatic malaria cases of 8.8% (CI 95% 7.0%-10.9%).

A total of 54 malaria positive subjects elected to be treated with Pyramax® (3-day course) and a single dose of primaquine. The study is ongoing with data from 46 individuals (cohort 1) and 6 from cohort 2. The study findings will be presented at the meeting.

EXposure to a multi-channel malaria SBC program among gold miners in Guyana

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Early diagnosis and prompt treatment is critical for malaria elimination. Guyana’s Ministry of Health has implemented a program to increase access to testing and treatment in remote gold mining communities where malaria is endemic. The program interventions cut across the social ecological model, including distribution of insecticide-treated nets and training community members as volunteer malaria testers (VMT) who can administer rapid diagnostic tests, provide treatment for individuals with uncomplicated cases of malaria, and conduct ongoing monitoring. In partnership with the USAID-funded Breakthrough ACTION project, the VMT program is complemented by a National Malaria Social and Behavior Change (SBC) strategy and a multi-channel SBC intervention, Little Mosquito Big Problem (LMBP), that utilizes culturally relevant activities and messaging to increase miners’ perceptions of malaria risk and self-efficacy to sleep under LLINs, test for malaria, and complete treatment. A robust mixed methods evaluation of this program examined pre- and post-intervention changes in malaria knowledge, attitudes, and practices among gold miners in the study areas. The evaluation included cross-sectional surveys with approximately 1,200 adult miners in 2019 and 2022. At post-test 77% of miners recalled at least one LMBP campaign component and a third of the miners knew about the VMT program. Multivariable logistic regression analysis controlling for miner’s sociodemographic characteristics and mining context found that exposure was associated with higher knowledge, progressive attitudes towards malaria prevention and treatment, self-efficacy for malaria-related behaviors, positive self-image, and availability of social support. The results highlight gains made in Guyana to address malaria in remote areas. Care-seeking behaviors were reported by slightly more than half of all miners, and as such, there continues to be the need for multi-level programs for malaria elimination and expanded reach of the program.
CONSIDERATIONS FOR MEDICATION SAFETY FOR MASS
DRUG ADMINISTRATION FOR PLASMODIUM FALCIPARUM
MALARIA ELIMINATION

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The use of mass drug administration (MDA) for malaria can have a large
impact - ranging from rapidly decreasing morbidity and mortality during
malaria outbreaks to potentially contributing to malaria elimination with
multiple rounds. These two scenarios may carry different risk-benefit
outcomes because of their differing levels of malaria transmission, yet there
are few published reports on the implementation of safety measures in MDA
campaigns notably for malaria elimination. A medication delivered to a large
population should be both operationally appropriate and safe to justify its
use in MDA. The ideal medication for MDA would meet the following criteria:
efficacious in clearing all stages of the parasite, long prophylactic effect
against new infections, a single dose regimen per round, safe in pregnancy,
safe in common chronic medical conditions, minimal risk of drug-drug and
drug-food interactions, low potential for drug hypersensitivity reactions, and
associated with only minimal reports of adverse drug reactions (ADRs). In
addition, rigorous screening algorithms to assess an individual’s eligibility
to participate may be implemented during the MDA campaign to further
minimize the risk of adverse events. In 2020, the National Malaria Control
Program in Haiti administered sulfadoxine-pyrimethamine (SP) and a single
low dose of primaquine (PQ) to 42,249 people. We implemented a medical
eligibility screening algorithm that found self-reported rates (preliminary data)
of kidney or liver failure (1.92%), SP allergy (0.28%), PQ allergy (0.39%),
currently taking other medications that required additional review before
administering SP or PQ (4.58%); and among women 15-49 years old, known
or possible pregnancy (9.44%), currently breastfeeding (19.92%).
Additional screening questions or testing were administered as needed.
For ADR monitoring, passive pharmacovigilance was implemented that
detected four cases of Stevens-Johnson syndrome. A review of MDA
screening procedures identified no errors. An analysis of our campaign
procedures and experience will provide lessons learned to increase the
safety of MDA campaigns.

ELUCIDATING INTERSEASON RESIDUAL PLASMODIUM
INFECTION IN HUMANS AND WILD MOSQUITOES
TO GUIDE THE SUCCESSFUL IMPLEMENTATION OF
INTERVENTIONS FOR MALARIA ELIMINATION

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In the efforts of malaria control, multiple interactive strategies need to
be deployed in the field. Because of specific mechanisms and action of
each strategy, it is critically important to assess the main epidemiological
parameters of malaria transmission in natural setting. The responsibility
of human and vector as the main Plasmodium reservoirs in the parasite
transition between two malaria seasons is unclear. Here, we aim to
investigate the seasonality of Plasmodium infection during the dry
season emphasizing on the potential role of human and vector in Burkina
Faso. A longitudinal survey was performed during an entire dry season of
6 months for P. falciparum infection combining molecular and microscopy
analysis in school age-group of children who were classified into 4 cohorts.
The first group consisted of confirmed-uninfected individuals, the second,
malaria submicroscopic infections, the third, asymptomatic infected-
children with low P.f densities (to monitor parasitemia progress), and the
fourth group included malaria asymptomatic cases which were treated
with antimalarial drug. In parallel, we examined the spatial and temporal
distribution of the mosquito vectors and their Plasmodium infection status.
Following approximately 1000 children revealed that humans remain the
main parasite reservoir between malaria seasons; with more than 50% of
P.f-asymptomatic carriers remain infected all dry season during which the
vector was quasi-absent. Advanced molecular analysis using Plasmodium in
blood samples will provide more information about the parasite
genotypes. Interestingly, 95% of negative individuals for P. falciparum
infection (naturally or by antimalarial treatment) remains uninfected until
the start of the transmission season, implying that intervention such as mass
drug administration in the absence of the vector could be more beneficial
for malaria control.

KNOWLEDGE AND PERCEPTIONS OF NATIONAL
GUIDELINES FOR THE CASE MANAGEMENT OF MALARIA
IN PREGNANCY AMONG HEALTHCARE PROVIDERS AND
DRUG DISPENSERS IN THE CONTEXT OF MULTIPLE FIRST-
LINE THERAPIES IN WESTERN KENYA: A MIXED METHODS
STUDY

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Emerging resistance to artemether-lumefantrine (AL) in Africa prompted the
pilot introduction of multiple first-line therapies against malaria in the general
population in western Kenya, potentially exposing women of childbearing
age to antimalarials with unknown safety profiles in the first trimester.
We undertook a mixed-methods study to explore the knowledge and
perceptions of malaria treatment guidelines for pregnant women among
healthcare providers and drug dispensers. Structured questionnaires were
administered to 174 providers across 50 health facilities and 40 drug
outlets. In-depth interviews (IDIs) were conducted with 33 purposively
selected healthcare providers, drug dispensers, and health managers.
Transcripts were coded by content analysis using the WHO health system
building blocks framework. Descriptive analyses and Chi-square tests
were used to report differences in proportions. There was a greater
awareness of guidelines in health facilities (83/134 [62%]) versus drug
outlets (16/40 [40%]; p=0.023), and more staff in health facilities had been
trained on malaria in pregnancy in the last year (49% vs 20%, p=0.002).
Lack of training on malaria case management was also evident from the
IDIs with drug outlet providers, who did not know the national malaria
treatment guidelines and reported a lack of pregnancy tests. Knowledge of
recommended antimalarials for treatment in the first trimester (72% vs 90%,
p=0.02), second/third trimesters (70% vs 84%, p=0.07), and for severe
malaria (50% vs 71%, p=0.02) was less common in drug outlets than health
facilities. Providers reported using AL instead of quinine in the first trimester
due to the side effects and unavailability. Patient preference was a major
factor in the antimalarials prescribed. Health managers reported a lack of
supervision of drug outlets due to insufficient funds. Almost all providers
reported drug stock-outs, with quinine most affected. Improved training,
regulation, and monitoring of drug outlet providers is warranted, in addition
to regular supply of malaria commodities and pregnancy test kits to ensure
adequate management of malaria in pregnancy.
MALARIA AMONG CHILDREN IN THE AHANTA WEST DISTRICT, GHANA

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Malaria is a persistent problem in sub-Saharan Africa, particularly among children. The World Health Organisation (WHO) has set a goal to reduce cases by 50% and mortality by 90% by 2025. However, the asymptomatic carriage of parasites continues to pose a challenge. Rapid diagnostic tests (RDTs) are recommended for surveillance due to their ease of use and low technical requirements. In this study, we evaluate the performance of three RDTs for detecting Plasmodium falciparum and Pan Plasmodium in asymptomatic children in the Ahanta West Municipality of Ghana in December 2022. We collected demographic and medical information using a structured questionnaire and performed venepuncture to obtain peripheral blood for the malaria RDTs. The study included 113 participants with a mean age of 12.10 ± 2.3; 70 (61.9%) were girls, and 25 (22.1%) reported receiving malaria treatment in the three months prior to data collection. Malaria positivity rates were 44.2% for Bioline, 49.6% for NxTek, and 52.2% for First Response, with an overall prevalence of 53.1%. No significant associations were found between asymptomatic malaria positivity and study variables. Our findings suggest a high prevalence of asymptomatic malaria even during the dry season, which may hinder current elimination strategies and contribute to the transmission of malaria. Additionally, individuals with asymptomatic malaria may act as reservoirs for malaria parasites, underscoring the need for continued surveillance and treatment.

EPIDEMIOLOGICAL STUDY TO ESTIMATE MALARIA PREVALENCE AND USE OF CONTROL MEASURES IN AN AREA WITH PERSISTENT TRANSMISSION IN SENEGAL

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While there is evidence of malaria burden among under-five, limited data on malaria prevalence and use of control measures are available across all age groups in Senegal. As the country is shifting from control to elimination, there is a need to better understand malaria distribution across all age groups in order to guide future interventions. A cross-sectional survey was conducted in four health posts in the Saraya health district, an area with persistent malaria transmission during the 2021 transmission season. A multistage random sampling technique was used to select households and individuals over 6 months of age who consented to participate. Socio-demographic data, household assets, and use of preventive measures were collected using an electronic questionnaire. Malaria parasites were screened by microscopic examination of blood smears, and hemoglobin levels were measured using a portable hemoglobinometer (Hemocue 301TM). Logistic regression was used to identify factors associated with malaria infection. Overall, 1759 participants were enrolled and the falciparum prevalence was 20.5%. There is no statistically significant difference between the prevalence of under-five children (20.5%) and adolescents (26.6%), (p = 0.52), nor between 5-10 years old children (26.6%) and adolescents (24.7%), (p = 0.76). F. falciparum accounted for 99.2% of the malaria infection, and 69% were asymptomatic. The odds of malaria was associated with the location of Khosanto (aOR = 1.97, 95% CI: 1.35-2.88) and the primary education level (aOR = 1.64, 95% CI: 1.08-
Malarial trends during the COVID-19 pandemic in the city province of Kinshasa / DR Congo

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Malaria is one of the scourges that concern the world with high morbidity and mortality. In 2019, 212 million cases of malaria were recorded worldwide with 429,000 deaths. The African region is the most affected and accounts for 85% of all cases and 91% of deaths recorded worldwide (WHO 2019). In DR Congo, malaria is the leading cause of hospitalization among children under 5 and pregnant women. Added to this is the occurrence of the COVID-19 pandemic with the highest lethality (2.6%) (Epidemiological Bulletin 2020). We want to know if the COVID-19 pandemic has an impact on the proportions of malaria in the city of Kinshasa. II. We carried out a cross-sectional study using data from the annual reports of the PNLP / DR Congo edition 2019 and 2020. In 2019, about 2,249,789 suspected cases of malaria were registered, 1,270,497 confirmed cases (56%), 119,009 serious cases and 973 deaths. In 2020, 2,148,169 suspected cases, 1,169,841 (54.5%) confirmed cases, death. The results of this study revealed no significant difference. The increase in deaths during the pandemic period is justified by the fact that at the start of this pandemic, the population had deserted the health structures. In conclusion, COVID-19 appears to have no impact on the proportions of malaria cases. Large-scale studies across the country need to be conducted to confirm these findings.

Factors associated with active private health provider follow-up of Plasmodium vivax patients treated with primaquine in Myanmar

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Recorded cases of Plasmodium vivax have risen in Myanmar since 2019, with 39342 cases reported in 2022, almost double that of 2021, Myanmar aims to eliminate P. vivax by 2030. Elimination will require patient adherence to radical cure with primaquine (PQ), which has hitherto proven difficult due to the 14-day dosing regimen. Adherence can be supported by active provider follow-up. We developed a suite of tools to support malaria case providers, including an NMCP-approved direct observation treatment form, patient counseling form and case management guide. A cross-sectional mixed-method study was conducted with private general practitioners (GPs) and trained informal private outlet (PO) providers to investigate knowledge, attitudes, and practices related to P. vivax treatment and explore opinions of the new tools. A total of 216 GP and 204 PO providers completed a quantitative survey, which was followed by qualitative in-depth interviews (IDIs) with 10 GP and 6 PO. Descriptive and logistic regression analyses were conducted for survey data and deductive content analysis performed on qualitative data. Providers had excellent knowledge of P. vivax treatment, with 99.1% of GPs and 94.1% of POs correctly citing the national guidelines. However, only 25.5% of GP and 79.4% of PO reported that they usually followed-up patients who were treated with PQ. Multivariate regression identified the following significant determinants of patient follow-up: providers aged above 60 yrs (aOR=0.39, p=0.016), provider education above high school (aOR=0.15, p=0.001) and providers in rural areas (aOR=1.9, p=0.019). IDIs revealed transportation and connectivity at patients’ location and poor patient awareness of the importance of adherence as challenges to patient follow-up. Providers valued the new tools and believed that they would help improve treatment adherence and monitoring PQ side effects. However, providers could not complete the counseling form for every patient when their case load was high. The study highlighted additional customized program support is needed to support providers with active patient follow up per treatment guidelines.
The burden of malaria in Kenya is not uniformly distributed, with the Lake-endemic region in Western Kenya having a prevalence of 19% compared to the national average of 6%. There is limited data on repeat malaria infections in this region. We sought to characterize repeat malaria infections epidemiologically and identify associated factors among outpatients. We reviewed medical records from 1st June 2021 through 31st May 2022 in nine public health facilities in three counties in the Lake-endemic region. The study population was patients diagnosed with confirmed malaria. Temporal, demographic and diagnostic data were abstracted using a standardized template from outpatient and laboratory registers. A repeat malaria infection was defined as confirmed parasitemia in a patient fourteen days after treatment initiation. The outcome variable was &gt;1 confirmed malaria episode in the same patient. Analysis was performed using Epi Info v7.2.5.

We calculated means and medians for continuous variables, frequencies and proportions for categorical variables. Chi-square test was used to measure the association between repeat infections and independent variables and factors with p &lt;0.05 were considered statistically significant. We analyzed 26,133 records; 12% (3,132/26,133) were repeat malaria cases, 84% (1,993/3,132) were aged &lt;15 years and 27% (844/3,132) &lt; 5 years. The majority, 87% (2,728/3,132), had one repeat infection, and 15.2% (476/3,132) had more than one episode. The median duration to repeat episodes was 146 days (IQR:51-341). Overall, diagnosis by microscopy was 57% (1,783/3,132) and 43% (1,335/3,132) by RDT. Healthcare workers adhered to national treatment guidelines in 88.2% (1,683/1,909) cases representing 90.6% (727/802) among &lt;5 years and 86.4% (956/1,107) among 5-14 years. We found an association between diagnosis and patients &lt;5 years (cOR 1.5, 95% CI 1.1-2.1), Persons aged &lt;15 years had the highest burden of repeat infections. The malaria lake-endemic counties should enhance laboratory surveillance and treatment for repeat malaria infections in children.
TARGETING MALARIA CONTROL EFFORTS IN MALAWI: OUTPUTS AND RECOMMENDATIONS FROM A WORKSHOP ON BURDEN STRATIFICATION FOR THE 2023-2030 STRATEGIC PLAN

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Malawi’s National Malaria Control Programme (NMCP) is developing a new strategic plan for 2023-2030 to combat malaria and recognizes that a blanket approach to malaria interventions is no longer feasible. To inform this new strategy, the NMCP set up a task force comprising 18 members from various sectors, which convened a meeting to stratify the malaria burden in Malawi and recommend interventions for each stratum. The burden stratification workshop took place from November 29 to December 2, 2022, in Blantyre, Malawi, and collated essential data on malaria burden indicators, such as incidence, prevalence, and mortality. Workshop participants reviewed the malaria burden and intervention coverage data to describe the current status and identified the districts as a appropriate administrative level for stratification and action. Two scenarios were developed for the stratification, based on composites of three variables. Scenario 1 included incidence, prevalence, and under-five mortality, while Scenario 2 included total malaria cases, prevalence, and under-five mortality. Results of these two scenarios are presented in the next steps for the NMCP are to develop four burden strata (highest, high, moderate, and low) for each scenario, resulting in a final list of districts assigned to each stratum. The task force concluded with 10 districts in the highest-burden stratum (Nkhotakota, Salima, Chirula, Dowa, Mchinji, Nkhotakota, Nkhotakota, Ntchisi, Machinga, Phalombe, and Chiradzulu). These districts were recommended for interventions to be implemented at the local level. Overall, this burden stratification workshop provides a critical foundation for developing a successful malaria strategic plan for Malawi.
for malaria and other health conditions. These data are recorded in CHV Activity Registers (CHV-AR) which was digitized using ocular character recognition technology (ScanForm) to enhance quality, timeliness, and data use of information collected in this register. We analyzed data collected between January 2021–December 2022 from 35 health facility OPD ScanForm registers (stratified by age to <5yrs, 5–14yrs and 15+yrs) and CHV-ARs from the corresponding 39 CUs covering 307 villages with a total population of about 128,926 people in Rarienda sub-county, western Kenya. We reviewed the number of persons tested for malaria and calculated test positivity rates (TPR). Overall, 229,511 malaria tests were performed, including 203,208 at OPD (5yrs=40,326, 5–14yrs=63,321, 15+yrs=99,435) and 26,598 in the community (<5yrs=7,770, 5–14yrs=9,453, and 15+yrs=9,080). The overall TPR was 56.4% at OPD (<5yrs=51.9%, 5–14yrs=69.3%, 15+yrs=48.1%) and 81.1% in the community (<5yrs=85.3%, 5–14yrs=82.5%, 15+yrs=76.0%). Community cases accounted for 11.5% of all malaria tests but 16.1% of all positive cases, highlighting the high TPR in the community. Appropriate provision of artemisinin-based combination therapy for test positive cases was 92.4% at OPD and 95.1% at the community. Despite significant utilization of health facilities, the burden of symptomatic malaria in the community remains high. Our data suggest that CHVs appropriately follow testing and treatment guidelines and reach a population in need. Further support for the CCM should be considered to increase the reach of CHVs.

**5422** IMPLEMENTING HIGH QUALITY COMMUNITY CASE MANAGEMENT AND DATA REPORTING: LESSONS FROM THE FIELD IN SIAYA, WESTERN KENYA

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Malaria is still a major cause of mortality and morbidity in western Kenya, despite implementation of recommended malaria control interventions. Community case management of malaria (CCM), implemented by Community Health Volunteers (CHVs), is a key strategy for timely identification of fever cases and management. However, inadequate data capture tools, training and supervision affect the quality of data. We aimed to track access to diagnostics, treatment, and referral services offered by CHVs using scannable registers to enhance accurate data capture and reporting. In 2021, we deployed scannable registers in 2 sub-counties, in Siaya County, western Kenya. 914 CHVs covering 975 villages in 89 community units (CU) attached to 95 health facilities were trained on the use of scannable registers and senior MoH staff trained on supervision and data quality review, including summary dashboards. 34 CUs up from 22 CUs in Alego Usonga began reporting during the implementation period (out of 50). In Rarienda, which has received support since 2021, all CUs are reporting (n=39). There was an increase in the number of malaria positive cases reported by CHVs, from 8653 in 2021 to 12955 in 2022. While cases seen at the health facility decreased from 61370 to 51842, the proportion of overall cases seen by a CHV increased from 12.4% in 2021 to 20%. More frequent (Monthly) data reviews at CU level as opposed to quarterly at sub-county level improved reporting timeliness. We uncovered gaps in data collection, review, tallying and reporting. Delayed reports prevented action on critical interventions e.g., restocking malaria testing and treatment commodities. Multiple registers collecting similar indicators presented challenges for CHVs. Therefore, streaming existing data tools and ensuring partner coordination could reduce CHV workload and improve efficiency. Routine data reviews provide opportunities for supportive supervision and could improve quality of care and data. Integrating and automating tallies from CHV line-listed data into DHIS2 could enhance data quality and facilitate analyses for more targeted malaria control interventions.

**5424** DYNAMICS OF SUBMICROSCOPIC MALARIA INFECTION IN SOUTHERN BENIN

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Since 2016, malaria cases have increased; the largest annual increase of 13 million cases was observed between 2019 and 2020 during the first year of the COVID-19 pandemic according to WHO. In Benin, malaria transmission occurs throughout the year with a peak during the rainy season. Since 2000, there has been a progressive increase in the number of new cases of malaria in Benin, despite the various control strategies in place. According to Health Statistical Yearbook 2021, malaria is the first cause (44.6%) of outpatient consultations in public health facilities. Malaria remains a major public health problem in Benin, which is struggling to control morbidity and mortality. Benin, has set a target for malaria elimination to be realized in Kenya.

**5423** NON-RANDOM DISTRIBUTION OF PLASMODIUM SPECIES INFECTIONS AND ASSOCIATED CLINICAL OUTCOMES IN CHILDREN 3-17 YEARS OF AGE IN THE LAKE VICTORIA REGION, KENYA, 2012-2020

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Plasmodium falciparum (Pf), P. malariae (Pm), and P. ovale (Po) infections are endemic in Kenya. However, plasmodium species mixed infections (co-infections) have not been well documented. We assessed the distribution of co-infections and associated clinical features in the Lake Victoria region of Kenya. Twelve school-based cross-sectional surveys were conducted in Mfangano island and Ungoye village between 2012-2020. Peripheral blood was collected on filter paper for dried blood spots from children aged 3 to 17 years. Plasmodium infection was determined by microscopy and nested polymerase chain reaction (nPCR). The multiple-kin lottery (MKL) model calculated the expected distribution of plasmodium infections in the population and compared it to observed values using a chi-squared test ($\chi^2$); a p-value of 0.05 was considered statistically significant. Multivariate logistic regression model generated adjusted odds ratios (aOR) adjusting for age, sex, school, and survey year and 95% confidence intervals (CI) to assess any association between co-infections, and fever (axillary temperature above 37.5°C), splenomegaly (clinically palpable spleen), and anemia (hemoglobin below 11 g/dl) all measured on the day of the survey. The plasmodium prevalence by nPCR was 41.3% (6849/16563). Among all infections (6849), Pf, Pm, and Po mono-infections were 60.1%, 4.1%, and 2.4%, respectively, Pf-Pm, Pf-Po, Pf-Pm-Po co-infections were 21.5%, 3.4%, 0.2%, and 6.9%, respectively. MKL modeling revealed non-random distributions with frequencies of Pf-Pm and Pf-Po-Pm co-infections higher than expected ($\chi^2$=2130, p<0.001). Pf co-infections with Pm and or Po were associated with a decreased risk of fever (aOR 0.70, 95% CI 0.56-0.87; p=0.03). Co-infections with Pf were associated with splenomegaly (aOR 2.79, 95% CI 2.13-3.64; p<0.001) and anemia (AOR 1.23, 95% CI 1.00-1.50; p=0.04) compared to single-species infections. Plasmodium co-infections were common and non-randomly distributed. Prompt diagnosis and adequate treatment of plasmodium co-infections are urgently needed for malaria elimination to be realized in Kenya.

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86.49% were asymptomatic in the dry season and 82.95% in the rainy season. The proportion of individuals with asymptomatic infections was 34.30% and 49.88% in the dry and rainy seasons respectively. Whether in the dry or rainy season, individuals between 5 and 15 years of age have the largest infectious reservoir (50.8% in the dry season and 70.1% in the rainy season) followed by those over 15 years of age (41.1% in the dry season and 60.3% in the rainy season) and then by those under 5 years of age (21.6% and 42.5%). In univariate polytomous logistic regressions, the effect size for age was high in both years and significant ($p < 0.001$). During the rainy season, the risk was greater for 5–15 year olds ($OR: 3.43$ [95% CI: 2.44 - 4.81]) than for those over 15 years old ($OR: 2.00$ [95% CI: 1.42 - 2.81]). Our results confirm the ubiquity of the asymptomatic reservoir in the dry and rainy seasons. Age, sex, season and the use of preventive measures played a role in explaining the asymptomatic infections.

5425

HIGH PROPORTION OF LOW PARASITAEMIA AND SUBMICROSCOPIC MALARIA INFECTIONS IN HONDURAN MOSKITIA

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Efforts towards malaria elimination in Honduras have achieved substantial progress and reductions in the incidence of cases in the country. La Moskitia, a region with the highest concentration of malaria infections in the country, reported less than 800 cases in 2020. However, achieving and sustaining malaria elimination requires the implementation of highly sensitive tests that can detect low-density parasitemia to identify asymptomatic and submicroscopic carriers. In this study, we implemented photoinduced electron transfer polymerase chain reaction (PET-PCR) and assessed its performance versus light microscopy and conventional nested PCR (nPCR) on 309 whole blood samples collected from febrile subjects using a passive surveillance approach at the Puerto Lempira hospital in Gracias a Dios, Honduras. Different diagnostic performance metrics were calculated including sensitivity, specificity, negative and positive predictive values, kappa index, accuracy, and ROC. Malaria prevalence was estimated at 19.1% by Light Microscopy (LM), 27.8% by nPCR, and 31.1% by PET-PCR with 40 and 13 cases missed by LM and nPCR, respectively. The sensitivity of LM and nPCR was 59.6% and 80.8% using PET-PCR as the reference test. LM showed a kappa index of 0.67, with a moderate level of agreement. This study identified a prevalence of 12% submicroscopic malaria among febrile cases in the Honduran Moskitia. Potential asymptomatic malaria cases are not routinely tested, and, therefore, the actual prevalence in this population could be even higher. Asymptomatic malaria represents a major challenge to malaria elimination in Honduras. Therefore, highly sensitive molecular surveillance tools such as PET-PCR that maximize malaria diagnosis are needed to reduce overall malaria burden, and to secure the progress that Honduras has achieved in the fight against malaria.

5426

A PRELIMINARY ANALYSIS OF HEALTH BEHAVIORS AND ACCESS TO CARE FOR SEVERE MALARIA DISEASE AT SUSSUNDENGA-SEDE HEALTH CENTER

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Severe malaria disease prevention is influenced by access to care, individual health behaviors, and navigation of care at health centers. Severe malaria case management rarely considers the complexity of factors and environments that cause increased morbidity and mortality associated with severe malaria disease. There is limited research about severe malaria case management in Western Mozambique, especially in rural, high transmission settings. Our aim was to quantify access to care and use of malaria prevention behaviors among individuals seeking care at the Sussundenga-Sede health center in Sussundenga, Mozambique, a rural village bordering Zimbabwe in Manica Province. We conducted a time-matched case control study from April 2022–2023. We used systematic sequential sampling to enroll 120 individuals with severe malaria disease and 120 individuals with non-malaria disease who are hospitalized at the Sussundenga-Sede health center. Cases were defined as a hospitalization with malaria tested by blood smear or positive malaria rapid diagnostic test (RDT) and one or more severe malaria symptoms. Controls were defined as a hospitalization without malaria tested by a negative blood smear or negative malaria RDT and not seeking care for conditions related to an accident. Eligible participants were: 1) older than 3 months; 2) full time residents in Manica Province; 3) had the capacity to provide consent; and 4) presented to Sussundenga-Sede health center within 72 hours of enrollment. The study excluded military members, children younger than 3 months, and pregnant women. All consenting participants completed a survey about their neighborhood level access to care, malaria prevention behaviors, and process to seek care at Sussundenga-Sede health center. The survey included a medical records abstraction tool to record severity of disease and treatment. The findings of this preliminary analysis will provide additional insight into community access to care and identify malaria health behaviors that impact seeking care for severe malarial disease.

5427

HEAVY SCHISTOSOMA MANSONI INFECTION IS ASSOCIATED WITH REDUCED RISK OF PLASMODIUM INFECTION IN SCHOOLCHILDREN IN LEMFU, DEMOCRATIC REPUBLIC OF THE CONGO

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Schistosoma mansoni and Plasmodium spp. coinfection is common in the tropics. Studies on the interactions between these parasites have yielded conflicting results in the sense that S. mansoni infection is associated either with reduced or increased concurrent Plasmodium spp. infection intensity. The lack of data on the interaction between these two diseases in the Democratic Republic of the Congo (DR Congo) motivated the undertaking of the present study. An analytical cross-sectional study was conducted in two elementary schools in Lemfu. The diagnosis of S. mansoni and Plasmodium infections was made by Kato-Katz and Giemsa-stained blood drop smears techniques, respectively. The association between both infections was determined by the Chi-square test of independence. Out of 216 schoolchildren involved in the study, 93 (43.1%) were concurrently infected with both S. mansoni and Plasmodium spp., of whom 52 (56.52%) and 23 (24.7%) had light malaria parasite burden and heavy S. mansoni infection, respectively. Half (13) of those with heavy schistosomiasis had light malaria infection. Forty-two (45.16%), 35 (37.63%), and 16 (17.2%) schoolchildren had S. mansoni-P. falciparum, S. mansoni-P. malariae and S. mansoni-P. falciparum-P. malariae, respectively. Twelve-years old subjects with heavy schistosomiasis had about 6 times less risk of having concurrent malaria infection than those with light S. mansoni infection, especially with P. malariae. S. mansoni-Plasmodium spp. coinfection is common in the tropics, including DR Congo where it constitutes a public health problem. Effects of S. mansoni on Plasmodium spp. seems to be dependent on the S. mansoni parasite burden and the age of the human hosts. Further studies are needed to exclude possible influence of the endemicity and transmission levels for both schistosomiasis and malaria.

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ASYMPTOMATIC AND SUBMICROSCOPIC MALARIA INFECTIONS IN SUGAR CANE AND RICE DEVELOPMENT AREAS OF ETHIOPIA

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Water resource development projects such as dams and irrigation schemes have a positive impact on food security and poverty reduction but might result in an increased prevalence of malaria. Therefore, we assessed asymptomatic and sub-microscopic malaria in sugarcane and rice development areas of Ethiopia. We conducted two cross-sectional surveys in dry and wet seasons in irrigated and non-irrigated clusters of Arjo sugarcane and Gambella rice development areas of Ethiopia in 2019. A total of 4464 and 2176 blood samples were collected from Arjo and Gambella. A subset of 2244 microscopy-negative blood samples was analyzed by polymerase chain reaction (PCR). Multivariate logistic regression was used to estimate the association between risk factors and malaria infection. The prevalence of malaria infection by microscopy was 2.0% (88/4464) in Arjo and 6.1% (133/2176) in Gambella. In Gambella, the prevalence was significantly higher in rice-irrigated clusters (10.4% vs 3.6%) than in non-irrigated clusters, but no difference was found in Arjo (2.0% vs 2.0%). Educational level was a risk factor associated with infection in Arjo [adjusted odds ratio (aOR): 3.2; 95% confidence interval (CI) (1.27-8.16)] and in Gambella [aOR: 1.7; 95%CI (1.06-2.82)]. While the duration of stay in the area for < 6 months [aOR: 4.7; 95%CI (1.84-12.15)] and being a migrant worker [aOR: 4.7; 95%CI (3.01-7.17)] were risk factors in Gambella. Season [aOR: 15.9; 95%CI (6.01-42.04)], no ITN utilization [aOR: 22.3; 95%CI (7.74-64.34)] were risk factors in Arjo, while irrigation [aOR: 2.4; 95%CI (1.45-4.07)] and family size [aOR: 2.3; 95%CI (1.30-4.09)] were risk factors in Gambella. Of the 1713 and 531 randomly selected smear-negative samples from Arjo and Gambella analyzed by PCR the presence of Plasmodium infection was 1.2% and 12.8%, respectively. P. falciparum, P. vivax and P. ovale were identified by PCR in both sites. In conclusion, of Plasmodium infection was 1.2% and 12.8%, respectively. P. falciparum, P. vivax and P. ovale were identified by PCR in both sites. In conclusion, malaria transmission is still significant in the studied areas and proper health education for at-risk groups residing or working in such development corridors is needed.

SPATIO-TEMPORAL DISTRIBUTION OF MALARIA CASES IN MUTASA DISTRICT FOLLOWING MALARIA CONTROL INTERVENTION BETWEEN 2017 AND 2023

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Over the last decade, the incidence of confirmed malaria has declined significantly in Mutasa District. Despite a relatively good national case reporting system, detailed maps of malaria distribution have not been publicly available. In this study, monthly surveillance records over the period 2017 - 2023 of malaria burden data by PfHRP2 based rapid diagnostic tests confirmed malaria parasite positive blood specimen, were used to produce maps of malaria distribution across the District. The maps show that Plasmodium falciparum malaria incidence has a marked variation in distribution over the District. The incidence of P. falciparum malaria follows a spatial heterogeneity pattern. In the north of the district, malaria shows one seasonal peak in the period April- May of the year, whereas towards the south, the malaria cases are sporadic. This paper provides maps of

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P. falciparum malaria incidence distribution in Mutasa district resolution, which may be useful to health professionals, travelers in their assessment of malaria risk in Mutasa. As incidence of malaria changes over time, regular updates of these maps are necessary.

5431

SPATIAL DYNAMICS OF MALARIA TRANSMISSION

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Malaria transmission dynamics are complex due to variations in space, time, heterogeneity, stochasticity, and other exogenous forces like the weather. These variations affect mosquito ecology and malaria transmission dynamics through blood feeding. From the Ross-Macdonald model, several individual-based models have been developed and analyzed but with little emphasis on the analysis of spatial dynamics and uncertainty. Modeling and analyzing real systems can become computationally overwhelming with parametric challenges and increasing factors from dimension, interactions, and system processes. This always leads to the development of simple but unrealistic models that give limited room for robustness. This study aims to provide a modular framework as an alternative approach to dealing with complexity that is analytically tractable. It also provides algorithms to understand mosquito ecology, parasite dispersal, mosquito dispersal on a spatial landscape, and human stratification by behavior, travel, age, sex, bed net usage, and care-seeking among others. The framework further provides a platform for quantifying and synthesizing transmission that occurs at a particular time and place by keeping track of the mosquito and human position. As a case study, we provide a three-habitat, two patches, and two human stratified model describing mosquito ecology and malaria dynamics in the modular framework. This is analyzed with steady states and reproductive numbers to prove mathematical consistency and biologically meaningful output. From this study, it is noted that the modular framework makes it easy to develop and extend this existing model to incorporate other factors including exogenous forcing, drug resistance, and vector control among others. It is also easy to modify the functional response and some basic parameters that may affect the outcome while maintaining robustness. We intend to show a model of malaria transmission in Uganda at the district level built using this framework.

5432

EPIDEMIOLOGICAL PROFILE OF PLASMODIAL SPECIES IN SYMPTOMATIC SUBJECTS IN THE CITIES OF BANDUNDU AND KIKWIT, KWILU PROVINCE, DEMOCRATIC REPUBLIC CONGO OF THE

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Malaria is a parasitosis transmitted to humans by the bite of infected female Anopheles. In 2020, approximately 220 million cases were reported by WHO country members. The Democratic Republic of Congo (DRC) is the second most affected African country, with 15,272,767 cases reported. Three of these plasmodial species, Plasmodium falciparum, P. malariae, and P. ovale are reported and their distribution in the cities of Bandundu and Kikwit is poorly known. This study aims to provide reliable and updated data on frequency and distribution of the plasmodial species in the study cities to improve management of malaria. To determine the frequency and distribution of the plasmodial species circulating in the towns of Bandundu and Kikwit. A cross-sectional study was conducted. We collected thick and thin blood smears from patients admitted in the health facilities to determine parasite load and identify the plasmodial species involved. Measures of central tendency and dispersion of study variables were determined. The association between test positivity and study variables was tested by logistic regression and the non-parametric Kruskal-Wallis test. The parasite prevalence was 33.1% in both cities. The study revealed 2 plasmodial species circulating in the region; P. falciparum 94.3% of cases and P. malariae 5.7% of cases. We found a statistically significant association between gender, overnight stay under mosquito nets and malaria positive test of respectively OR=0.668, p=0.009 and OR=0.59, p=0.023. In conclusion, the study identified two plasmodial species, P. falciparum and P. malariae that circulate in Bandundu and Kikwit. P. falciparum being the most frequent.

5433

METHODOLOGY TO ESTIMATE DISTRIBUTION OF MALARIA CASES AMONG CHILDREN IN SUB-SAHARAN AFRICA BY SPECIFIED AGE CATEGORIES

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Children in Sub-Saharan Africa (SSA) remain the most vulnerable to malaria infections and death, thus a better understanding of distribution of malaria within this subpopulation is important for the enrolment of representative populations into clinical trials. To estimate the distribution of Plasmodium falciparum (Pf) malaria among children by specified age categories (0 to <2 years, 2 to < 6 years, 6 to < 12 years, ≥12 years) in SSA. We employed data on the number of cases and incidence rates of PF malaria by age group from the Institute of Health Metrics and Evaluation (IHME 2019) for 11 countries located in SSA. Different statistical distributions were fitted to observed data. The best fitting statistical distribution was used to estimate the percentage of individuals within the age bands of interest. We found that the best-fitting distribution of Plasmodium falciparum (Pf) malaria cases by prespecified age categories was derived using a combination of a log-Normal and Weibull distribution. According to this distribution of Pf malaria was 15.4% for ages 0 to <2 years, 30.5% for 2 to < 6 years, 17.6% for 6 to <12 years, and 36.5% for ≥12 years based on data from selected countries in SSA. In conclusion, our results have important implications for the current drive by regulators to ensure the representativeness of real-world populations in clinical trials evaluating the safety and efficacy of medication exposure. The theoretical distributions of Pf malaria reported in this study will help guide researchers in ensuring that children are appropriately represented in clinical trials and other interventions aiming to address the current burden of malaria in SSA.

5434

PREVALENCE OF ASYMPTOMATIC AND SUBMICROSCOPIC MALARIA INFECTIONS AMONG HIV PATIENTS IN YAOUNDE, CAMEROON

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Malaria remains a major public health problem in Cameroon despite the scale-up of interventions. Asymptomatic carriers are important reservoirs
for transmitting malaria parasites to susceptible human hosts. Malaria and HIV infections are known to interact bi-directionally and synergistically with each other. Hence, this study aimed to determine the prevalence of asymptomatic and submicroscopic malaria infections among HIV patients in Yaounde, Cameroon. A cross-sectional hospital-based study was conducted among HIV patients attending the antiretroviral clinic at the District Hospital Cité Verte, Yaounde. In 2018, Malaria parasite detection was done using microscopy and rapid diagnostic test (RDT). Parasite DNA was extracted from the Whatman 903 filter papers spotted with whole blood. Molecular detection was done by nested PCR (n-PCR) targeting the 18S rRNA. A total of 240 asymptomatic samples were successfully genotyped. The prevalence of asymptomatic malaria infections by microscopy, RDT and n-PCR was 23.8% (57/240), 10.8% (26/240), and 16.3% (39/240), respectively. Submicroscopic malaria infections were identified in 14.5% (26/179) of the study participants. Moreover, the sensitivities, specificities and overall diagnostic accuracies using n-PCR as the standard method were: microscopy (52.90%, 78.61% and 71.67%) and RDT (33.33%, 93.53% and 83.75%). The Cohen’s Kappa value for microscopy, rapid diagnostic test, and n-PCR was 0.448 (P<0.0001). The performance of RDT was higher (AUC=0.634, P=0.008) when compared with microscopy (AUC=0.573, P=0.152). Among the Plasmodium species identified, Plasmodium falciparum accounted for 50.0% (17/34) of asymptomatic infections, followed by P. malariae and P. ovale with 26.5% (9/34) and 23.5% (8/34), respectively. This study reported a moderate prevalence of asymptomatic and submicroscopic malaria infections among HIV patients. It was observed that RDTs performed better than microscopy in the identification of asymptomatic malaria infections. There is a need to reinforce training in microscopy due to the high number of false positives registered.

5435

EXPLORING THE COST EFFECTIVENESS OF PROACTIVE CASE DETECTION IN HARD-TO-REACH, HIGH INCIDENCE COMMUNITIES FROM A COHORT STUDY IN SOUTHEAST MADAGASCAR

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Passive, untargeted malaria control strategies may fail to reach and manage cases in hard-to-reach communities. Investments in proactive approaches targeted at these communities may enable progress towards malaria elimination goals. However, questions remain as to cost-effectiveness and scalability. We studied a cohort of 500 households (2485 individuals) from a high-incidence district (Mananjary) in southeast Madagascar. Households were defined as hard-to-reach if they had low access to treatment at healthcare facilities, community case management, and prevention coverage. Beginning in July 2021, bimonthly testing and treatment were provided to enrolled households via mobile clinic. At baseline, less than 3% of infected individuals received treatment from a healthcare facility or community health worker, and prevalence varied from 15.3% to 60.4% among communities. From the monthly screening data (23,632 total observations to date), we estimate cost-effectiveness in terms of the cost per individual screened, cost per infection treated, and explore potential savings from cases averted. During follow-up, we observed a mean decline in prevalence of 58.6% among enrolled households to date. We leverage this empirical data and mathematical modeling to explore scalability from two perspectives. First, we model susceptibility of proactive case detection approaches to disruptions, such as extreme weather events. Second, we model the extent and sustainability of declines in incidence achievable at varying levels of coverage at the community level. The optimal screening frequency and coverage to maximize cost-effectiveness will be presented. Results demonstrate the need to invest in more proactive case management in hard-to-reach areas to achieve basic control goals and set foundations for elimination efforts.

5436

GENETIC DIVERSITY AND GENOTYPE MULTIPLICITY OF PLASMODIUM FALCIPARUM INFECTION IN PATIENTS WITH UNCOMPLICATED MALARIA IN CHEWAKA DISTRICT, ETHIOPIA

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Genetic diversity in Plasmodium falciparum poses a major threat to malaria control and elimination interventions. Characterization of the genetic diversity of P. falciparum strains can be used to assess intensity of parasite transmission and identify potential deficiencies in malaria control programmes, which provides vital information to evaluating malaria elimination efforts. In this study, we investigated the P. falciparum genetic diversity and genotype multiplicity of infection in parasite isolates from cases with uncomplicated P. falciparum malaria in Southwest Ethiopia. A total of 80 P. falciparum microscopy and qPCR positive blood samples were collected from study participants aged six months to sixty years, who visited the health facilities during the evaluation of a therapeutic efficacy study of artemether-lumefantrine from September-December, 2017. Polymorphic regions of the msp-1 and msp-2 were genotyped by nested polymerase chain reactions (nPCR) followed by gel electrophoresis for fragment analysis. Of 80 qPCR-positive samples analyzed for polymorphisms on msp-1 and msp-2 genes, the efficiency of msp-1 and msp-2 gene amplification reactions with family-specific primers were 95 % and 98.8%, respectively. A total of 29 msp alleles (10 for msp-1and 19 for msp-2) were detected. In msp-1, K1 was the predominant allelic family detected in 47.7% (42/88) of the samples followed by Mad20 and RO33. For msp-2, the frequency of FC27 and IC/3D7 were 77% (57/74) and 76% (56/74), respectively. Eighty percent (80%) of isolates had multiple genotypes and the overall mean multiplicity of infection was 3.2 (95% CI: 2.87- 3.46). The heterozygosity index was 0.43, and 0.85 for msp-1 and msp-2, respectively. There was no significant association between multiplicity of infection and age or parasite density. The study revealed high levels of genetic diversity and mixed-strain infections of P. falciparum populations in Chewaka district, Ethiopia; reflecting both the endemicity level and malaria transmission remained high and more strengthened control efforts are needed in Ethiopia.

5437

ULTRA-DEEP AMPLICON SEQUENCING OF HIGHLY POLYMORPHIC NOBLE MARKERS OF PLASMODIUM FALCIPARUM SHOWS DECLINING OF MALARIA TRANSMISSION IN ETHIOPIA

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Plasmodium falciparum is the most serious, genetically complex, and fastest-evolving malaria parasite. This study was initiated to explore the population structure, to generate relatedness networks, multiple infections, and heterozygosis of the P. falciparum population in three malaria-endemic sites northern, eastern, and southern Ethiopia. The participants of the study were patients who were recruited for uncomplicated falciparum malaria therapeutic efficacy tests from October 2015 to December 2015 and November 2019 to December 2019. Quantitative real-time polymerase chain reaction (QRT-PCR)-confirmed Dry blood spot samples were analyzed by ultra-deep amplicon sequencing to detect P. falciparum amp-1, csp, opp, cpm, and msp7 genes. Population structure was analyzed using STRUCTURE software. Identity-by-state (IBS) was calculated as a measure
of parasite relatedness and used to generate relatedness networks. 183 infections were successfully sequenced by deep amplicon sequence. Five genes, ama1, csp, ccpp, cpmp and msp7 genes were successfully sequenced and respectively detected in 179 (97.6%), 181 (98.9%) and 179 (97.8%), 178 (97.3%) and 180 (97.4%) of the samples from the three sites. Multiple of infection (MOI) of each marker was 1.2, 1.06, 1.16, 1.14, and 0.99, respectively. The overall MOI was 1.38. The expected heterozygosity index (He) of each marker was 0.25, 0.15, 0.26, 0.24, and 0.26, respectively. No population structure was evident for suggesting high transmission and gene flow among the three sites (P > 0.05). In conclusion, low genetic diversity in the P. falciparum population and the overall declining trend were observed as demonstrated by the lower Multiple of infection and heterozygosis suggesting better progress in malaria control in the regions.

GENETIC DIVERSITY OF PLASMODIUM FALCIPARUM AND TRANSMISSION PATTERNS IN FOREST-GOING POPULATIONS IN SOUTHERN LAO PDR

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The Lao People's Democratic Republic (PDR) is approaching malaria elimination and the remaining cases are increasingly clustered in forest areas in the southern provinces. To assess parasite transmission patterns in this area, 53 Plasmodium falciparum (Pf) positive cases detected through test and treat campaigns between December 2017 and November 2018 were sequenced, targeting 180 diverse microhaplotypes. Two R packages, Moire and Dcofer, were applied to assess the complexity of infections (COI), within-host parasite relatedness, and inter-sample relatedness. Genomic data were integrated with survey data to investigate the associations between parasite genetic diversity and case characteristics. Parasite genomic analysis showed that 32% of the cases (17/53) were polyclonal infections (COI ≥ 2-3), and 68% (36/53) of cases were infected by a single parasite clone (COI = 1). Most of the polyclonal samples showed evidence of strong within-host relatedness (mean F = 0.7), suggesting that cotransmission rather than superinfection was primarily responsible for maintaining polyclonality in this low transmission setting. We identified five genomic clusters with high pairwise relatedness (F = 0.7-0.9) in forest fringe villages in Pathomphone (PT) district; this area had the highest test positivity and forest activity. There were four smaller clusters of 2-3 cases linking Moonlapamok (MP) and PT districts, with a lower degree of genetic relatedness (mean F = 0.58) or greater sampling time difference (> two months), suggesting cross-district transmission, possibly on a longer time scale. Among 53 positive cases, 81.1% (43/53) were infected by genetically linked parasites, and they were primarily male adults (median aged 27) detected through a focal test and treat intervention specifically targeting forest-goers. Transmission clusters identified in this study provide useful information for understanding malaria parasite transmission dynamics in this highly mobile forest-going population and targeting of remaining foci, where there is stronger evidence of ongoing local transmission.

CYP3A4 GENE VARIANTS AMONG RESIDENTS OF LAKE VICTORIA REGION, KENYA, 2013

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Precision medicine approaches in Africa need to be driven by native population data. Cytochrome P450 enzymes, the main drug-metabolizing enzymes in humans, therefore, must be better characterized in the African population. Cytochrome P450 3A4 (CYP3A4) significantly contribute to inter-individual variation in drug metabolism and is involved in the metabolism of about 30% of clinically used drugs, including the antimalarials lumefantrine and halofantrine. We explored CYP3A4 polymorphisms in residents of the Lake Victoria basin where malaria is highly endemic. We used 136 archived DNA samples collected in 2013 during malaria cross-sectional surveys from adults resident in the four islands (Takawiri, Mfangano, Ngocho, Kibugi, and Ngodo) in the Lake Victoria region and a coastal mainland (Ungoye). We used polymerase chain reaction

GENETIC DIVERSITY OF PLASMODIUM FALCIPARUM AND GENETIC PROFILE IN CHILDREN WITH ACUTE UNCOMPLICATED MALARIA IN CAMEROON

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Malaria is a major public health problem in Cameroon. Genotyping of malaria parasite population is essential for understanding the mechanism underlying malaria pathology and to determine parasite clones profile in infected individuals, for proper malaria control strategies. The objective of this study was to perform a molecular characterization of Plasmodium falciparum as is highly endemic. The study advocate for an intensification of the malaria control strategies in Cameroon
PLASMODIUM FALCIPARUM WITH PFHRP2 AND PFHRP3 GENE DELETIONS IN ASYMPOMATIC MALARIA INFECTIONS IN THE LAKE VICTORIA REGION, KENYA

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As an important component in early diagnosis and treatment of malaria, rapid diagnostic tests (RDTs) are easy to use and can provide results in as few as 15 minutes. For diagnosis of Plasmodium falciparum, RDTs targeting the P. falciparum histidine rich protein 2 (PFHRP2) are widely used. Recently, reports of P. falciparum strains lacking PFHRP2 and structurally similar PFHRP3 have led to concern about the usefulness and reliability of PFHRP2-based RDTs. The aim of this study was to detect the presence of P. falciparum with pfhrp2/pfhrp3 gene deletions in the area around Lake Victoria, Homa Bay County, Kenya. Dried blood spot samples were collected during four cross-sectional malaria surveys of school children between September 2018 and January 2020. RDT negative but PCR positive (n=448) samples were selected for analysis. PCR amplifications of two different single-copy genes (msp1 and msp2) indicated that 125 (28.1%) samples had sufficient P. falciparum DNA for detection of pfhrp2/3 gene deletions. PCR amplifications of the region between exons 1 and 2 of pfhrp2 and pfhrp3 showed that 11.2% (n=14), 7.2% (n=9), and 12.8% (n=16) of the examined samples harbored pfhrp2, pfhrp3, and pfhrp2/pfhrp3 double deletions, respectively. P. falciparum with pfhrp2/pfhrp3 double deletions were found in all surveys. While the presence of P. falciparum with pfhrp2 deletions in the study area was identified in 2014, this study reveals for the first time the presence of both parasites with pfhrp3 and parasites with pfhrp2/pfhrp3 double deletions in Kenya. Although the study was not designed to determine the prevalence of P. falciparum with pfhrp2/pfhrp3 deletions, the presence and persistence of these parasites highlight the need to monitor and evaluate the performance of PFHRP2-based RDTs currently in use.

GENETIC DIVERSITY AND MOLECULAR EVOLUTION OF PLASMODIUM VIVAX Duffy binding protein and Merozoite surface protein I in Northwestern Thailand

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The local diversity and population structure of malaria parasites vary across different regions of the world, reflecting variations in transmission intensity, host immunity, and vector species. This study aimed to use amplicon sequencing to investigate the genotypic patterns and population structure of P. vivax isolates from a highly endemic province in Thailand during 2015-2021. Amplicon deep sequencing was done on 70 samples for the 42-kDa region of PvMSP1 and domain II of PvDBP. Unique haplotypes were identified and a network constructed to illustrate genetic relatedness in northwestern Thailand. Overall, 16 and 40 unique haplotypes were identified in PvDBP1 and PmMSP142kDa, respectively. Nucleotide diversity was higher in PmMSP142kDa than in PvDBP1 (n=0.027 and 0.012) as well as haplotype diversity (Hd = 0.962 and 0.849). Significant positive values of neutrality tests were observed in both PmMSP142kDa (n=0.0027 and 0.012) as well as haplotype diversity (Hd = 0.962 and 0.849). Significant positive values of neutrality tests were observed in both PmMSP142kDa (n=0.0027 and 0.012) as well as haplotype diversity (Hd = 0.962 and 0.849). A lower recombination rate was found in PvDBP1, while the PmMSP142kDa showed strikingly higher levels of genetic differentiation (Fst) in northwestern Thailand versus other regions (0.2761-0.4881). Based on the two gene markers, the genetic diversity of P. vivax in northwestern Thailand was influenced by host immunity and evolved under the balancing selection. The PmMSP142kDa exhibited higher variation and showed genetically specific signatures in a certain area, while PvDBP1 is more likely to be a candidate for strain-transcending vaccines due to its fewer genetic polymorphisms. Findings from this study provide an understanding of P. vivax population structure and the evolutionary force on vaccine candidates.
at the next rainy season (RS), but finding a handful of such mosquitoes precluded any opportunities for quantitative essay or parameter estimation. This work uses an indirect (i.e., genetic) approach as a means to estimate the two seasons’ breeding sizes as well as the asexinating sizes, in particular, utilizing signals from temporal allele frequency dynamics. We mathematically derive that the magnitude of drift is dampened at early RS when previously asexinating individuals reappear. This has a severe impact on temporal effective population size (\( N_e \)), estimates, that the DS breeding size is overestimated by a factor of, where is the asexinating proportion when two samples are from consecutive RS. In fact, sampling the breeding individuals at three consecutive seasons starting from RS is sufficient to estimate the three sizes. This method does not require sampling asexinating individuals, which is the biggest challenge in most experiments. We apply the method to a published An. coluzzii dataset collected from Thierola, Mali between 2008 and 2010. The estimated breeding sizes are 658 and 61 for the first year, with an asexinating size of 580. While information is lacking for the second year, precise estimates are obtained for the DS size (59) and the composite parameter (51%). Further, the DS breeding sizes for both years are significantly larger than 0, suggesting the coexistence of reproducing and asexinating populations in the DS. Extensive simulations are run to verify our derivations.

5445

TRANSCRIPTOME ANALYSIS REVEAL MOLECULAR TARGETS OF INVASION PHENOTYPE DIVERSITY IN NATURAL PLASMODIUM FALCIPARUM ISOLATES FROM MALARIA ENDEMIC REGIONS OF CAMEROON

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Better understanding of the diversity of molecular interactions and mechanisms underlying RBC invasion phenotypes in natural endemic malaria parasites will facilitate target identification and prioritization for vaccine or drug development against blood stage infection in malaria. Current understanding of invasion ligands is limited to the well characterized erythrocyte binding (EBAs) and reticulocyte homolog (Rhs) gene families. To uncover the wider and complex repertoire of genes associated with invasion phenotype diversity, RBC invasion phenotypes and transcriptome profiles were simultaneously investigated in schizont-stage preparations of Plasmodium falciparum isolates from uncomplicated malaria cases from endemic sites in Cameroon. RBC invasion phenotypes were determined for 63 samples using two-color flow cytometry-based invasion assays against RBCs treated with standard proteases: Neuraminidase (Nm), Trypsin (Tp) and Chymotrypsin (Ch). The transcriptome profiles of a random set of 16 samples were determined by deep RNA sequencing on Illumina (ONT) handheld MinION device, targeting antimalarial resistance markers and the leading vaccine antigen, circumsporozoite protein (csp), in a multiplex PCR amplicon sequencing approach. Sample collection, sequencing and analysis was undertaken in Accra and the Upper East Region, using the latest ONT chemistry and a commercial gaming laptop computer. An open-access Nextflow informatics pipeline was developed for real-time genetic variant calling, calling nano-rare (the nanopore rapid analyses and variant explorer). The workflow was rapid, robust, accurate, affordable and straightforward to implement. We found that P. falciparum parasites in Ghana had become largely susceptible to chloroquine, with persistent sulfadoxine-pyrimethamine (SP) resistance, and no evidence of resistance to the current front-line antimalarial, artemisinin. Multiple single nucleotide polymorphism differences from the vaccine csp sequence were identified at high frequencies, though their clinical significance is uncertain. These results agreed closely with Illumina sequence data from the same regions. We will present further development and expansion of this ONT multiplex sequencing platform, including the addition of a new variant calling software that can detect minor variants in mixed infections, and extension of the method to dried blood spot samples. Overall, this ongoing study demonstrates the potential utility and feasibility of malaria genomic surveillance using nanopore sequencing, and provides methodological and analytical tools for its establishment in endemic settings.

5446

NANOPORE SEQUENCING FOR REAL-TIME GENOMIC SURVEILLANCE OF PLASMODIUM FALCIPARUM

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Malaria is a global public health priority causing over 600,000 deaths annually, mostly young children living in Sub-Saharan Africa. Molecular surveillance can provide key information for malaria control, such as the prevalence and distribution of antimalarial drug resistance. However, genome sequencing capacity in endemic countries can be limited. Here, we have implemented an end-to-end workflow for Plasmodium falciparum genomic surveillance in Ghana using the Oxford Nanopore Technologies (ONT) handheld MinION device, targeting antimalarial resistance markers and the leading vaccine antigen, circumsporozoite protein (csp), in a multiplex PCR amplicon sequencing approach. Sample collection, sequencing and analysis was undertaken in Accra and the Upper East Region, using the latest ONT chemistry and a commercial gaming laptop computer. An open-access Nextflow informatics pipeline was developed for real-time genetic variant calling, calling nano-rare (the nanopore rapid analyses and variant explorer). The workflow was rapid, robust, accurate, affordable and straightforward to implement. We found that P. falciparum parasites in Ghana had become largely susceptible to chloroquine, with persistent sulfadoxine-pyrimethamine (SP) resistance, and no evidence of resistance to the current front-line antimalarial, artemisinin. Multiple single nucleotide polymorphism differences from the vaccine csp sequence were identified at high frequencies, though their clinical significance is uncertain. These results agreed closely with Illumina sequence data from the same regions. We will present further development and expansion of this ONT multiplex sequencing platform, including the addition of a new variant calling software that can detect minor variants in mixed infections, and extension of the method to dried blood spot samples. Overall, this ongoing study demonstrates the potential utility and feasibility of malaria genomic surveillance using nanopore sequencing, and provides methodological and analytical tools for its establishment in endemic settings.

5447

GYPB DELETION VARIANTS (DEL1 AND DEL2) DISTRIBUTION AMONG GHANAIAN POPULATIONS AND RELATIONSHIP WITH MALARIA SUSCEPTIBILITY

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Glycoporphins play an important role in the mediating the invasion of erythrocytes by Plasmodium falciparum, and thus variation in the glycoporphin gene locus has implications for malaria susceptibility. In West Africa, the most common variants are deletions of the whole GYPB gene.
The allele frequencies of these GYPB large deletions have been previously estimated to be between 0.05-15% for GYPB DEL1 and DEL2 respectively in populations in Africa but the effect of these deletions on malaria susceptibility is still unknown. To understand this, we genotyped for GYPB DEL1 and DEL2 alleles to facilitate the identification of phenotypes for functional characterization of these variants and malaria disease outcomes. In this study, the distribution and the allele frequency of GYPB DEL1 and DEL2 among Ghanaian populations were determined using a high throughput assay for the identification of these deletions. We genotyped over 2000 samples from different ethnicities in Ghana. Overall, the allele frequency of GYPB deletions was observed to be 0.07% and 0.032% for GYPB DEL1 and DEL2 respectively. The highest allele frequencies for GYPB DEL1 and DEL2 were in the Zambra (41.67%) and Mo (20.59%). In addition, we observed that GYPB DEL1 or DEL2 allele was associated with absence of malaria parasites and self-reported absence of malaria re-infections. This is the first comprehensive large survey on the distribution of the Glycophorin B deletion variants using a high-throughput assay to genotype different populations. This allows for further experimental work to be done using these ethnic groups with relatively high GYPB DEL1 or DEL2 variants within the Ghanaian population, and for stratification of genetic association studies to understand the role of this region in malarial disease. This study will pave the way for GYPB DEL1 and DEL2 surveys in other malaria endemic populations in relation to malaria susceptibility.

5448

INCREASED FREQUENCY OF PFHRP2-DELETED PLASMODIUM FALCIPARUM IN THE PERUVIAN AMAZON IS NOT EXPLAINED BY SELECTION OF THE GENE DELETION

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Since the first report of the pfhrp2 gene deletion (pfhrp2- ) in Plasmodium falciparum parasites from the Peruvian Amazon, the deletion has become more frequent. Recently, this deletion has been reported in other countries within and outside of South America, threatening malaria control efforts. In regions where the deletion surpass 5% prevalence, WHO recommends using alternative diagnostic tools to hrp2-based RDTs. The use of RDTs has been suggested as a main factor driving the increase of pfhrp2- parasites in Ethiopia. However, those findings cannot be extrapolated to countries like Peru, where malaria diagnosis is mainly performed by microscopy. In this regard, using PCR, we evaluated if the frequency of the deletion has increased in samples collected between 2013-2017 (n=173). Subsequently, we performed the genomic analysis of Pf1 with time, for what we added to our genomic data (n=41), available genomes from additional years (in total=100, 2006-2018). Thus, we characterized the population structure and analyzed the genomes looking for signatures of selection. Our findings showed that pfhrp2- parasites increased to >76%. In addition, pfhrp2- and pfhrp2- parasites are two different clonal populations that would have expanded after a bottleneck around 2010, when PAMAFRO interventions ceased. We could not detect any signature of selection in the genomic region close to the deletion with Tajima’s D test neither with LD-based methods. However, parasites with and without pfhrp2 deletions share common regions with signals of be under balancing selection in genes of the var family, which are involved in parasite interaction with immune system of the human host. Furthermore, pfhrp2-deleted parasites presented unique candidate regions to be under selection that include a transcription factor involved in the gametocyte differentiation (AP2-G) and a gene suggested to be associated with artemisinin resistance (FP2A). These findings suggest that the pfhrp2 deletion has not been under selective pressure in Peru, and its increase in frequency could be consequence of this parasite’s drug sensitivity profile and/or increased transmission success.

5449

HYBRID CAPTURE SEQUENCING OF PLASMODIUM MALARIAE FROM TANZANIA

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Plasmodium malariae (Pm) is already prevalent in certain African regions and expected to become more prevalent as P. falciparum (Pf) is eliminated. Like other non-falciparum malaria species, Pm usually causes less clinically severe infections than Pf, but it can cause chronic infections. To better understand this neglected but widespread malaria pathogen, we performed hybrid capture sequencing of 42 Tanzanian Pm isolates collected as part of the Molecular Surveillance of Malaria in Tanzania (MSMT) project in 2021 and Transmission from Submicroscopic Malaria in Tanzania (TranSMT) project 2018-2021. These isolates span fourteen different regions representing malaria transmission strata ranging from very low (e.g. Kilimanjaro) to very high (e.g. Tabora), with regional Pm positivity rates ranging from 0.2% to 4.5%. Fourteen isolates (33%) are Pm mono-infections, while the others are mixed with Pf. Positive isolates were identified using a semi-quantitative Pm-specific 18S qPCR, with a CT of 35 as the upper limit for sequencing. Library preparation and hybrid capture were performed using a custom Twist Bioscience kit prior to sequencing on an Illumina NovaSeq 6000. This method enables us to generate high coverage sequences spanning the entire Pm genome. Initial experiments in six isolates showed enrichment, with an average of 78% of reads mapping to Pm (range 64% - 88%). Using this method, we are able to generate high-quality Pm sequences even in lower-density isolates and Pm/Pf mixed infections. Using these genome sequences, we will present the first in-depth, country-wide population genomic analysis of this understudied and poorly understood malaria species.

5450

EXPLORING HOW TRANSMISSION INTENSITY, SAMPLING, AND HUMAN MOBILITY IMPACT OUR ABILITY TO MEASURE GENETIC RELATEDNESS ACROSS PLASMODIUM FALCIPARUM POPULATIONS

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Malaria parasite genomic data are increasingly used to identify highly related infections which can illuminate epidemiological, spatial, or temporal factors associated with patterns of transmission. However, in settings with moderate to high transmission, measuring relatedness between infections is inhibited by complex infections, overall high forces of infection, and diverse parasite populations. It is not clear how these factors impact the ability to measure between-infection relatedness under various conditions including different sampling schemes, patterns of missing data, and levels of human mobility. Further investigation is required to determine which patterns of relatedness we expect to be able to reliably detect with high quality, densely sampled genomic data in a high transmission setting. We evaluated two identity-by-state measures of relatedness and applied them to ampiclon
deep sequencing data collected as part of a longitudinal cohort from an area of high transmission in Western Kenya. We observed evidence of temporal structure, but not of fine-scale spatial structure in the cohort data. To explore factors associated with the lack of spatial structure in these data, we constructed a series of simplified simulation scenarios using an agent-based model calibrated to entomological, epidemiological, and genomic data from this cohort study to investigate whether the lack of spatial structure observed in the cohort could be due to inherent power limitations of this analytical method. We further investigated how our hypothesis testing behaves under different sampling schemes, levels and mechanisms of missingness, transmission intensity, and patterns of human mobility. In high transmission settings, we found that identity-by-state measures of relatedness, even using frequently sampled, longitudinal infection data were likely underpowered to detect moderate levels of spatial structure in parasite populations. This has important implications for the use of parasite genomic data in illuminating patterns of transmission in areas of high endemicity.

5451

AMPLICON DEEP SEQUENCING REVEALS MULTIPLE GENETIC EVENTS LEAD TO TREATMENT FAILURE WITH ATOVAQUONE-PROGUANIL IN PLASMODIUM FALCIPARUM

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Atovaquone-Proguanil (AP) is used as treatment for uncomplicated malaria, and as a chemoprophylactic agent against Plasmodium falciparum. Imported malaria remains one of the top causes of fever in Canadian returning travellers. In this study, twelve sequential whole-blood samples before and after AP treatment failure were isolated from a patient diagnosed with P. falciparum malaria upon their return from Uganda and Sudan after partial compliance with AP as a chemoprophylactic agent. Targeted ultra-deep sequencing was performed on the cytb, dhfr, and dhps markers of treatment resistance before and during the episode of recrudescence. Haplotyping profiles were generated using three different approaches: msnp2-3D7 agarose and capillary electrophoresis, and ccpp using ampiclon deep sequencing (ADS). A complexity of infection (COI) analysis and haplotyping profiles were included. De-novo cytb Y268C mutants were observed during an episode of recrudescence 17 days and 16 hours after the initial malaria diagnosis and AP treatment, indicating treatment failure. No Y268C mutant reads were observed in any of the samples prior to the recrudescence, suggesting no preselected mutants within the limits of detection of electrophoresis and ADS haplotyping. SNPs in the dhfr and dhps genes were observed upon initial presentation. The haplotyping profiles suggest multiple clones mutating under AP selection pressure (COI=0) with a soft selective sweep. Significant differences in COI were observed by capillary electrophoresis and ADS compared to the agarose gel results. ADS using ccpp revealed the lowest haplotype variation across the longitudinal analysis compared to the msnp2 length-polymorphic marker. Our findings highlight the power of ultra-deep sequencing to provide a higher resolution to reveal the natural selection of a mutation as well as haplotyping profiles to understand P. falciparum infection dynamics.

5452

A CANDIDATE GENE ANALYSIS OF SEVERE MALARIA VARIANTS IN A COHORT OF MALIAN CHILDREN IDENTIFIES A NOVEL SUSCEPTIBILITY LOCUS IN CSMD1 GENE

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Plasmodium falciparum malaria is still a leading cause of child mortality in sub-Saharan Africa. Malaria infection can be asymptomatic or symptomatic, with sequelae ranging from no clinical signs to severe disease. Variations in malaria clinical outcomes are partly attributed to host genetic factors with estimated narrow-sense heritability of 23%. Here, we investigated associations of human candidate gene polymorphisms and risk of severe malaria (SM) in a cohort of Malian children. Based on our previous Genome-wide Association (GWAS) analysis, specific candidate genes (N=11) were selected for in-depth analysis. Selection criteria included gene-level GWAS scores, functional overlap with malaria pathogenesis, and evidence of association with protection or susceptibility to other infectious and inflammatory diseases. Twenty-two representative single nucleotide polymorphisms (SNPs) residing within these genes were selected primarily based on p-values in our previous GWAS studies as well as allele frequency in West African populations. The selected SNPs were genotyped using KASP technology in 477 DNA samples (87 children that had experienced SM and 390 that did not). Logistic regression analysis revealed that a common intron variant, rs13340578 in Cub and Sushi Multi Domain (CSMD1) gene, is associated with increased susceptibility to severe malaria in a recessive mode of inheritance (MAF = 0.42, OR=1.8, 95% CI = [1.78, 1.84], P= 0.029). The SNP is in linkage disequilibrium (LD) (r2=0.75) with a regulatory variant, rs59961277, located 888bp up-stream in the complement control (Sushi) domain of CSMD1. This finding suggests that modified regulation of complement may contribute to malaria disease severity. Further studies are needed to identify causal variants in this locus and the underpinning molecular mechanisms mediating SM.

5453

PROTECTIVE HUMORAL RESPONSE TO PLASMODIUM FALCIPARUM Pf27 AND ITS ORTHOLOG P. VIVAX PV27 ANTIGENS IN SERA FROM DANGASSA AND KOILA, TWO MALARIA ENDEMIC AREAS IN MALI

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We identified new Plasmodium falciparum (Pf) and P. vivax (Pv) proteins using Bioinformatic tools. Preliminary results showed that individuals sera from Malian donors reacted well with the couple Pf27/Pv27 antigens (42.7% vs 29.2%) about antigens tested, suggesting existence of crossreactivity between Pf and its ortholog Pv proteins and their potential as malaria vaccine candidates. This study aimed to assess the protective immunity (antibody responses) associated with Pf27/Pv27 proteins in sera from a cohort study in Mali. Samples and data on malaria episodes were collected during cross-sectional survey in December 2021 amongst 209 participants from Dangassa and Koila, two malaria endemic areas located approximately at 82 km south-west and 385 km northeast of Bamako, the capital city of Mali, respectively. Antigenic peptides covering orthologs Pf and Pv proteins were tested on sera using ELISA assay. Overall prevalence of antibodies anti-Pf27 vs Pf27 was 55.9% vs 64.7% in Dangassa and 54.2% vs 63.6% in Koila. In Dangassa, Pf27 seroprevalence (67.5% vs 42.4%, p = 0.02) and antibody levels (p = 0.016) were significantly higher in Pf infected children than in Pf uninfected children during malaria transmission season, while they were similar in adults. No significant variation in antibody levels against both proteins was observed in Koila. High level of antibodies against Pf27 in children and its association with protection against clinical malaria in Dangassa may warrant further investigation of Pf27 as a potential malaria vaccine candidate.
BROADLY REACTIVE ANTIBODIES TARGET SEVERE MALARIAL ANTIGEN TO NEUTRALISE PARASITE SEQUESTRATION

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Severe childhood malaria caused by Plasmodium falciparum is associated with the accumulation of parasite-infected erythrocytes in blood vessels and tissues. Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) family expressed on the surface of infected erythrocyte binds to the endothelial protein C receptor (EPCR), is associated with development of severe symptoms. Naturally acquired antibodies which block this interaction are found in people from malaria endemic regions and are expected to confer protection to severe malaria. Individuals developing protein against severe malaria harbour broadly reactive antibodies against EPCR binding PfEMP1s. In this study, through the characterisation of PfEMP1 specific B cell receptor and serum repertoire, we have isolated antibodies capable of neutralising PfEMP1 - EPCR interaction. The structural and sequence analyses have revealed unique epitopes of these antibodies capable of recognising high sequence diverse PfEMP1s and neutralise EPCR interaction.

THE CHEMOKINE RECEPTOR CXCR3 PLAYS A CRITICAL ROLE IN T CELL-MEDIATED PROTECTION FROM LIVER-STAGE PLASMODIUM INFECTION

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Plasmodium infection involves an asymptomatic liver stage that occurs in hepatocytes and a symptomatic blood stage that occurs in red blood cells. Immunization with whole sporozoite vaccines including genetically attenuated parasites (GAPs) that arrest in the liver can completely prevent the symptomatic blood stage. However, improving these GAPs requires a better understanding of anti-Plasmodium hepatic immunity. Activated T cells increase surface expression of CXCR3 and CXCR3+ liver-resident memory CD8 T cells (TRMs) are critical mediators of protection against infection following GAP vaccination. Hepatocytes also upregulate the CXCR3-binding chemokines CXCL9 and CXCL10 during liver-stage Plasmodium infection, indicating a role for CXCR3 in T cell-mediated protection. We find that GAP-immunized CXCR3/-/- mice exhibit impaired ability to control liver-stage infection at 40 days post-immunization. While flow cytometry analysis shows impaired recruitment of activated CD8 T cells into the livers of CXCR3/-/- mice early after GAP immunization, by day 40 GAP-immunized CXCR3/-/- and wildtype mice exhibit similar numbers of TRMs. This indicates that while CXCR3 is critical early after immunization, other factors may recruit T cells to the liver at later timepoints. However, given that CXCR3/-/- mice have impaired protection from challenge at 40 days post-immunization, CXCR3 must play other roles in TRM reactivation, recruitment, and/or function. Hepatic immunity is unique in that hepatocytes and resident immune cells are asymmetrically positioned across the tissue. Recent studies indicate that parasite development in the liver is not uniformly distributed. Thus, we hypothesize that CXCR3 modulates TRM positioning in the liver such that these cells are optimally placed to respond to future infections. Ongoing spatiotemporal studies will characterize parasite development, chemokine production, and TRM positioning to determine how chemokines impact GAP-induced immunity.

These findings about the role of CXCR3 in T cell-mediated protection from liver-stage Plasmodium could assist in the development of improved GAP vaccines.

IMMUNOLOGICAL PROFILING OF MALARIA PHENOTYPES IN ENDEMIC AREAS OF KENYA: A LONGITUDINAL COHORT STUDY

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Malaria is a life-threatening disease caused by Plasmodium parasites and transmitted via infected Anopheles mosquitoes. In 2021, an estimated 247 million cases and 619,000 deaths were registered worldwide, with Sub-Saharan Africa bearing the major burden. Clinical manifestations of malaria can vary and depend on a dynamic interplay between host, parasite, and environmental factors. Distinct “malaria phenotypes” can be categorized as asymptomatic, uncomplicated, or severe. The course of the infection is shaped by the host immune response. Within the adaptive immune system, Plasmodium-specific T and B cells play a crucial role in determining the progression from asymptomatic to severe malaria and the development of immunological memory. However, the dynamics and interactions between distinct immune populations in response to malaria remain poorly understood. To define the immunoprofile driving individual reactions, a heterogeneous population with a full spectrum of clinical phenotypes needs to be interrogated. To this purpose, we designed a longitudinal cohort study involving 300 individuals aged 5 to 65 years old from a highly malaria-endemic area of western Kenya. Our baseline surveys indicated a prevalence by RDT of 40.7% and the presence of both symptomatic and asymptomatic cases. 46.3% of these showed repeated infection at the following monthly visit. By analyzing field-transmission samples throughout a broad range of age groups, we aim to profile antimalarial immunity and stratify the cohort into distinct infection groups. Moreover, while cross-sectional studies do not allow to distinguish between asymptomatic or pre-symptomatic states, in longitudinal cohorts disease progression is tracked overtime, allowing asymptomatic cases to be defined more clearly and individuals categorized into a gradient of clinical phenotypes. By analyzing peripheral blood mononuclear cells (PBMCs) from the cohort via high-throughput mass cytometry (CyTOF), we will gain insights into distinct immunological signatures of the host antimalarial response, eventually leading to new vaccination and immunotherapeutic strategies.

OPSONICPHAGOCYTOSIS IgG5 TO ICAM1BINDING PLASMODIUM FALCIPARUM ERYTHROCYTE MEMBRANE PROTEIN 1 ARE ASSOCIATED WITH THE CLINICAL PRESENTATION OF MALARIA IN BENINESE CHILDREN

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Severe childhood malaria caused by Plasmodium falciparum is associated with the accumulation of parasite-infected erythrocytes in blood vessels and tissues. Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) family expressed on the surface of infected erythrocyte binds to the endothelial protein C receptor (EPCR), is associated with development of severe symptoms. Naturally acquired antibodies which block this interaction are found in people from malaria endemic regions and are expected to confer protection to severe malaria. Individuals developing protein against severe malaria harbour broadly reactive antibodies against EPCR binding PfEMP1s. In this study, through the characterisation of PfEMP1 specific B cell receptor and serum repertoire, we have isolated antibodies capable of neutralising PfEMP1 - EPCR interaction. The structural and sequence analyses have revealed unique epitopes of these antibodies capable of recognising high sequence diverse PfEMP1s and neutralise EPCR interaction.

The pathogenicity of severe malaria (SM) caused by Plasmodium falciparum is associated with the adhesion of P. falciparum-infected erythrocytes (EIs) to the microvasculature of infected individuals. This adhesion is mediated by the adhesion of P. falciparum-infected erythrocytes (EIs) to the microvasculature of infected individuals. This adhesion is mediated...
by PIEMP-1, which binds one of the most common receptors of the human host, intracellular adhesion molecule-1 (ICAM-1). Knowledge on the quantity and quality of IgG to such PIEMP-1 is limited. The acquisition of IgG specific for IEs, selected for expressing ICAM-1 binding PIEMP-1 and the IgG-mediated opsonic-phagocytosis of such IEs are unknown and the objective of this study was to provide such data. Plasma samples collected from Beninese children under the age of five years with either SM or uncomplicated malaria (UM) were used to measure antibody levels to two recombinant PIEMP-1 domains and their corresponding native proteins expressed on the surface of IEs. ELISA was used to measure PIEMP-1-specific antibodies to the domains, flow cytometric based assay was employed for IE surface reactivity and antibody-mediated opsonic phagocytosis to measure the functional properties of antibodies with respect to the selected PIEMP-1 expressing IEs. Levels of PIEMP-1-specific IgG to the domains and their corresponding IEs were similar in the two groups, SM and UM, suggesting similar exposure to P. falciparum parasites expressing those PIEMP-1 variants. Also, no differences in the reactivity to native IT4VAR13 were observed between all plasma at hospitalization and at convalescence. However, in qualitative assessment, the ability of antibodies to promote opsonic phagocytosis of IEs by monocytes (THP-1 cells) was significantly higher in children with UM at hospitalization on PFD1235w expressing parasites. The findings indicate similar P. falciparum malaria exposure in children with either SM or UM and supports the presence of antibody dependent phagocytosis as a measure of antibody effector function and its association with protection against SM in children.

**COMPOSITION OF PRE-TRANSMISSION SEASON STOOL MICROBIOTA IS ASSOCIATED WITH RESISTANCE TO MALARIA IN OLDER MALIAN CHILDREN**

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Gut microbiota composition is known to alter the susceptibility of mice to Plasmodium infection; however, the impact of gut microbiota on the severity of malaria in humans is less well defined. We collected stool samples from a cohort of 180 Malian children aged six to ten years at multiple time points before, during, and after a Plasmodium transmission season, analyzed them using 16S rRNA gene sequencing, and examined the relationship between fecal microbiota composition and the prospective susceptibility of the children to symptomatic Plasmodium infection. We found that microbiota composition of the pre-transmission season sample from ten-year-old children was significantly associated with whether the children were resistant or susceptible to the development of malaria during the study period. No relationship was observed in younger children, who overwhelmingly developed febrile symptoms when infected, or in samples taken during or shortly after the transmission season, possibly due to obfuscation by additional season-dependent factors. In order to examine the potential causal role of gut microbiota, germ-free mice were colonized with the fecal samples from either resistant or susceptible children and then infected with Plasmodium. Mice that received fecal samples from resistant children displayed significantly better control of their parasitemia compared to mice that received fecal samples from susceptible children, demonstrating that gut microbiota play a causal role in malaria severity in Malian children. The specific mechanisms and bacteria associated with susceptibility to malaria are currently being investigated.

**EVALUATING THE IMPACT OF NATURAL KILLER CELL PHENOTYPE, MALARIA DIVERSITY AND TRANSMISSION, AND ERYTHROCYTE POLYMORPHISMS ON ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY**

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In recent years, the ability of natural killer (NK) cells to mediate antimarial immunity through antibody-dependent cellular cytotoxicity (ADCC) has become increasingly appreciated. Our group recently reported that an atypical population of NK cells prevalent in malaria-exposed children has enhanced ADCC activity against opsonized infected erythrocytes superior to that of conventional NK cells. However, ADCC was only evaluated in wild-type erythrocytes infected with laboratory-adapted P. falciparum, despite the high prevalence of hemoglobin polymorphisms in endemic areas and antigenic diversity of field isolates. Furthermore, extensive cellular phenotyping to find features associated with enhanced cytotoxicity and malaria exposure have not been conducted. This work aims to characterize the impact of parasite diversity, erythrocyte polymorphism, NK cell phenotypes, and malaria exposure on the ability to mediate ADCC against infected erythrocytes. We have already found that HbAS erythrocytes and plasma from high-transmission areas enhance ADCC in comparison to HbAA erythrocytes and low-transmission plasma. Experiments in progress are characterizing NK cell phenotypic traits associated with ADCC capacity in malaria-exposed Ugandans in comparison to malaria-naïve North Americans. Peripheral blood mononuclear cell (PBMC) samples from the PRISM (Program for Resistance, Immunology, Surveillance, and Modelling of Malaria) observational study cohort in Nagongera, Uganda and malaria-naïve North Americans are stimulated with infected erythrocytes or pro-inflammatory cytokines analyzed for phenotypic markers and activation using multiparameter flow cytometry. Killing of infected erythrocytes by NK cells is assessed using a colorimetric assay that quantifies released hemoglobin in cell supernatant. These results will help improve the translatability of future research by creating experimental conditions that better reflect malaria-endemic settings.

**BIOCHEMICAL AND BIOINFORMATIC CHARACTERIZATION OF SURFACE EXPRESSED HYPERVARIABLE PROTEIN FAMILIES (RIFIN AND STEVOR) ASSOCIATED WITH PATHOGENESIS AND ACQUIRED IMMUNITY TO PLASMODIUM FALCIPARUM INFECTION**

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Malaria, caused by the infectious parasite Plasmodium falciparum (PF), is a vector borne disease, responsible for approximately 619 thousand deaths and 247 million cases worldwide in 2021, with 94% cases in sub-Saharan Africa. Pathogenesis of malaria infection is dependent on parasite and host factors, and geographic and social factors, causing different clinical outcome and disease severity. Parasite virulence is partly caused by evasion of the human host immune system during the blood stage of infection. Sequestration and cytoadherence are characteristic PF virulence factors, enabled by parasite-derived proteins expressed on the surface of infected erythrocytes (IEs). These proteins are antigenic and are associated with acquired immunity to Pf. IE surface-expressed antigens are associated with antigenic variability, called Variant Surface Antigens (VSA). VSA are acquired immunity to Pf. IE surface-expressed antigens are associated with antigenic variability, called Variant Surface Antigens (VSA).
translocated from blood stage Pf to the IE surface via protein trafficking through Maurer’s cleft, a parasite-derived membranous structure, and are expressed on the IE membrane. RIFIN and STEVOR are VSA protein families encoded respectively by 180 rif and 40 stevor gene copies per parasite. Members of each family differ mostly in their hypervariable region, exposed to the circulation and possessing antigenic epitopes. Both variable domains are associated with Pf exposure and potentially clinical outcome. Seroreactivity and recognition to both protein families are age and exposure dependent, with higher reactivity in adults and higher domain recognition in individuals with clinical malaria than in subpatent infections. This study is the first to successfully express isolated domains of RIFIN and STEVOR proteins as recombinants, characterize their antigenicity, expand understanding of the Pf proteome, by investigating novel markers for exposure to Pf and develop a library of variants to explore the breadth of antibody responses in participants following mass drug administrations (MDA) in clinical trials (MATAMAL NCT04844905; MASSIV NCT03576313) to explore the impact of the trial interventions on naturally acquired immunity to Pf.

5461
CRYO-EM REVEALS THE STRUCTURAL BASIS OF EPITOPE SELECTIVITY AND PROTECTION FROM MALARIA INFECTION IN A FAMILY OF POTENT ANTI-PFCSP ANTIBODIES

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The generation of high-quality antibody responses to PfcSP, the primary surface antigen of Plasmodium falciparum sporozoites, is paramount to the development of an effective malaria vaccine. Here we present an in-depth structural and functional analysis of a panel of potent antibodies targeting the PfcSP major repeat region (NPNA) and the highly potent mAb L9, which targets the PfcSP minor repeats (NPNN). Each of these mAbs is encoded by the IGHV3-33 heavy chain germline gene, which belongs to one of the most prevalent and potent antibody families induced in the anti-CSP immune response in humans. Cryo-EM reveals a remarkable spectrum of higher-order Fab-CSP structures stabilized by homotypic interactions, many of which correlate with somatic hypermutation. Notably, the homotypic interface in the L9-CSP complex is distinct from the panel of NPNA-specific mAbs; this is the first observation of homotypic interactions in a PICSP epitope outside of the NPNA region. In vitro and in vivo data demonstrate the key role of these mutated homotypic contacts for high PICSP avidity and in protection from P. falciparum malaria infection. Importantly, correlation analyses of mAb binding kinetics with in vivo protective efficacy indicates high PICSP affinity is insufficient to confer potency, highlighting a potential structural determinant of protection. Overall, these data emphasize the importance of anti-homotypic affinity maturation in the frequent selection of IGHV3-33 antibodies and inform next-generation PICSP vaccine design.

5462
ANTIBODY PROFILES AGAINST NON-MALARIA PATHOGENS DISPLAYED IN PLASMODIUM VIVAX-INFECTED INDIVIDUALS FROM THE PERUVIAN AMAZON

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In this study, we screened IgG antibody responses to serological markers (seromarkers) from malaria and other non-malaria pathogens endemic in tropical areas, in Plasmodium vivax (Pv)-infected individuals from the Peruvian Amazon. Between 2018 - 2021, a nested case-control study was conducted in the Peruvian Amazon, for a comparison of immune responses between Asymptomatic (Asym, n=28) and Symptomatic (Sym, n=30) Pv-infected individuals vs control endemic individuals (n=30). Asym individuals had no common malaria symptoms but remained infected with Pv during a 3-week follow-up (p<0.0001). Control endemic individuals had no history related to malaria during the last three years and no history of comorbidities or chronic infections. Serum samples from all individuals were processed to measure exposure (IgG antibodies responses) against 24 antigens from a validated panel of seromarkers for neglected tropical diseases, using Multiplex Bead Assay (Luminex). Results: Pv-infected individuals showed significantly high IgG antibody responses against PvMSP119 protein and seromarkers for Chikungunya and Filariasis (p < 0.0001); Strongyloides, Taeniasis, and Neurocysticercosis (p < 0.01); and Giardiasis and Trachoma diseases (p < 0.05) compared to endemic controls. Interestingly, IgG antibody levels against seromarkers for DENGUE serotype 1, 2, 3, and 4 were lower than endemic controls (p < 0.0001). No significant difference was found for IgG responses between Asym and Sym Pv-infected individuals. Furthermore, using a classical cut-off, we were able to determine a higher proportion of Pv-infected individuals exposed to Chikungunya (98% vs 67%), Filariasis (76% vs 37%), Strongyloides (69% vs 30%), Neurocysticercosis (66% vs 23%), and Taeniasis (47% vs 17%) in comparison to endemic controls respectively. In conclusion, this study demonstrates that Pv-infected individuals in the Peruvian Amazon are more exposed to other tropical infectious diseases than endemic control individuals.

5463
PLACENTAL MALARIA MODULATES NEONATAL DENDRITIC CELLS’ PHENOTYPE AND FUNCTION: A CROSS SECTIONAL STUDY IN BENIN

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Malaria in Pregnancy (MiP) is characterized by the accumulation of Plasmodium falciparum (Pf)-infected red blood cells in the placenta and leads to adverse outcomes in infant. They have a shorter delay to first malaria infection and a higher number of malaria and non-malaria infections in infancy. This indicates that not only infant’s adaptive but also innate immunity is modulated by maternal infection. Several studies demonstrated that immune responses were modulated at birth depending on the timing of infection during pregnancy (recent-active, chronic-active or past MiP). There are strong in vitro and ex vivo evidences that Pf is also able to modulate Dendritic Cells (DCs). In that sense, our team showed that neonatal DCs are partially activated in case of MiP. The mechanisms responsible for this modulation and the capability of those DCs to induce (or not) a strong T cell response have not been established. In our cross sectional study, we have purified DCs from cord bloods of 50 naturally-exposed individuals and phenotyped the DC subtypes (cDC1, cDC2 and pDC). We confirmed the partial activation of neonatal DCs with a lower expression of CD80 in

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**Assessment of Host Clinical Parameters and Parasite Determinants Responsible for Disease Severity**

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Plasmodium falciparum is known to cause severe malaria but since the last decade, *P. vivax* is also seen to be causing severe malaria in clinical isolates. In the present study clinical parameters, cytokine profile (SOD-1, TNF-α, IL-10, IL-6, and IFN-γ), integrin gene, and parasite molecular marker (vir genes, drug resistance genes, and msp3 genes) were analyzed in *P. vivax* clinical infections. A total of 169 *P. vivax* samples were collected and categorized into severe vivax malaria (n=106) and non-severe vivax malaria (n=63) according to WHO severity criteria. Samples were diagnosed with microscopy, RMAAT, and 18S PCR. We measured host biomarker levels of interferon (IFN-γ), superoxide dismutase (SOD-1), interleukins viz. (IL-6, IL-10), and tumor necrosis factor (TNF-α) in patient plasma samples by ELISA for pro- and anti-inflammatory cytokines in severe malaria. Severity was assessed and correlated with the integrin, drug resistance, Pfmsp3, and vir gene using PCR, RT-PCR and sequencing analysis. In our study, thrombocytopenia and anemia were major symptoms in severe *P. vivax* patients. The levels of cytokines (IL-10, IL-6, and TNF-α) and the antioxidant enzyme SOD-1 were significantly higher in severe vivax patients. Molecular genotyping of Pfmsp3α genes has four major genotypes out of which Type B (1.5kb) was predominant in severe and Type C (2kb) was predominant in non-severe samples. For drug resistance genes severe isolates had more mutation than non-severe isolates. Vir-14 gene indicates a significantly higher expression level in the severe samples and Vir-10 related gene showed a higher level of expression in non-severe vivax samples. In conclusion, higher levels of inflammatory cytokine can serve as baseline data for evaluating severe *P. vivax* infections which will be helpful in developing an effective diagnostic biomarker for understanding the immunopathology of *P. vivax* malaria.

**The Direct Binding of Plasmodium Vivax AMA1 to Erythrocytes Defines a RON2-Independent Invasion Pathway**

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Plasmodium vivax is a major human malaria species and is the most geographically widespread disease accounting for approximately 2.5 billion people living under threat of infection. Nonetheless, the study of *P. vivax* has been greatly impeded due to the absence of an in vitro cultivation system, with only two host proteins, Duffy blood group antigen and Transferrin receptor 1, identified as binding partners for *P. vivax* Duffy Binding Protein (PvDBP) and Reticulocyte Binding Protein 2b (PvRBP2b), respectively. The increase, thus, in Duffy-negative infections and lack of *P. vivax*-specific vaccine candidates make the discovery of more ligand/receptor combinations an important task. In this study, we used a transgenic parasite in which Plasmodium falciparum parasites were genetically modified to express *P. vivax* apical membrane antigen 1 (PvAMA1) protein in place of *P. falciparum* apical membrane antigen 1 (PfAMA1) protein. *P. vivax* transgenic parasites expressing PfAMA1 indicating that PfRON2 had no role in the invasion of PfAMA1 transgenic parasites. Interestingly, PvRON2p blocked the invasion of PvAMA1 transgenic parasites in a dose-dependent manner. We found that recombinant PvAMA1 domains 1 and 2 (PfAMA1) bound to reticulocytes and normocytes indicating that PvAMA1 directly interacts with erythrocytes during the invasion, and invasion blocking of PfRON2p may result from it interfering with PvAMA1 binding to erythrocytes. It was previously shown that the peptide containing Loop1a of PvAMA1 (PvAMA1 Loop1a) is also bound to reticulocytes. We found that the Loop1a peptide blocked the binding of PfAMA1 to erythrocytes. PfAMA1 Loop1a has no polymorphisms in contrast to other PfAMA1 loops and may be an attractive vaccine target. We thus present the evidence that PvAMA1 binds to erythrocytes in addition to interacting with PfRON2 suggesting that the *P. vivax* merozoites may exploit complex pathways during the invasion process.
ELEVATED FERRITIN, SEVERE MALARIA, AND ACUTE KIDNEY INJURY

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Ferritin is a multimeric iron-storage protein expressed by different tissues to either efficiently bind and sequester iron (spleen and liver) or respond to free iron associated oxidative stress (kidney). Ferritin levels are increased in severe malaria but the clinical implication and importance of this pathway in severe malaria pathogenesis is poorly understood. Children <5 years of age with severe malaria (n=594) and community children (n=120) were enrolled at two sites in Uganda and followed for one year. Ferritin was measured at enrolment in all children and one month follow-up in severe malaria survivors. A ferritin threshold of ≥ 5000ng/mL was considered elevated. At enrolment, 22.4% of children with severe malaria had elevated ferritin levels and clinical risk for elevated ferritin included acute kidney injury and signs of hemolysis (severe anemia, jaundice, blackwater fever). Elevated ferritin was associated with increased mortality with 11.3% of children with elevated ferritin dying compared to 6.1% of children with normal ferritin levels (p=0.04). However, ferritin was no longer related to mortality when adjusted for acute kidney injury. At one month follow-up, none of the severe malaria survivors had elevated ferritin but the median ferritin levels remained higher compared to community children (p<0.0001). The presence of elevated ferritin on admission was associated with increased post-discharge mortality OR 3.32 (95% CI 1.89 to 5.81), p<0.0001 independent of acute kidney injury or persistent kidney disease. We further explored the relationship between elevated ferritin and pathways of host response on admission. Elevated ferritin was strongly associated with increases in markers of hemolysis and depleted heme-hemoglobin scavengers (p<0.001 for all) but was not associated with the acute phase response. These findings suggest that ferritin increases in the context of acute kidney injury in response to free iron from hemolysis. Further, ferritin may be a useful biomarker to identify children with severe malaria who have higher risk of post discharge mortality.

IMPACT OF SEASONAL MALARIA CHEMOPREVENTION ON MALARIA PREVALENCE AND IMMUNITY AMONG CHILDREN IN NORTHERN BENIN

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Malaria is still a major cause of morbidity and mortality despite all efforts to control the disease. The burden of death is still particularly marked in young children in sub-Saharan Africa. A new preventive strategy was recently developed to strengthen the fight against pediatric malaria in areas of seasonal transmission called Seasonal Malaria Chemoprevention (SMC). The Ministry of Health in Benin, as in other countries with this epidemiological facies, initiated the implementation of this strategy in 2019. However, and as often, the countries have not provided the means to assess the impact of this program. This study aims to evaluate the effects of SMC with Sulfadoxine-Pyrimethamine (SP) and Amodiaquine (AQ) on malaria incidence and consequence as well as on the acquisition and maintenance of anti-malarial immunity in treated children. We conducted a cross-sectional study on children aged 6 to 59 months living in two villages in northern Benin, subjected or not to SMC. Sociodemographic and clinical data as well as repeated blood samples were collected from 440 children (before, during and after SMC treatment). Samples were analyzed for malaria infections by RDT, microscopy and PCR. Anti-malaria immunity was assessed to investigate the repertoire of antibody responses to critical antigens using a panel of 28 antigens targeting PIERP1, GLURP and MSP1 by Luminex assay. Despite the implementation of the SMC, the prevalence of malaria remained similar in treated and untreated villages. During the follow up, malaria prevalence by PCR rather increased during SMC in both villages and decreased, after the SMC in the treated village. No significant impact of SMC was observed on the dynamics and level of antibodies against PIERP1 and other malaria antigens suggesting that in an area of high seasonal malaria transmission, SMC implementation do not impact acquisition and maintenance of anti-malaria immunity.

NEUREGULIN 1 DECREASES HEME-INDUCED INFLAMMATION IN INDUCED PLURIPOTENT STEM CELLS- DERIVED ENDOThelial CELLS FROM CHILDREN WITH INTRAVASCULAR HEMOLYSIS

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Hemolysis associated with malaria and sickle cell disease SCD leads to activation of vascular endothelial cells and this modulates migration and adhesion of inflammatory cells. Both diseases result in systemic elevation of free heme leading to oxidative stress and subsequent inflammation and damage to vascular endothelial cells. Previous studies have used cobalt protoporphyrin IX, PPiX to induce heme scavengers to decrease proinflammatory cytokine production in hemolysis associated diseases. However, development of appropriate models to test interventions against heme induced damage to host cells have been limited. Recent studies have revealed that a neuroprotective factor Neuregulin1, NRG1 mediates human brain vascular endothelial cell protection. We differentiated patient derived induced pluripotent stem cells iPSCs into endothelial cells ECs and used them as a model to assess vascular endothelial integrity during exposure to free heme. We hypothesized that NRG1 can modulate heme induced vascular inflammation and endothelial cell dysfunction. Blood and urine samples were used to generate iPSCs from children with SCD and malaria presenting at Korle Bu Teaching Hospital in Accra Ghana. iPSCs derived endothelial cells iPSC-ECs were assessed CD 31 and CD144 as expected. Biomarkers associated with vascular injury Ang1&Ang2, proinflammatory chemokine and cytokines CXCL10 TNFα IL 1 IL 6 IL 10 and brain derived neurotropic factor BDNF, were assessed in plasma and compared. iPSC-ECs controls were treated with heme, PPiX and NRG1. Patient derived iPSCs from HbSS and HbAS children differentiated to ECs displayed decreased growth and cellular dysfunction under heme treatment. Moreover proinflammatory factors CXCL10 Ang2/Ang1 and BDNF increased in plasma of SCD children infected with Plasmodium falciparum. In addition, PPiX and NRG1 increased survival and proliferation of iPSC-ECs and reduced endothelial dysfunction. In conclusion our patient derived EC could be used as a model for assessing individualized vascular injury and inflammation during SCD or malaria pathogenesis and determined that NRG1 can be used to increase EC survival

IDENTIFICATION OF BIOMARKERS ASSOCIATED WITH MALARIA IN PREGNANCY AND CLINICAL CORRELATION WITH OUTCOMES

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In most tropical areas, pregnant women are at increased risk of malaria, as a consequence of the massive sequestration of parasitized red blood cells.
in the placenta. Malaria in pregnancy (MiP) may alter placental functions leading to anemia, prematurity, and low birth weight. Although there are several tools to diagnose malaria infection during pregnancy, there is currently no test to assess placental dysfunction. Various biomarkers associated with placental dysfunction that are of high specificity should be studied in the context of MiP to evaluate their predictive value. Plasma and dried blood spots from a recent diagnostic clinical trial "LAMPREG" which enrolled 2500 women in Ethiopia will be used for samples and clinical data. To evaluate if there is a differential host response between symptomatic and asymptomatic women, we will measure the plasma level of the following inflammatory markers: CRP, WVF, hepcidin. Also, the gene expression of multiplex cytokines panels will be studied using droplet digital PCR in order to assess the host inflammatory response. Additionally, a metabolomic approach will be implemented to map the host response during MiP. Metabolomics will be performed using targeted and untargeted approaches, across several liquid chromatography mass spectrometry platforms. We will conduct analysis for both small molecules and lipids, to obtain a full metabolite coverage. We will use the manufacturer's built-in databases for putting the identification of metabolites at the tandem mass spectrometry level.

5471

HISTOPATHOLOGICAL CHARACTERISTICS OF DISCRETE BRAIN REGIONS DURING PLASMODIUM FRAGILE EXPERIMENTAL CEREBRAL MALARIA IN A NONHUMAN PRIMATE MODEL

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Cerebral malaria (CM) is a diffuse encephalopathy associated with the detection of Plasmodium spp. in blood. The prognosis of CM is poor, with 15-20% of cases being fatal and resolved cases often resulting in persistent neurological deficits. P. falciparum has been previously used to model P. falciparum infection due to its ability to replicate severe malaria in rhesus macaques (RMs). Prior work identified the presence of P. falciparum in cerebral vasculature of RMs but did not explore immune involvement or parasite burden within discrete cerebral compartments. We therefore hypothesized that P. falciparum infection of RMs would result in CM pathology and differential sequestration of infected red blood cells (iRBCs) in the vasculature of distinct brain regions. An adult male RM (n=1) was intravenously inoculated with 2x10^6 P. falciparum parasites. Peripheral parasitemia and anemia were quantified by Giemsa staining of thin blood smears and assessment of parasitophorous vacuole (PV), which is delineated by a membrane (PVM). At necropsy, parasites were formalin fixed and paraffin embedded and stained with hematoxylin and eosin. Parasite burden in each brain region was determined by paraffin sections. Parasite burden was greatest in the cerebellum (16%), followed by the parietal lobe (11%), frontal lobe (10%), medulla (10%), temporal lobe (9%), basal ganglia (9%), occipital lobe (8%), and pons (6%). HZ was observed in iRBCs within the capillaries and white blood cells (WBCs), indicative of an ongoing immune response.

Taken together, these data indicate that P. falciparum infection of RMs is a suitable model for examination of CM pathogenesis. Additional studies are underway to identify the role of WBC infiltration in CM pathogenesis.

5472

COMPARISON OF PLASMODIUM FALCIPARUM GROWTH IN VITRO AND IN VIVO IN HUMANISED MICE

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In vitro culture has been used to study Plasmodium falciparum erythrocytic cycle for years, allowing the characterisation of genes and testing of therapies. However, it is known that P. falciparum undergoes genetic changes under continuous culture and there are limitations in the host-parasite interactions that can be explored in vitro. Humanised mice (HuMice) are immunodeficient mice that can be engrafted with human cells and tissues. They are the only small animal model that can be used to study P. falciparum in situ and may provide a unique opportunity to model human malaria infection. We have successfully established P. falciparum infections in HuMice and have used two distinct approaches to determine parasite changes in vitro and in vivo. Here we compare parasite growth and gene expression in vitro, and in vivo in the presence (His-RBC-HuMouse model) or absence (RBC-HuMouse model) of a human immune system. DRAG and NSG humanised mice were engrafted with either 1 human RBCs (iRBCs) alone to maintain infection in vivo (RBC-HuMouse model); or 2) with human immune cells and iRBCs to determine the effect of the human immune system (His-RBC-HuMouse model). We established two different His-RBC-HuMouse models to characterise changes in parasite growth in vivo. First, we used stem cells to reconstitute the human immune system (SC-His-HuMouse model), allowing the development of functional human immune system cells including B and T cells. Secondly, we established a new approach using PBMCs (PBMC-HIS-HuMouse) allowing for the reconstitution of mature immune cells. Using these approaches, we aim to use RNA-Seq to determine the gene expression of P. falciparum in these models and identify genes implicated in parasite growth in vivo. We also aim to evaluate how predictive in vitro assays for the assessment of therapeutics are in these models using monoclonal antibodies. Finally, we discuss the opportunities and limitations of each approach and outline considerations for the selection of models appropriate to research questions.

5473

GENERATION OF A PLASMODIUM BERGHEI LINE EXPRESSING A HALOTAGGED PARASITOPHOROUS-VACUOLE MEMBRANE PROTEIN TO STUDY TARGETED PROTEIN DEGRADATION DURING LIVER STAGE MALARIA

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As malaria continues to be a global public health concern, the need for the development of novel therapeutics becomes more prominent in the face of antimicrobial resistance and difficult-to-treat infections. During the liver stage of malaria, parasites infect hepatocytes and reside within an intracellular parasitophorous vacuole (PV), which is delineated by a membrane (PVM)
SEVERE MALARIA ASSOCIATED ACUTE KIDNEY INJURY
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Malaria is a leading cause of acute kidney injury (AKI) and a risk factor for persistent kidney disease. We leveraged a prospective cohort of children with severe malaria to identify biomarkers on admission and at one-month follow-up associated with persistent and chronic kidney disease (CKD). Between 2014 and 2017, we enrolled 600 children aged 6 months to 4 years admitted to two tertiary hospitals in Uganda. AKI was defined using the Kidney Disease: Improving Global Outcomes (KDIGO) criteria.

Drivers of Long-lasting Insecticide-treated Net Utilization and Parasitaemia Among Under-five Children in 13 States with High Malaria Burden in Nigeria

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Although Nigeria has made some progress in malaria control, there are variations in progress across administrative levels. This study investigated the factors associated with and utilisation of long-lasting insecticide-treated net (LLIN) and parasitaemia among under-five children in 13 States with high malaria burden. Data from the 2015 Nigeria Malaria Indicator Survey and 2018 Demographic and Health Survey were analysed. The 13 study states were stratified into two based on whether they had increased or reduced parasitaemia between 2015 and 2018. Random-effects logit models were fitted to identify independent predictors of LLIN utilisation and parasitaemia. A set at 0.05. LLIN was used by 53.4% of 2844 children, while parasitaemia prevalence was 26.4% in 2018. Grandchildren (AOR=5.35, CI: 1:09-26.19) were more likely to use LLIN while other relatives (AOR=0.32, CI: 0.11-0.94) were less likely compared to direct children of household-heads. Furthermore, LLIN use was more common in children whose mother opined that only weak children could die from malaria (AOR=1.83, CI: 1.10-3.10); more likely among children whose mothers obtained net from ANC or immunisation clinics (AOR=5.30, CI: 2.32-12.14) and campaigns (AOR=1.77, CI: 1.03-3.04). Children aged 24-59 months compared to 0-11 months (AOR=1.78, CI: 1.28-2.48), those in whom fever was reported (AOR=1.31, CI: 1.06-1.63) and children of women with no formal education (AOR=1.89, CI: 1.32 - 2.70) were more likely of parasitaemia. The likelihood of parasitaemia was higher among children from poor households compared to the rich (AOR=2.06, CI: 1.24-3.42). The odds of parasitaemia were 98% higher among rural children (AOR=1.98, CI: 1.37-2.87). In conclusion, the key drivers of LLIN utilisation and parasitaemia were mainly related to socioeconomic factors. These should be targeted as part of integrated malaria elimination efforts.

Uptake of Four or More Doses of Intermittent Preventive Treatment of Malaria During Pregnancy with Sulfadoxine Pyrimethamine (IPTp-SP) in Zambia: A Secondary Analysis of the 2018 Malaria in Pregnancy Survey Data

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In Zambia approximately 25% of pregnant women show evidence of placental infection at delivery. The Zambian government is implementing the malaria in pregnancy (MIP) policy including intermittent preventive treatment of malaria during pregnancy with sulfadoxine pyrimethamine (IPTp-SP). However, the latest (2018) malaria indicator surveys (MIS) showed low uptake of four doses of IPTp-SP at 5%. This study determined the prevalence and predictors of the uptake of four or more doses of IPTp-SP in Zambia. We conducted a secondary analysis of the 2018 MIS dataset comprising 4, 044 women (15-49 years). The survey covered all ten provinces of Zambia. Only 1,381 (34%) women who delivered in 2018 or after the new IPTp-SP policy was introduced were included in our final sample. Descriptive statistical analysis was carried out to summarise participant characteristics and IPTp-SP uptake.
logistic regression was carried out to determine association between the explanatory and outcome variables. Explanatory variables with a p-value less than 0.20 on univariate analysis were included in the multivariable logistic regression model and crude and adjusted odds ratios along with their 95% CIs, p-value <0.05 were computed. Only 7.6% of the participants received IPTp-SP 4+. The province of residence and wealth quintile were significantly associated with uptake of IPTp-SP doses; participants from Luapula (aOR=8.72, 95%CI [1.72–44.26, p=0.009]) and Muchinga (aOR=6.67, 95%CI [1.19–47.47, p=0.031]) provinces were more likely to receive IPTp-SP 4+ compared to those from Lusaka province. Conversely, women in the highest wealth quintile were significantly less likely to receive IPTp-SP 4+ doses compared to those in the lowest quintile (aOR=0.32; 95%CI [0.13–0.79, p=0.014]). In conclusion, these findings confirm low uptake of four or more doses of IPTp-SP in the country. Preventive strategies should target women in urban provinces with low malaria transmission to ensure adherence to the IPTp-SP guidelines. Interventions should include health messages with emphasis on the new policy of four or more doses of IPTp-SP.

5477

PREVALENCE OF MALARIA CLINICAL PHENOTYPES DURING ROUTINE CONSULTATION IN HOSPITAL DISTRICT HEALTH OF COMMUNE 4, MALI

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Seasonal Malaria Chemoprevention (SMC) significantly has reduced malaria incidence in target population since its implementation in Mali in 2012. However, the recent studies have demonstrated a change in the epidemiology of malaria in the context of SMC, constituting an obstacle to its long-term implementation in Mali. This study aims to determine the prevalence of severe malaria during routine consultation at hospital district of commune 4 from July to December 2022. Sociodemographic and clinical phenotype data on malaria were collected on tablets. SPSS software version 22 was used for data analysis and Chi2 test for comparison of qualitative variables with a significance threshold at 5%. A total of 559 patients aged 0 to 15 years were enrolled. Age group 5 to 15 was the most represented (51.7%) and severe malaria according to WHO definition frequency was 40%. Clinical malaria phenotype varied significantly according to age groups (48.9% in under 4 years vs. 39.4% in older children aged 5 to 15 years, p = 0.02). Severe malaria was dominated by convulsion (53.3%), followed by obtundation 48.4%, severe anaemia (41.3%), coma (34.6%), and respiratory distress (21.1%). Our data show a high prevalence of severe malaria and the need to explore association between severe malaria and Plasmodium falciparum genetic diversity in areas under SMC.

5478

ASSOCIATIONS BETWEEN ANOPHELES VECTOR DENSITY AND MALARIA INCIDENCE IN TWO ADJACENT UGANDAN DISTRICTS WITH AND WITH OUT INDOOR RESIDUAL SPRAYING

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Five years of Indoor Residual Spraying (IRS) with Bendiocarb and Actellic from 2014-2019 reduced malaria incidence among children in the Tororo district of Uganda from 2.96 to 0.040 episodes/person/year. A switch to clothianidin-based IRS formulations in 2020 was associated with a resurgence of malaria to pre-IRS levels. This study explores associations between Anopheles vector density and malaria incidence in two adjacent districts with and without IRS. Data was collected from September 2020 through January 2023 in 661 participants from 61 households in Tororo (IRS) and 23 households in Busia (without IRS). Individuals were followed at a study clinic and malaria was defined as fever and a positive thick blood smear. Mosquitoes were collected via CDC light traps set every two weeks in participant sleeping rooms. The exposure of interest was the mean vector density from the previous month. The outcome was whether an individual had at least one case of malaria per month. Mixed effects Poisson regression with a log-link was used to estimate the relative risk of malaria adjusted for age. In the IRS district, >1 An. funestus mosquito captured per collection was associated with a 41.8% increase in risk of malaria the subsequent month (95% CI: 24.6-61.2%; p<0.001), but was not associated with malaria in the non-IRS district. Interestingly, >1 An. gambiae s.l. mosquito was associated with a 21.9% decrease in malaria risk in the IRS district (95% CI: 10.7-31.8%; p<0.001), compared to 41.6% increased risk in the non-IRS district (95% CI: 11.3-80.1%; p=0.005). Future work will classify An. gambiae s.l. mosquitoes to the sub-species level. These findings suggest that ongoing IRS in Uganda may be leading to selection pressures with heterogeneous responses by vector species. These changes might be contributing to the malaria resurgence observed in IRS districts like Tororo which had previously achieved very low transmission.

5479

A GEOSTATISTICAL ANALYSIS OF USE OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY AMONG PREGNANT WOMEN IN NIGERIA

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Malaria infection during pregnancy is a major public health problem in Nigeria, with substantial risks for the mother, her foetus, and the neonate. Intermittent preventive treatment of malaria in pregnancy (IPTp) is a full therapeutic course of antimalarial medicine given to pregnant women at routine antenatal care (ANC) visits to prevent malaria and maternal and foetal anaemia. In line with the World Health Organization’s recommendation, Nigeria, in 2014, adopted the use of at least three doses of Sulfadoxine-pyrimethamine (SP), the recommended medicine for IPTp in the country, during ANC visits. However, data from the 2018 Nigeria Demographic and Health Survey (NDHS) and the 2021 Malaria Indicator Survey (MIS) indicate that only 17% and 31% of pregnant women respectively took the recommended dose. Relying on data from the 2013, 2018 NDHS and 2021 MIS, we used a model-based geostatistical approach within a Bayesian framework to estimate trends in IPTp-SP usage across the subnational levels of Nigeria and to determine specific locations with recorded improvements in 2018 and 2021 when compared with 2013. We further compared the pattern of usage at the subnational levels, among pregnant women who sought ANC at public and private healthcare provider. Our findings highlights Nigeria States with lagging improvements in IPTp-SP usage and the need for locally tailored interventions that will improve usage of the preventive measure across the country.
MALARIA PREVALENCE IN CHILDREN WITH A HISTORY OF EXPOSURE TO SEASONAL MALARIA CHEMOPREVENTION & EXIT FROM THE TARGET: RESULTS OF A CROSS-SECTIONAL STUDY IN SOUTHERN SENEGAL

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Seasonal malaria chemoprevention (SMC) was adopted in Senegal in 2013 and implemented to determine the prevalence of malaria in children within a history of SMC exposure and out of target. This cross-sectional study was conducted between September and December 2016 in the region of Kédougou, Kolda, Tambacounda and Sédhiou in southern Senegal. The study population was children who had exited the SMC target. The inclusion criteria were to be aged 1-14 years, to have taken SMC at least once and to be apparently healthy with a negative RDT for malaria. A questionnaire was administered and a blood sample taken for a mixed smear and blotter paper for each participant. A real-time PCR of the 18S gene were performed for Plasmodium falciparum identification. A total of 226 children, with a mean age of 11.8 (+/- 0.8) years and a sex ratio (MF) of 1.5, were recruited in this study. The proportions of children with a history of taking SMC were 33.6% for 2013, 87.6% for 2014, 39.5% for 2015 and 0.9% for 2016. The number of children having taken at least 3 monthly cycles of SMC was higher in 2013, 2014 and 2015 with 51.3%, 83.3% and 82.7%, respectively. The prevalence of malaria by PCR 19.46%. The proportions of positives were higher in children aged 11 (34.09%) and 12 (47.73%). Children who have been protected by SMC become potential reservoirs again when they leave the target. Extension of this strategy to all the population is urgently needed.

ASSOCIATION BETWEEN BEDNET USE AND MALARIA PREVALENCE BY AGE GROUP IN RARIEDA SUB-COUNTY, WESTERN KENYA, 2015-2020

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Malaria remains a leading cause of illness in western Kenya, particularly among school-aged children (5-14yrs). Identifying whether lack of insecticide-treated bednet (ITN) use by this age group is a factor driving high prevalence is important to guide targeted public health interventions. From April 2015-March 2020, year-round household surveys were conducted in Rarieda sub-county to assess malaria prevalence and ITN use. An average of 1,250 households (HH) were randomly selected to be surveyed each year. A questionnaire was administered to each HH member (or caregiver) to collect data on sleeping structure characteristics, ITN ownership and use, and self-reported fever in the past 2 weeks. Participants were tested with a pLDH/HRP2-based malaria rapid diagnostic test (RDT) irrespective of the presence of fever. Prevalence ratios were obtained by modified Poisson regression. Overall, 21,837 questionnaires and malaria tests were administered in 6,419 enrolled HH. ITN access (≥ 1 ITN/2 people per HH) was 41.2%. Reported ITN use the night before the survey was 81.0%:

Malaria prevalence was 29.7%; 32.0% in <5yrs, 46.1% in 5-14yrs, 15.8% in 15+yrs and 19.0% of cases reported fever in the past 2 weeks (25.1% in <5yrs; 19.8% in 5-14yrs; 15.8% in 15+yrs). Not sleeping under an ITN the night before the survey was associated with higher malaria risk (41.6% vs 27.1%, PR=1.5, 95% CI 1.4 - 1.6, p=0.001). This was significant (p=0.001) in all age groups (<5yrs: PR=1.6 [95% CI: 1.3 - 1.7]; 5-14yrs: PR=1.2 [95% CI: 1.1 - 1.3]; 15+yrs: PR=1.2 [95% CI: 1.1 - 1.3]). Fever in the past 2 weeks was associated with higher RDT positivity versus those without a fever (42.9% vs 27.7%, P=1.6, 95% CI 1.5 - 1.6, p<0.001) in all age groups (<5yrs: PR=1.7 [95% CI: 1.5 - 1.9]; 5-14yrs: PR=1.5 [95% CI: 1.4 - 1.6]; 15+yrs: PR=1.4 [95% CI: 1.3 - 1.6]). ITN access remains well below globally accepted targets in western Kenya. Non-use of ITNs was associated with malaria among all ages. Children 5-14yrs had the highest malaria prevalence and reported the lowest ITN use. Improving ITN access and use could help to reduce malaria prevalence in this age group.

THE PRESS TOUR: AN OUT-OF-THE-BOX APPROACH TO IMPROVE MALARIA MESSAGING IN MADAGASCAR

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Malaria caused 2.4 million cases and >500 deaths in Madagascar in 2021. Behaviors like using bednets and promptly seeking healthcare can reduce morbidity and mortality and the media can be a powerful channel for sensitizing the public, but journalists do not typically receive malaria-related training. We aimed to train journalists to increase the quality and quantity of malaria messaging in Madagascar. In Nov 2021, PMI Madagascar, the US Embassy Public Affairs’ Office, and the National Malaria Program held a 5-day workshop for 18 journalists (7 TV, 4 radio, 5 written, 2 online) followed by 5 days of field visits to communities to learn more about malaria-related activities and concerns. In Feb 2023, we evaluated the results of this training by emailing a questionnaire to each journalist to collect the number of and references for products (articles, broadcasts, and posts on malaria) and the estimated reach per product type and outlet 12 months before and 12 months after the training, and perceptions of changes in their malaria-related knowledge and reporting quality. All 18 journalists answered the quantitative questions, while 13 answered the qualitative questions. The median number of malaria-related products per journalist increased from 1 (IQR 0-2) to 3 (IQR 2-5.8), increasing the estimated reach per journalist from a median of 15,500 (IQR 0-306, 300) to 326,185 (IQR 65,000-1,425,000) people. Among 13 journalists who responded, all reported that their approach changed from copying press releases to investigative reporting and individual storytelling. Other self-reported improvements included writing skills (10/13; 77%), knowledge of public health (11/13; 85%), knowledge of malaria (12/13; 92%), understanding of the US government role in malaria control (12/13; 92%), and importance of a community health approach to malaria control (9/13; 69%). This training was a simple way to help sensitize the public through key malaria messaging, by improving the quantity and likely the quality of malaria reporting via mass media. The results spurred the Ministry of Health to plan a similar press training on immunization and family planning in 2023.
AN EXAMINATION OF NATIONAL SURVEYS AND PROGRAM REVIEWS TO DOCUMENT ACHIEVEMENT OF ANTENATAL CARE AND IPTP TARGETS IN NIGERIA

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In 2012, the World Health Organization updated the Intermittent Preventive Treatment of malaria during pregnancy (IPTp) coverage indicator to a minimum of three doses. In 2014, Nigeria set the national target of 100% of women attending ANC to receive IPTp. This study reviewed national survey data for antenatal care (ANC) attendance and IPTp provision from the 2013 and 2018 Demographic Health Surveys (DHS) and the 2015 and 2021 Malaria Indicator Surveys (MIS). Extracted from the national malaria program reviews (MPR) of the National Malaria Strategic Plans (NMSP) of 2014 and 2019 were explanations of program implementation issues. ANC4 attendance and IPTp uptake (1st and 3rd doses) were compared using descriptive statistics. The 2015 MIS did not document ANC 4th visit, so attendance in the remaining surveys was 51%, 57%, and 52% (X2=160.0, df2, p <0.0001). The slow increase of ANC attendance and drop in 2021 meant that opportunity to acquire three IPTp doses was not possible for most women. Over the four surveys, IPTp1 increased from 23% to 64% to 76%, then dropped to 42% to 44% to 52%, and finally dropped to 44% to 45% to 49%. IPTp3 rose from 6% to 19% to 28%, and then dropped to 16.6% before increasing to 31% (X2= 1755, df3, p<0.0001). The MPR reports identified four factors inhibiting achievement of the ANC and IPTp targets including insecurity (terrorism, civil unrest), poor integration of malaria in pregnancy into reproductive and maternal health programs, inadequate procurement and stock-outs of SP, and logistical hurdles (lack of vehicles and fuel). By not meeting ANC4 and IPTp1 targets, limits were set for IPTp2 uptake. As other researchers have suggested, NMSPs embody global targets and may not reflect local realities. Local governments, who deliver the bulk of ANC and IPTp services, must be part of the process of setting and planning how to achieve targets.

EFFECT OF BEDNETS USE ON UPTAKE OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY; FURTHER ANALYSIS OF THE 2019 GHANA MALARIA INDICATOR SURVEY DATA

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Malaria in pregnancy constitutes a persistent public health threat in malaria-endemic areas of Africa, with substantial adverse maternal and foetal outcomes, due to low uptake of malaria preventive measures. The combined use of multiple interventions against malaria such as bednets and intermittent preventive treatment of malaria in pregnancy with sulphadoxine-pyrimethamine (IPTp-SP) is greatly recommended by WHO, to achieve maximum impact. However, the use of one intervention may influence the uptake of other interventions. This study explored the effect of bednet use on the uptake of IPTp-SP in Ghana. The data source was the 2019 Ghana Malaria Indicator Survey. Stata version 16.0 was used for data analysis. Weighted frequencies and proportions highlighted participant variables. Bednet use and IPTp-SP uptake were considered the main independent and outcome variables. Survey-adjusted bivariate and multivariate logistic regression analyses were done at 95% confidence interval and 5% level of significance. A total of 2308 women who had children within the last 12 months were involved in the analysis. Participant characteristics were such that 34.4%, 32.5%, and 33.1% of respondents were aged 15-24 years, 25-34 years and 35-49 years respectively with a mean age of 29.70 years (SD = 9.65). The national uptake of 1 dose and at least 3 doses of IPTp-SP were 91.7% and 65.0% respectively. While, bednet ownership stood at 80.0%, use of bednets among pregnant women was 52.5%. The combined use of bednet and IPTp-SP was 49.0%. The determinants of uptake of at least 3 doses of IPTp-SP were attending ≥4 ANC visits [aOR = 2.59, 95% CI: 1.67, 4.03; p = 0.006] and exposure to messages on malaria in last 6 months [aOR = 1.34, 95% CI: 1.07, 1.67; p = 0.011]. The use of bednets was strongly associated with twice the odds of uptake of IPTp-SP among pregnant women [aOR = 2.02, 95% CI: (1.23, 3.15); p = 0.003]. The combined use of bednets and uptake of IPTp-SP among Ghanaian women is low. Maternal use of bednet positively enhances their uptake of IPTp-SP. Interventions to improve uptake of multiple doses of IPTp-SP should also emphasize the use of bednets to achieve maximum impact.

POSITIVE EFFECTS OF INDOOR RESIDUAL SPRAYING (IRS) IN MALARIA PREVENTION IN NGOMA DISTRICT

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In last years Rwanda has faced different health problems due to some diseases that were leading to the death and suffering, among them, we found Malaria. After the government of Rwanda identified this rising in prevalence of Malaria developed policies to reduce number of death and people affected by Malaria. The method that was used is spray of medication (IRS indoor residual spraying) in all houses that was done in 2019 and 2020. Ngoma district is in the Eastern province of Rwanda between Krehe and Kayonza districts as well as in other part of the country the spray was done, hence it gave outstanding results in that period. This abstract describes the way malaria spraying medication was effective in prevention of Malaria in Ngoma district. The prevalence of simple malaria, severe malaria and death from malaria before home spraying and after showed there was tremendous reduction. The number shows that in two years before home spraying (2017-2018) simple malaria cases were 4 406 225 cases, severe malaria were 24 946 cases and deaths from malaria were 812 cases while after IRS the number decreases tremendously at the extent simple malaria were 1 350 479 cases, severe malaria were 7 250 cases and number of death were about 255 cases. Percentage of simple malaria cases incidence drop down from 69.35% to 30.65% while the percentage of severe malaria cases reduced from 70.94% to 29.06% and the deaths percentage of malaria cases drop down from 68.60% to 31.40% All these show that IRS should be a continuous measure to be implemented as it gave effective results.

MENTORSHIP-BASED TASK-SHIFTING APPROACH FOR COMMUNITY HEALTH OFFICERS IMPROVES ANTENATAL CARE ATTENDANCE AND INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY SERVICES IN LOWER-LEVEL FACILITIES IN GHANA

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Inadequate numbers of midwives at the sub-district level remain a barrier to providing antenatal care (ANC) services in Ghana. A 2019 Community-based Health Planning Services (CHPS) policy implementation assessment revealed that only 15% of CHPS zones have resident midwives to provide ANC services, leaving out most communities. Community Health Officers (CHOs) who operate CHPS zones are trained mainly to offer essential maternal and child health services. Although CHOs have some knowledge of ANC services, they lack adequate skills to provide ANC, including malaria in pregnancy (MIP) interventions such as intermittent preventive treatment of
malaria during pregnancy (IPTp) in the communities they serve. PMI Impact
Malaria collaborated with the National Malaria Elimination Program to build
on other CHO capacity-strengthening efforts by introducing a mentorship-
based task-shifting training approach to address the challenge. CHOs in
CHFS zones without midwives received a practical-based 3-day training
to gain skills in history taking, documentation, physical examination, ITN
distribution, IPTp services, and case management of MIP. This training
was followed by a period of mentorship by midwives from nearby facilities
who were paired with the CHOs, to serve as mentors to support the CHOs
in developing competence in ANC. The district mentors made facility
supervisory visits 4 and 12 months after the training. A total of 185 CHOs
were trained on IPTp services and case management of MIP with an overall
knowledge gain of 16.5% points (average pre-test score 72.5% and post-
test 89%). CHOs mobilized pregnant women through intensified education
during the home visit and outreach services. They provided basic ANC to
pregnant women with the support of midwives’ mentors, increasing ANC
attendance from 52,117 to 58,044 in 18 months. IPTp1 coverage increased
from 59.9% to 75.2%, and IPTp3 increased from 29.1% to 56.5% within
the same period. This pilot demonstrates that through carefully designed
mentorship-based task-shifting initiatives, CHOs can be supported to
provide ANC and IPTp services at peripheral health facilities.

5487

PAYMENT SYSTEM FOR COMMUNITY ACTORS DURING
THE 2021 AND 2022 INSECTICIDE-TREATED NET (ITN)
MASS DISTRIBUTION CAMPAIGNS IN MADAGASCAR

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To prevent malaria in districts at risk, Madagascar conducted mass
ITN distribution campaigns in 2021 (all 101 higher-burden districts) and
2022 (10 of 13 elimination districts). More than 91,000 community
actors (authenticating agents, community health volunteer mobilizers,
storekeepers, and distribution agents) and local supervisors were mobilized
to implement the distribution activities. During the previous mass campaigns
(2010, 2013 and 2015), community actors’ allowances were sent by
bank to district level and payment was made through the health facility
responsibility party. In 2018, the campaign’s national coordination committee
(NCC) attempted to process payment by mobile money, but it took two
years, from November 2018 to December 2020, for community actors to
get paid because of long delay in setting up the mobile payment system
and far distance between payment place (cash point) and beneficiaries.
During the 2021 and 2022 campaigns, to ensure timely payment of
community actors, the NCC set up a hybrid payment system: i) proximity
payment through civil society organizations, targeting 91,546 community
actors in 2021 and 6,020 in 2022; and ii) payment by mobile money directly
to 6,232 local supervisors in 2021, and 20 in 2022. As a result of this hybrid
payment, 91,510 (99%) of 91,546 community actors (2021) and 5,978
(99%) of 6,020 actors (2022) who were paid via proximity payment and
6,220 (99.8%) of the 6,232 local supervisors (2021) and all 20 supervisors
in 2022 who were paid via mobile money received their allowances within
one month of completing their work. This payment system substantially
shortened the lag between work and payment and allowed direct payment
of beneficiaries without intermediaries. Through this successful experience,
the National Malaria Program and its partners plan to scale up and
standardize this approach for other ITN distribution activities that involve
community actors.

5488

IMPACT OF KNOWLEDGE, ATTITUDES, AND PRACTICES
REGARDING LONG-LASTING IMPREGNATED NETS ON THE
PREVALENCE OF MALARIA INFECTION AMONG CHILDREN
UNDER FIVE YEARS OF AGE IN THE DODJI-BATA DISTRICT
OF SOUTHERN BENIN

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For good effectiveness of LLINs, several factors must be taken into
account, including effective use, integrity, durability, and, in general,
knowledge, attitudes, and practices towards LLINs. The purpose of this
study was to assess household knowledge, attitudes, and practices
regarding LLINs and to determine the impact of these elements on parasite
prevalence among children under 5 years of age. Data were collected
using an administered questionnaire based on the MillDA User. Overall
“knowledge, attitude, practice” scores are calculated and ranked using
Bloom’s threshold. Relationships between the independent variables and
the prevalence of malaria infection are compared using a chi-2 statistical
test. Of 402 children seen in the households, 190 subjects were female and
203 subjects were male. The age range of children from 12 to 59 months
was the most represented. In the households surveyed, 97.96% recognized
mosquito nets as a means of controlling malaria. Of the children selected,
89.80% owned a net and 89.94% of these children had spent the night
before the survey under the net. Nearly 7 out of 10 households used nets
throughout the year; however, the number of people occupying a net was
more than 2 in 90% of the cases, which makes the nets less effective
and negatively influences the malaria infection rate in this community. The
malaria infection rate was 11%. Physical inspection of the fabric integrity
of the LLINs seen in the households revealed a proportion of 25% with
holes, while 40% of the nets seen were from the 2020 campaign and
were of the Olyset Plus type. The overall knowledge, attitude, and practice
regarding LLINs were satisfactory in the study area. However, reinforcement
of communication in the community must be done for a change of behavior
such as the provision of mosquito nets for children under 5 years of age
in the households. Continuation of net distribution strategies must be
effective to ensure adequate coverage of households with nets to avoid an
exaggerated number of people occupying a net. Ongoing monitoring and
evaluation of the use and durability of LLINs should be conducted to limit
misuse to reduce malaria infection rates.

5489

DETERMINANTS OF MISSED OPPORTUNITIES FOR
PERENNIAL MALARIA CHEMOPREVENTION TAKING CUES
FROM INTERMITTENT PREVENTIVE TREATMENT OF
MALARIA IN PREGNANCY, VITAMIN A SUPPLEMENTATION
AND VACCINATION DELIVERY AMONG CHILDREN 0-24
MONTHS UNDER PROGRAMMATIC CONDITIONS: A
SYSTEMATIC REVIEW

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Despite a World Health Organization recommendation for the use of
perennial malaria chemoprevention (PMC) in endemic areas, currently
only Sierra Leone implements PMC at scale. A major bottleneck to policy
uptake was the limited efficacy of the original 3-dose schedule, exacerbated
in real-world settings by poor implementation and coverage. Missed
opportunities for PMC uptake during immunization clinics are an important
contributor to poor coverage. This review, planned for June 2023, will
systematically identify and synthesize evidence on possible determinants
of missed opportunities for PMC drawing valuable lessons from literature on
the implementation of intermittent preventive treatment in pregnancy (IPTp),
vitamin A supplementation (VAS), and childhood vaccination. Searches will
include electronic bibliographic databases, grey literature and bibliographies
of retrieved articles. Inclusion criteria will include: quantitative and qualitative or mixed methods research studies; studies assessing determinants of missed opportunities for IPTp, IPTi, VAS or vaccination; conducted in sub-Saharan Africa; reports and peer-reviewed or grey literature. Search concepts will include ‘Intermittent preventive treatment’, ‘Factor’, ‘Barrier’, ‘Determinant’, ‘Infant’, ‘Routine immunization clinic’, ‘Malaria’, ‘Coverage’, ‘Vitamin-A supplementation’, ‘Missed opportunity’, ‘Policy uptake’ etc. Lists of titles and abstracts of articles retrieved will be exported to Endnote library and duplicates removed, and eligible full articles will be retrieved. Quality assessment of the studies will be done using relevant checklists to deal with the risk of bias. A descriptive analysis of the selected studies will be done with a narrative synthesis to bring the findings together. Data extraction will be aided by a tool and verified by a second reviewer. Pre-existing themes used by the authors will be identified and stratified based on user or provider perspectives. This review will contribute to the development of a conceptual framework for addressing missed opportunities for PMC to facilitate uptake and scale up.

5490

LOST TO FOLLOW-UP AND LOW INTermITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCYAT ANTEnatal CARE SETTINGs IN LIBERIA

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In 2018, Liberia implemented WHO-recommended intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine. The 2020 Liberia Demographic and Health Survey found that 87% of women with a pregnancy in the last four years attended at least four antenatal care (ANC) visits during their most recent pregnancy; 40% of them reported taking at least three IPTp doses (IPTp3). To assess the gap between high ANC attendance and low IPTp3 uptake, we conducted a retrospective review of ANC records of women 15–49 years old who initiated ANC between November 2020 and January 2021 at 33 randomly selected ANC sites in Bong, Nimba, and River Gee Counties. All selected ANC sites were public health facilities (clinic, health center, hospital). A woman who had ANC1 at a given site and never returned to that site was defined as lost to follow up (LTFU). We conducted multivariable logistic regression to investigate factors associated with LTFU. Among 1724 women registered for ANC1, 50% returned to the same site for ANC2, 32% for ANC3, and 19% for ANC4. Overall, IPTp3 uptake was 30% and 9% (141/1644) of women tested at ANC1 were diagnosed with malaria. Among the 878 women who returned for ANC2 services at the same site, 38% completed ANC4; of those 59% received IPTp3. LTFU ranged from 25% in River Gee to 47% in Nimba and 70% in Bong. Women with malaria were more likely to be LTFU (aOR= 1.9; 95% CI:1.3-2.9). Maternal age (aOR=1.0; 95% Cl:0.98-1.3), parity (aOR=1.0; 95%, Cl:0.9-1.1), ANC site (clinic aOR=1.0 [95% Cl:0.8-1.4], and health center vs hospital (aOR=0.9 [95% CI:0.6-1.2]) were not associated with LTFU after ANC1. Compared to those attending ANC at hospitals, women attending ANC at clinics were more likely to receive IPTp3 (aOR=3.4; 95% Cl: 1.8-6.6). Retention in ANC was low, which impedes optimal delivery of IPTp. To improve ANC retention, it is important to better understand and address the factors that prevent women who initiate ANC from attending all recommended visits. It is also critical to understand factors contributing to missed opportunities at ANC to provide IPTp, particularly in hospital settings.

5491

THE ROLE OF COMMUNITY LEADERS IN SEASONAL MALARIA CHEMOprevention: BUILDING STRATEGIES TO COMMUNITY ENGAGEMENT IN NORTHERN MOZAMBIQUE

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Seasonal malaria chemoprophylaxis (SMC) is a highly efficacious and effective intervention to prevent malaria infections in areas where the malaria burden is high, and transmission is seasonal. SMC is implemented in northern Mozambique since 2012, administering monthly courses of sulfadoxine plus pyrimethamine and amodiaquine (SPQA) to children aged 3–59 months. Sensitization and mobilization are carried out before the distribution by community leaders, members recognized as authorities by the community, using a door-to-door approach. A qualitative study was conducted in 2021 in Nampula to assess acceptability of SMC. The presented work describes the contribution of community leaders to the implementation of the SMC campaign. Twenty key informant interviews were conducted with key stakeholders at community, provincial, national and district level and twenty focus group discussions (FGD) were carried out with caregivers of children who received SPQA, community distributors (CD) and CDIs supervisors. Thematic analysis was performed using Maxqda10 software, and themes were identified and categorized following prevalent topics. Community distributors reported that, ‘community leaders went to the community to inform’ caregivers about SMC medicines, thus ‘they [caregivers] accepted to receive the medicine’. District officials perceived that ‘the population joined in because of the leadership’ guiding and informing the community. Community leaders talked about the role of community leaders who ‘recommended us to participate once the campaign started’ and explained the benefits of SMC for children. Participants at community and district level reported that, community mobilization led by community leaders and their direct involvement in the SMC campaign helped deliver accurate information to communities and contributed to their acceptance of the intervention. Community leaders should be recognized authorities that can help further promote dissemination of information on, and acceptance of SMC.

5492

COVERAGE AND FACTORS ASSOCIATED WITH UTILIZATION OF PYRETHROID-PIPERONYL BUTOXIDE TREATED NETS IN MALARIA ENDEMIC REGION, WESTERN KENYA

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Increased resistance to pyrethroid based Long-Lasting Insecticidal Nets (LLINs) informed WHO recommendation to deploy PIPB based LLINs. Kenya adopted use of PBO nets in endemic areas, though coverage is not known. We determined coverage and factors associated with utilization of PBO nets in a malaria endemic county, Western Kenya. We conducted cross-sectional study with multi-stage sampling in Matayos Sub-County, Busia County. Data were collected using questionnaire from June-July 2022. Data was analyzed by Stata version 16. Universal coverage was defined as ownership of one PBO net for two household members. Proper utilization was defined as sleeping under a mosquito net the previous night, net usage on all days of the week, hanging the net adequately. Data was collected on net ownership, access, utilization. Proper utilization of nets was the dependent variable. We calculated measures of central tendency and dispersion for continuous variables and proportions for categorical variables. Chi-square was used to test for association between dependent and independent variables.
Variables with a p-value < 0.05 were considered statistically significant. A total of 402 participants were interviewed; mean age was 41.2 years (±16.7 years); 268 (66.7%) resided in rural areas, 313 (77.9%) were female, 287 (71.4%) were married, 181 (45%) had formal education and 348 (86.6%) had informal occupations. Among all respondents, 347 (86.3%) had nets, 92.5% (222/347) were PBO and 261 (64.9%) households attained universal coverage. The odds of a household utilizing a PBO net if the household head had informal occupation was 71% less than households whose head had formal occupations (aOR=0.29, 95% CI=0.11-0.78). The odds of households that had not attained universal coverage utilizing a PBO net was 99.9% less than households that had attained universal coverage (aOR=0.01, 95% CI=0.01-0.03). Universal coverage of PBO nets was below the national target. Informal occupation and universal coverage were found to be associated with the utilization. We recommend continuous distribution of nets through additional innovative channels.

5493
PSYCHOSOCIAL FACTORS INFLUENCING INSECTICIDE-TREATED NET USE AND CARE IN LIBERIA
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In Liberia, which has year-round malaria transmission, ownership and use of insecticide-treated nets (ITN) is a key strategy for malaria control. To assess the association of psychosocial determinants with consistent ITN use (i.e., daily) and net care (i.e., tying up nets when not in use), Breakthrough ACTION and the National Malaria Control Program conducted a Malaria Behavior Survey in 2021. Standard questionnaires were administered to 5822 individuals (4677 women and 1145 of their male partners) from 3719 households. Psychosocial factors, including attitudes towards nets, confidence in one’s ability to use a net every night, and perceptions of others’ use of nets (norms), were assessed based on agreement with a series of statements. Responses were scored (1 for agreement, 0 for uncertainty, and -1 for disagreement) and summed into factor-specific scales. Scale scores greater than 0 defined presence of relevant psychosocial factors. In 2454 households with at least one net (66%), 4192 (72%) and 4308 (74%) respondents reported consistent use and tying up nets when not in use, respectively. In multivariate regression, factors associated with consistent net use included favorable attitudes towards ITN use (OR: 2.1, 95% CI: 1.6-2.7), confidence in one’s ability to use a net every night (OR: 7.4, 95% CI: 5.8-9.5), and perceived community use (OR: 1.4, 95% CI: 1.1-1.6). Factors associated with net care included knowledge that a net can prevent malaria (OR: 1.3, 95% CI: 1.1-1.6) and favorable attitudes towards both net use (OR: 2.1, 95% CI: 1.7-2.8) and net care (OR: 2.3, 95% CI: 1.2-4.3). Net sufficiency (1 net per 2 household members) was not found to be associated with the utilization. We recommend continuous distribution of nets through additional innovative channels.

5494
PREVENTION OF MALARIA IN PREGNANT WOMEN AND ITS EFFECTS ON MATERNAL AND CHILD HEALTH, THE CASE OF CENTRE HOSPITALIER DE KINGASANI II IN THE DEMOCRATIC REPUBLIC OF THE CONGO
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During pregnancy, malaria causes life-threatening outcomes to the mother and the newborn. The strategies to control malaria during the pregnancy rely on management malaria cases and anemia, and preventive measures such as intermittent preventive treatment with sulfadoxine-pyrimethamine (IPT-SP) and the use of insecticide-impregnated mosquito nets (ITN). Therefore, this study aimed to provide updated data of the benefit of ITN and IPT-SP on the birth weight of the newborn and the hemoglobin level of the mother. This cross-sectional analytical study was conducted among 467 women in labor in the Maternity of Centre Hospitalier de Kingasani II, in Democratic Republic of the Congo. Data collection was conducted using a structured questionnaire that was pre-tested in a face-to-face interview. The chi-square test was used to compare the proportions. Multivariate analysis (logistic regression) was also used to identify variables significantly associated to IPT-SP compliance, ITN use, low birth weight and to maternal anemia, with the 95% of the confidence interval. The ITN ownership rate was 81% (95%CI: 77-84) and the ITN use rate was 66% (95%CI: 62-70). Sixty-five percent (95% CI: 60-69) reported having received at least three doses of IPT-SP. Mothers who used ITN had a higher hemoglobin level compared to those who did not (9.4mg/dl IQ: 8.7-9.9 versus 11mg/dl IQ: 9.8-12.2, p=0.026). The non-use of the ITN was associated with low birth weight (aOR=3: 2.1-6.2; p<0.001) and anemia in pregnant women (aOR =2.41: 1.16-5.01; p=0.01). The use of the ITN and taking at least 3 doses of IPT-SP are associated with good birth weight. The number of doses of IPT-SP received during antenatal care is associated with the maternal hemoglobin level in the third trimester of pregnancy. Additional strategies to improve IPT-SP coverage and compliance may reduce maternal anemia associated with malaria during pregnancy.

5495
INCREASED MALARIA INCIDENCE FOLLOWING IRRIGATION PRACTICES IN THE ENDORHEIC RIFT VALLEY BASIN OF SOUTH ETHIOPIA
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Water resource development practice such as irrigation is key to ensuring economic growth and food security in developing countries. However, vector-borne diseases like malaria spread linked to such development have been a concern. The study was done to determine the impact of irrigation on malaria incidence and vector mosquito abundance in southern Ethiopia. Eight years of malaria morbidity data were extracted from the medical registers of health facilities, and mosquitoes were surveyed in both irrigated and non-irrigated settings. Malaria incidence, case distribution across age and sex, seasonality, parasite proportion, and mosquito density were analyzed and compared between irrigated and non-irrigated settings. The result showed that annual mean malaria incidence was 6.3 higher in the irrigated (95% CI: 0.7 – 33.6) than in the non-irrigated settings (95% CI: 1.2 – 20.6). Although a remarkable decline in malaria incidence was observed for three successive years (2013 - 2017), a significant resurgence between 2018 and 2020 was noted following the introduction of irrigation schemes. The densities of adult Anopheles mosquitoes were 15-fold higher in the irrigated settings compared to non-irrigated. Higher malaria incidence coupled with enhanced adult Anopheles density in the irrigated villages has important implications for designing tailored control interventions in such development settings.
UNBOUND PIPERAQUINE EXPOSURE IN CHILDREN AND PREGNANT WOMEN RECEIVING DIHYDROARTESMININ-PIPERAQUINE AS MALARIA CHEMOPREVENTION

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Dihydroartesminin-piperazine (DHA-PQ) is highly effective for malaria chemoprevention, but standard dosing is based on pharmacokinetic (PK) data from non-pregnant adults, which may not be optimal for children and pregnant women. We previously reported that PK exposure of PQ is reduced significantly in the context of childhood development, pregnancy, and efavirenz (EFV)-based antiretroviral therapy. However, as PQ is >99% protein-bound, it is important to assess potential alterations in protein binding during childhood development and pregnancy which may lead to changes in the pharmacokinetically effective drug fraction (fu) relative to total PQ. We therefore investigated the fu of PQ in children, pregnant women, and those receiving EFV to inform PK interpretation of changes in total drug exposure. Plasma samples from 0 to 24 hr after the third chemoprevention dose of DHA-PQ were collected in children at 32 and 104 wks of age, pregnant women at 28 wks gestation receiving or not receiving EFV-based antiretroviral therapy, and women 34-54 wks post-partum not receiving EFV (control adults). Unbound PQ was quantified via ultrafiltration and liquid chromatography-tandem mass spectrometry, with fu calculated as PQunbound/PQtotal. The geometric mean fu was 27% (p<0.001), 38% (p<0.0001), and 23% (p<0.0001) greater in children at 32 and 104 weeks of age, and pregnant women receiving EFV, respectively, compared to that in control adults. The fu did not differ between pregnant and control adults (p=0.66). Altered PQ fu is potentially due to developmental changes in children impacting protein concentrations and binding capacity and PQ displacement from plasma proteins by EFV. These results indicate that an increase in PQ fu modestly compensates for the significant decrease in total PQ exposure we previously reported. This appreciation should be considered if optimizing dosing guidelines based on total PQ PK results. Further study during the terminal elimination phase (e.g. on day 28 post-dose) would help better characterize unbound PQ exposure and thus the overall efficacy of PQ for malaria chemoprevention in these special populations.

RECEIPT OF SEASONAL MALARIA CHEMOPREVENTION BY AGE-INELIGIBLE CHILDREN AND ASSOCIATED FACTORS IN NINE IMPLEMENTATION STATES IN NIGERIA

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Seasonal malaria chemoprevention (SMC) has rapidly been scaled up in Nigeria, reaching nearly 27 million children in 2022. As part of implementation quality standards, SMC community distributors are expected to ensure that only age-eligible children (aged 3 - 59 months) receive SMC medicines during the monthly distribution cycles. However, difficulties in determining children's age and caregivers' desire for older children to receive protection from malaria, among other factors, may lead to older children receiving SMC medicines. We extracted data from a 2022 end-of-round SMC household representative surveys and analyzed data of 3,299 caregiver-child pairs sampled from nine SMC-implementing states in Nigeria. Mixed-effects multivariable logistic regression models were fitted to explore the association between receipt of SMC by age-eligible children and covariates. The mean age (±SD) of the children was 6.4 (1.4) years, 30.3% (95% CI: 27.8 - 32.9) of whom received at least one dose of SMC medicines in 2022. The majority (60.6%) of the over-age children who received SMC medicines were aged 5-6 years, while 19.5% were 7-year-olds and the rest (19.9%) were aged 8-10 years. We observed higher odds of an age-ineligible child receiving SMC among caregivers who had poor knowledge of SMC age of eligibility (OR: 1.8, 95% CI: 1.2 - 2.6, p=0.002), compared with those who had good knowledge of the age of eligibility. Notably, higher odds of receipt of SMC were also found among age-ineligible children whose caregivers had high confidence in SMC (OR: 2.3, 95% CI: 1.3 - 4.2, p=0.007), compared with those whose caregivers had low confidence in SMC. It was also found that age-ineligible children whose caregivers were older had lower odds of receiving SMC than those whose caregivers were younger. The study shows that a substantial proportion of age-ineligible children received SMC, with important implications for SMC implementation fidelity, effective coverage, impact and cost-effectiveness. The findings underscore the need for prioritizing programmatic quality improvement strategies to minimize the administration of SMC to ineligible children.
Malaria poses a greater risk to children under the age of five years due to its high morbidity and mortality. Nigeria contributes 27 percent to the malaria burden and 32 percent to the malaria deaths. Insecticide - Treated Nets (ITNs) use has been proven to be an effective preventive intervention for the control of malaria. We examined the relationship between the utilization of ITNs and the prevalence of malaria in children under five in Nigeria. Data were drawn from the most recent (2021) Demographic and Health Surveys (DHS). Logistic regression was used to analyze and establish the relationship between ITN use and malaria positivity (from blood smear result), controlling for wealth index, urban/rural residence, child's age in months, sex of the child, ITN use, geographic region, sex of head of household, age and education level of the mother. The study included 10,717 children under five with a blood smear test result: 27% urban and 73% rural. Only 38% of children under five used an ITN while 40% tested positive for malaria. Of note, the child under five sleeping under a net was not associated with malaria positivity (AOR: 1.06; 95% CI: 0.91-1.22). Factors associated with malaria positivity included increasing wealth quintile (AOR ranging from 0.18 to 0.59), geographic regions (AOR ranging from 1.47 to 1.63, 95% CI), child age in months (AOR: 1.02; 95% CI: 1.02-1.03), child's sex (AOR: 0.87; 95% CI: 0.78-0.96). Study findings suggest that malaria positivity may be influenced by multifaceted factors from the individual to the community/regional level. Community-level ITN use may be a better correlate of malaria prevalence than individual net use in Nigeria. Observed socioeconomic differences including wealth quintile, region, and maternal education level suggest that there may be underlying inequities among children under five that need to be addressed with targeted interventions in order to ensure malaria prevention in a heterogeneous setting like Nigeria.