9TH FUTURE of MALARIA RESEARCH

SYMPOSIUM | HYBRID

OCTOBER 13, 2023 • 8:30AM - 4:30PM EDT

ABSTRACT BOOK

JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH

Malaria Research Institute

UNIVERSITY of MARYLAND SCHOOL OF MEDICINE
CENTER FOR VACCINE DEVELOPMENT AND GLOBAL HEALTH

Future of Malaria Research Symposium

Organizers: Emily Stucke, Stephanie Rankin-Turner, Emma Rowley, Rubayet Elahi
Advisors: Miriam Laufer and David Sullivan

Friday, October 13, 2023

PROGRAM

07:45 am	**Registration |** Monument Street Entrance BSPH; **Coffee (**Gallery/Wall of Wonder)
08:30 am	**Welcome Address | Jane Carlton,** Johns Hopkins Malaria Research Institute Director, and **Miriam Laufer**, Malaria Research Program Director, University of Maryland School of Medicine| Sheldon Hall (w1214)
08:40 am	**Symposium Overview | Rubayet Elahi**, Johns Hopkins Bloomberg School of Public Health | Sheldon Hall (w1214)
SESSION 1	Moderator: **Rubayet Elahi,** Johns Hopkins Bloomberg School of Public Health (w1214)
08:45 am – 09:30 am	**Opening Keynote: Dibyadyuti Datta**, Indiana University School of Medicine "Brain injury and blood-brain-barrier dysfunction in pediatric severe malaria"
09:30 am – 09:45 am	**Benjamin Rice**, Princeton University "Hitting hotspots: Probing mass treatment approaches to reducing malaria transmission using Madagascar as a case study"
09:45 am – 10:00 am	**Mary Gebhardt**, Johns Hopkins Bloomberg School of Public Health "Exploring the spatial and temporal overlap in human and vector behavior in Nchelenge District, Zambia"
10:00 am – 10:15 am	**Anne Martin,** Johns Hopkins Bloomberg School of Public Health "Malaria case and focus investigation in a pre-elimination context in southern Zambia: a pilot project to assess impact and implementation outcomes"
10:15 am – 10:45 am	**Coffee Break** (Gallery/Wall of Wonder); Poster presenters set-up in Feinstone Hall (e2030)
SESSION 2	Moderator: **Emma Rowley**, University of Maryland School of Medicine (w1214)
10:45 am – 11:00 am	**Maria Nikulkova**, New York University "Understanding host-pathogen interactions of *Plasmodium vivax from* Ethiopia using integrative multi-omics"
11:00 am – 11:15 am	**David Anaguano**, University of Georgia "*Plasmodium falciparum* requires two rhoptries to invade host red blood cells"
11:15 am – 11:30 am	**Bing Guo,** University of Maryland School of Medicine "Benchmarking Identity-By-Descent Callers for *Plasmodium falciparum"*
LIGHTNING TALKS	**Zoom**: **Live streamed**
11:40 am – 12:15 pm	**Session A | Molecular approaches against malaria |** Live streamed in Sheldon Hall (w1214) Moderator: **Emily Stucke,** University of Maryland School of Medicine **Session B | Vectors and epidemiology |** Live streamed in Anna Baetjer room (w1030) Moderator: **Stephanie Rankin-Turner,** Johns Hopkins Bloomberg School of Public Health
12:15 pm – 01:00 pm	**Lunch | Feinstone Hall (e2030)**; Poster presenters set-up in Feinstone Hall (e2030)

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SESSION 3	Moderator: **Emily Stucke,** University of Maryland School of Medicine (w1214)
01:00 pm – 01:45 pm	**Afternoon Keynote: Filipa Rijo-Ferreira**, University of California, Berkeley "Circadian rhythms in hosts, malaria parasites and their vectors"
01:45 pm – 02:00 pm	**Kieran Tebben,** University of Maryland School of Medicine "Gene expression analyses reveal the mode of action of artesunate on *P. vivax* parasites"
02:00 pm – 02:15 pm	**Jakob Rupar**, Hesperos, Inc. "Implementation of a human cell-based Malaria-on-a-Chip phenotypic disease model for drug efficacy evaluation"
02:15 pm – 02:30 pm	**Steven Tan**, Walter Reed Army Institute of Research "Immune responses in mice immunized with *Plasmodium falciparum* mRNA liver stage antigens: antibody, CD4 Tfh, CD8 TRM and CD8 TEM cells"
02:30 pm – 02:40 pm	**Closing remarks |David Sullivan**, Johns Hopkins Bloomberg School of Public Health (w1214)
POSTER SESSION	
02:45 pm – 04:30 pm	**In-person Poster Session and Reception| Feinstone Hall** (e2030)

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LIGHTNING TALKS

SESSION A: Molecular approaches against malaria

Moderator: **Emily Stucke,** University of Maryland School of Medicine (Live streamed in w1214)

Faith Onditi, National Institute of Health, United States
"*Aotus nancymae* as a novel non-human primate model of chronic placental malaria"

Nadia Domingo, Rutgers - The State University of New Jersey, United States
"Fibrinogen activates microglia through CD11b binding and mediates neuropathology in hyperinflammatory experimental cerebral malaria"

Jenna Dick, University of Minnesota, United States
"Loss of Siglec-7 correlates with enhanced Natural Killer cell function and protection from malaria symptoms"

Georgina Agyekum, University of Ghana, Ghana
"Identification of immunodominant T cell epitopes within *Plasmodium falciparum* merozoite surface protein-11 (PFMSP-11)"

Savannah Watson, University of Pretoria, South Africa. "Hit-to-lead optimization of SQ109 analogues towards improved transmission-selective activity of *Plasmodium falciparum*"

Tosin Opadokun, McGill University, Canada, "Characterization of extracellular vesicles from *Plasmodium falciparum* infected red blood cells reveals distinct proteomic and transcriptomic profiles"

Sudipta Das, Indian Institute of Chemical Biology-CSIR, India, "M-O-M mediated denaturation resistant P2 tetramer on the infected erythrocyte surface of Malaria parasite imports serum fatty acids"

SESSION B: Vectors and epidemiology

Moderator: **Stephanie Rankin-Turner**, Johns Hopkins Bloomberg School of Public Health (Live streamed in w1030)

Susan Cilene Paredes Fernandez, University of Antwerp, Belgium
"Convergent-parallel approach to investigate malaria in indigenous communities under the COVID-19 pandemic"

Abraham Kpirikai, University of Ghana, Ghana
"Population genetic analysis of *Plasmodium falciparum* erythrocyte bindingantigen-175 (PfEBA-175) gene in Ghana"

Sahib Gul Afridi, Abdul Wali Khan University, Pakistan
"Merozoites surface proteins based genetic variations in *Plasmodium falciparum* and *Plasmodium vivax* field isolates from Nowshera district of Pakistan"

Mukuma Lubinda, Macha Research Trust, Zambia
"Spatiotemporal correlation of malaria intensity and vector abundance in a pre-elimination setting of Choma district, Southern Zambia"

Limonty Simubali, Macha Research Trust, Zambia
"Impact of volatile pyrethroid spatial repellant (VPSR) on the abundance of outdoor biting anophelines in a low malaria transmission setting, Southern Zambia"

Melina Campos, University of California Davis, United States
"Spatial population genetics of *Anopheles coluzzii* in oceanic islands"

Issiaka Sare, Institut de Recherche en Sciences de la Santé (IRSS), Burkina Faso
"Investigating the efficacy of native strains of Metarhizium from Burkina Faso on *Anopheles coluzzii* mosquitoes in both larval and adult stages for integrated vector management"

Dawit Hawaria Logita, Hawassa university, Ethiopia
"First report of *Anopheles stephensi* from Southern Ethiopia"

KEYNOTE ADDRESS

Opening Keynote | 8:45 – 9:30 am | Sheldon Hall (w1214)

Dibyadyuti Datta, Ph.D.
Assistant Professor
Indiana University, School of Medicine
Email: ddatta@iu.edu

Dr. Datta's research focuses on the pathophysiology of brain injury in pediatric cerebral malaria (CM), non-CM severe malaria, and other nervous system disorders relevant in malaria-endemic countries including traumatic brain injury (TBI). Her work spans the following areas: 1) Establishing specific, noninvasive prognostic biomarkers of brain injury to predict adverse neurologic outcome in pediatric severe malaria; 2) Investigating mechanisms of brain injury and barrier dysfunction using an *in vitro* stem cell-derived model of the blood-brain-barrier in CM; 3) Identifying biomarkers and risk factors associated with persisting neurodisability in pediatric TBI in Uganda.

"Brain injury and blood-brain-barrier dysfunction in pediatric severe malaria"

Severe *falciparum* malaria is a major driver of childhood mortality and persisting neurodisability in Africa. Without crossing the blood-brain-barrier (BBB), malaria parasites can significantly impact brain function resulting in cognitive impairment, whether a child presents with clinical signs of neurologic complications, as seen in cerebral malaria, or without, as in severe malarial anemia. Biomarkers of systemic inflammation, endothelial activation, and organ dysfunction have been identified as risk factors for mortality and cognitive impairment in severe malaria. Less is known about the role of brain injury biomarkers that reflect CNS damage and can differentiate between neuronal, axonal, or astroglial injury, although these biomarkers have been associated with adverse neurologic outcomes in acute pediatric disorders such as TBI. To address this gap in knowledge, my research is focused on identifying biomarkers and studying mechanisms of brain injury and BBB dysfunction in severe malaria. My approach includes: 1) using a highly sensitive digital detection assay for protein biomarker analysis in clinical pediatric severe malaria samples, and 2) a physiologically relevant *in vitro* model of the BBB in cerebral malaria, comprised of induced pluripotent stem cell derived brain endothelial cells, neurons, and astrocytes, co-cultured with parasite infected RBCs. My goal is to synthesize clinical and laboratory-based findings towards a better understanding of the pathogenesis of brain injury in severe malaria. The overarching goal is that this work will form the foundation for translational clinical research to develop targeted therapeutic interventions to prevent or reduce brain injury and future neurodisability in survivors of severe malaria.

KEYNOTE ADDRESS

Afternoon Keynote | 1:00 – 1:45 pm | Sheldon Hall (w1214)

Filipa Rijo-Ferreira, Ph.D.

Assistant Professor
University of California, Berkeley
Email: filipaferreira@berkeley.edu

Filipa is an Assistant Professor of Public Health and Molecular and Cellular Biology at the University of California, Berkeley and an Investigator at the Chan Zuckerberg BioHub. Filipa earned a PhD from University of Porto, Portugal followed by postdoctoral training at the University of Texas Southwestern Medical Center at Dallas, USA. The Rijo-Ferreira lab takes an integrated approach to study circadian rhythms in parasitic diseases, in particular Malaria and Sleeping sickness. The lab employs technical methodologies spanning from next-generation sequencing to cellular and behavioral assays to investigate the interactions of these parasites with their hosts and vectors. The Rijo-Ferreira Lab is committed to science communication and efforts to engage children primarily from underrepresented minorities in the exciting world of the life sciences. Filipa is a NIH Pathway to Independence awardee, a recipient of the Brown-Goldstein Excellence in Postdoctoral research award, a STAT Wunderkind, and a Searle Scholar.

"Circadian rhythms in hosts, malaria parasites and their vectors"

Earth rotation shaped the biology of many organisms. Animals, plants, and microbes evolved circadian rhythms to anticipate the predictive daily rhythms of their environment. Multiple parasitic diseases show daily rhythms, a contribution of both hosts and parasite biology. Malaria, a disease that kills over half a million people each year is one of such infections, in which infected individuals experience periodic fevers. We have recently shown that parasite biology is intrinsically rhythmic, with rhythms persisting in the absence of host rhythmic cues. Remarkably, rhythms in malaria parasite biology exist beyond their presence in the mammalian host, to when they infect their mosquito vector. For malaria transmission, sporozoite parasites need to leave the salivary glands of the mosquito and be deposited in the skin of the mammalian host, upon the mosquito blood meal. Here we uncover the rhythmic biology of both the salivary gland of mosquitos and the parasites within them. These findings provide insight into the co-evolution of the clocks of hosts, malaria parasites and their vectors with potential impact to the transmission of this disease.

ORAL TALKS

ORAL TALKS | SESSION 1
Sheldon Hall (w1214)
Moderator: **Rubayet Elahi,** Johns Hopkins Bloomberg School of Public Health

1. Hitting hotspots: Probing mass treatment approaches to reducing malaria transmission using Madagascar as a case study

Benjamin L. Rice[1,2], Estelle Raobson[3,4], Sylviane Miharisoa[5], Mahery Rebaliha[2,6], Joseph Lewinski[6], Amy Wesolowski[7], C. Jessica E. Metcalf[1,8]

[1] Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ, USA. [2] Madagascar Health and Environmental Research (MAHERY), Maroantsetra, Madagascar. [3] Mention Zoologie et Biodiversité Animale, University of Antananarivo, Antananarivo, Madagascar. [4] Association Vahatra, Antananarivo, Madagascar. [5] Institut Pasteur Madagascar, Antananarivo, Madagascar. [6] Multisectoral Malaria Project, Catholic Relief Services, Baltimore, MD, USA. [7] Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA. [8] Princeton School of Public and International Affairs, Princeton University, NJ, USA.

Additional active approaches to malaria control are warranted in areas where moderate to high malaria transmission persists despite existing, primarily passive, control measures. Mass drug administration (MDA) or mass test and treatment (MTaT) strategies to reduce the parasite reservoir have been proposed. From previous studies of these strategies, however, difficulties were observed in achieving a significant and lasting effect on transmission. The WHO, as a result, currently does not recommend their application and calls for further research into the optimal intervention frequency and feasibility. To further investigate the potential use of these active approaches in high burden geographies, we leverage data from a newly completed prospective cohort study from southeast Madagascar (*n* = 20,718 observations). First, using our study design of monthly active case detection with immediate treatment of positive individuals, we estimate spatial and temporal variation in the force of infection of *Plasmodium falciparum*. We then used these estimates to infer the maximum time interval between MDA or MTaT interventions allowable to maintain prevalence below a given target across the variation in infection rate we observed. In terms of feasibility, we use mathematical models to explore the vulnerability of MDA and MTaT based approaches to disruption, using, as an example, heterogeneity in the force of infection we observed before and after the tropical cyclones that impacted Madagascar in February 2022 and February 2023. We estimate the potential benefit of pre-disruption, proactive measures and discuss implications for climate and other disruptions across the endemic range of malaria.

2. Exploring the spatial and temporal overlap in human and vector behavior in Nchelenge District, Zambia

Mary E. Gebhardt[1], Brian Chirwa[2], Mohamed Bayoh[2], Mbanga Muleba[3], William J. Moss[4], and Douglas E. Norris[1]

[1] The W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA. [2] PMI Evolve Project, Abt Associates, Lusaka, Zambia. [3] Tropical Diseases Research Centre, Ndola, Zambia. [4] Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA.

After a decade of repeated insecticide-treated net (ITN) distributions and annual indoor residual spray (IRS) campaigns using insecticides with different modes of action, communities in Nchelenge District, Zambia, still suffer from holoendemic malaria transmission. The Southern and Central Africa International Center of Excellence for Malaria Research (ICEMR) has documented little impact of IRS on malaria prevalence and anopheline counts. One hypothesis for this lack of effect is that anophelines are exhibiting non-traditional foraging behaviors, biting early and outdoors. To investigate anopheline biting patterns, the U.S. President's Malaria Initiative Vectorlink Project performed monthly human landing catches (HLCs) indoors and outdoors in Nchelenge District from 2020-2022, and the ICEMR performed cross-sectional household surveys from 2022-2023 to elucidate human night-time behaviors. A proportional analysis was performed to identify spatial and temporal behavioral overlap between humans and vectors. Through direct measures of exposure (HLCs), almost 40% and 50% of bites from *Anopheles funestus* and *An. gambiae* occurred outdoors, with peak biting between 03:00-05:00. After adjusting for human behavior, 95% of bites were estimated to occur indoors, with an average of 91 bites/person/night. Individuals who use an ITN were estimated to receive only 10 bites/person/night, but 45% of those bites likely occurred outdoors during the early evening and morning. ITN coverage was only 54%, which is estimated to prevent 47% of total population-level exposure. Increasing ITN coverage to 90% could prevent up to 80% of exposure events in Nchelenge District; however, with such intense biting pressure, outdoor transmission will remain a barrier to malaria control.

3. Malaria case and focus investigation in a pre-elimination context in southern Zambia: a pilot project to assess impact and implementation outcomes

Anne Martin[1], Japhet Matoba[2], Caison Sing'anga, [2] Harry Hamapumbu[2], Mukuma Lubinda[2], Xinjue Chen[1], Ben Katowa[2], Michael Musonda[2], Tamaki Kobayashi[1], Edgar Simulundu[2] and William J. Moss[1] for the Southern and Central Africa International Centers of Excellence for Malaria Research

[1] Johns Hopkins Bloomberg School of Public Health, Baltimore USA. [2] Macha Research Trust, Zambia.

Choma District, located in Southern Province, Zambia is a very low transmission malaria setting; 2021 malaria microscopy prevalence in under-five s was 3.3%. That year, the National Malaria Elimination Centre (NMEC) began implementing malaria case investigation (MCI), also known as 1-3-7, a surveillance strategy initially implemented in China and southeast Asia. 1-3-7 requires a passively detected malaria case, an "index" case, be reported within 1 day, classified as locally transmitted or imported within 3 days, and investigated in its community with further follow-up and response within 7 days. This work describes a hybrid-type-2 implementation-effectiveness study of 1-3-7 in two health center catchment areas in Choma District beginning in 2022. To measure effectiveness, 1-3-7 was deployed in a randomly-selected half of the zones in the study area; community-based surveys measure malaria prevalence in index-case communities 7 and 35 days after index case diagnosis such that it may be compared across 1-3-7, "intervention", and non-1-3-7, "control" zones through multi-level logistic regression. To measure implementation outcomes, survey data, programmatic metadata, and semi-structured interviews with health care workers, are used to describe fidelity, feasibility, acceptability, equity, efficiency of the intervention. We will present the statistical results of the effectiveness aim and the descriptive results of the implementation aim. The results of this work will be used to inform broader scale up of MCI across low-transmission settings in Zambia.

ORAL TALKS | SESSION 2
Sheldon Hall (w1214)
Moderator: **Emma Rowley,** University of Maryland School of Medicine

4. Understanding host-pathogen interactions of *Plasmodium vivax* from Ethiopia using integrative multi-omics

Maria Nikulkova[1], Biniam Lukas[2], Daniel Tesfaye[2], Taye Teka[2], Tirusew Tolessa[2], Youssef Idaghdour[3], Delenasaw *Yewhalaw*[2], and Jane M. Carlton[4]

[1]New York University. [2]Tropical and Infectious Diseases Research Center (TIDRC), Jimma University, Ethiopia. [3] New York University – Abu Dhabi, [4]Johns Hopkins Bloomberg School of Public Health.

During the blood stage, the *Plasmodium* and host transcriptional and metabolic activity become highly intertwined as both try to ensure their survival. Previous metabolomic and gene expression studies have focused on *P. falciparum* and highlighted differences in immunoglobulin production, proinflammatory cytokines, RBC surface proteins, and host lipid and steroid production during infection. However, there have been few studies on human-*P. vivax* interactions. We collected multi-omic datasets from 148 individuals enrolled in a cross-sectional study at three health facilities in the Jimma area, southwest Ethiopia, in October 2022. Blood samples were collected and tested for *Plasmodium* infection by RDT and PCR, plasma underwent global metabolite profiling by LC-MS/MS or GC-MS, total DNA was sequenced by low pass Illumina whole genome sequencing, and transcriptomes were generated by dual RNA-seq. Of the 148 individuals examined, 62 were infected with *P. vivax,* 29 with *P. falciparum,* 13 with co-infections and 44 uninfected controls. A total of 867 metabolites were identified from global metabolite profiling and 888 lipids from complex lipid profiling. Of the metabolite perturbations associated with *P. vivax* infection, a decreased abundance of cholesterol ester and lysophosphatidylcholine was observed. Previous studies reported the inability of *P. falciparum* to produce its own cholesterol and its reliance on host cholesterol and lysophosphatidylcholine (LPC) for sexual stage regulation, suggesting that *P. vivax* similarly utilizes these host compounds for its own biological processes. Integrative analyses of this rich, high-dimensional multi-omics dataset is revealing distinct metabolic and gene expression signatures during the erythrocytic stage of *P. vivax* infection.

5. *Plasmodium falciparum* requires two rhoptries to invade host red blood cells

David Anaguano[1,2], James Blauwkamp[3], Manuel Fierro[1,2,#], Sabrina Absalon[3], and Vasant Muralidharan[1,2]

[1] Department of Cellular Biology, University of Georgia, Athens GA. [2] Center for Tropical and Emerging Global Diseases (CTEGD), University of Georgia, Athens GA. [3] Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis IN.

[#]Current address: Department of Biomedical Sciences, Iowa State University, Ames IA

Malaria is a global and deadly human disease caused by the apicomplexan parasites of the genus *Plasmodium*. The disease's clinical manifestations are closely linked to the proliferation of parasites within human red blood cells (RBCs). Establishment of infection within the human host begins with the invasion of RBCs by *P. falciparum*. This invasion is mediated by the secretion of effectors from specialized secretory organelles, most notably a pair of club-shaped organelles localized to the apical end of merozoite-stage parasites, termed rhoptries. In my research, I investigated the function of one of these effectors, namely Rhoptry Neck Protein 11 (RON11). RON11 contains seven transmembrane domains and a putative single calcium-binding EF-hand domain, localized towards the parasite's cytoplasm. By generating RON11 conditional knockdown mutants, I demonstrated that the reduction of RON11 inhibits parasite growth by preventing merozoite invasion of red RBCs. Using ultrastructure expansion microscopy (U-ExM), I observed a unique phenotype in the absence of RON11, characterized by fully developed merozoites featuring single rhoptries. More intriguingly, RON11 depletion does not hinder merozoite attachment, nor does it impede the release of rhoptry effectors into the RBC during invasion. However, it does block internalization of merozoites. When observing RON11 during rhoptry biogenesis, I noted RON11's participation in the formation of the second rhoptry pair in the final stages of schizogony, which coincided with merozoite segregation. In summary, these data collectively suggest that RON11 plays a pivotal role in generating two rhoptries and is crucial for completing the process of merozoite internalization into the RBC.

6. Benchmarking Identity-By-Descent Callers for *Plasmodium falciparum*

Bing Guo[1,3], Michele D. Spring[2], Mariusz Wojnarski[2], Brian A. Vesely[2], Joana C. Silva[1], Norman C. Waters[2], Shannon Takala-Harrison[3], and Timothy D. O'Connor[1]

[1] Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, USA. [2] Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand. [3] Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD USA.

Genomic surveillance is important for identification of at-risk populations for targeted intervention in support of malaria control/elimination efforts. Identity-by descent (IBD) is one population genetic metric used to study genetic relatedness, effective population size (Ne), migration and population structure, and drug resistance-related selection in *Plasmodium falciparum* (Pf). Despite the wide use of IBD as a metric of genetic relatedness, a comprehensive evaluation of IBD callers in Pf has not been performed, and valuable information conveyed by individual IBD segments is not always well exploited. To date, existing IBD quality assessments either focus on the human genome (rather than Pf), use a loose definition of IBD accuracy or are biased toward long segments from pedigree-based simulations. In this study, we performed population-based simulations that account for the high recombination rate and shrinking Ne of Pf and examine IBD segments spanning a wide length distribution. We benchmarked several IBD callers, including probabilistic (*hmmIBD*, *isoRelate*), Identity-By-State-based (*hap-ibd*, *TPBWT*), or other (*Refined IBD*, tree-based) methods, by comparing inferred IBD with genealogy-based true IBD. Our simulations suggest that *hmmIBD* tend to be less biased for the determination of pairwise total IBD, length-specific population total IBD, and IBD-based demography estimates while remaining sensitive to IBD positional enrichment due to positive selection. Ongoing work will focus on the validation of benchmarking results in an empirical dataset including MalariaGen Pf7 by evaluating the ability of different IBD callers to capture known selection signals and generate Ne and population structure estimates consistent with existing knowledge for each geographic region.

LIGHTNING TALK | Session A: Molecular approaches against malaria
Live streamed in Sheldon Hall (w1214)
Moderator: **Emily Stucke,** University of Maryland School of Medicine

7. *Aotus nancymae* as a novel non-human primate model of chronic placental malaria

Faith I. Onditi[1,2], Jennifer Howard[1], Steven Nadakal[1], Holly Torano[1], Justin Doritchamou[1], Alicia Spiegel[1], Rhea Stevens[1], Bob Morrison[1], Jillian Neal[1], Jake Raitin[1], Tarik Ouahes[1], Sachy Orr-Gonzalez[1], Lynn Lambert[1], and Patrick E. Duffy[1*]

[1] Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health. Bethesda, MD, USA. [2] Malaria Program, Department of Tropical and Infectious Diseases, Kenya Institute of Primate Research. Nairobi, Kenya.

Malaria-related pregnancy complications are a global health concern, with *Plasmodium falciparum* and *P. vivax* parasites causing most cases. The lack of suitable non-human primate (NHP) models hinders drug and vaccine development for placental malaria (PM). Recently, *Aotus nancymaae*, a new world NHP, was developed as an acute model of PM, but failed to replicate the intervillositis (IVS) observed in chronic human PM. Therefore, we aimed to optimize a chronic PM model describing parasite sequestration and IVS in pregnant *A. nancymaae* infected with *Pf*-CS2 for 7-17 days. The presence of parasites and IVS was determined through placental histology, while cytokine levels in the mother's peripheral blood plasma were measured using multiplex assay. The effect of chronic infection on the development of protective antibody (Ab) responses was evaluated by measuring antibodies binding to VAR2CSA and *P. falciparum*-infected red blood cells. Our findings demonstrated that infections lasting 12 or more days caused IVS in the placenta, with variation in cytokine and Ab responses among *Aotus*. Similarly, infections lasting 12 days or more showed increased IL-10, IL-12, IFNγ, TNF-α, and VAR2CSA-reactive and iRBC-binding Ab levels. We conclude that this recently developed NHP model for chronic PM provides a unique platform for the preclinical development of PM drugs and vaccines.

8. Fibrinogen activates microglia through CD11b binding and mediates neuropathology in hyperinflammatory experimental cerebral malaria

Nadia D. Domingo[1,2], Olivia D. Solomon[3], Florentin Aussenac[2], Difernando Vanegas[6], Paula Villareal[2], Lorenzo Ochoa[3], Andrew S. Mendiola[3], Lucinda Puebla-Clark[2], Sandra Cardona[6], Matthew J. Flick[7], Astrid Cardona[6], Katerina Akassoglou[5] Gracie Vargas[4], and Robin Stephens[1, 2]

[1] Center for Immunity & Inflammation, Rutgers New Jersey Medical School, Newark, New Jersy, USA. [2] Department of Internal Medicine, Division of Infectious Diseases, University of Texas Medical Branch, Galveston, Texas, USA. [3] Institute of Translational Sciences, Human Pathophysiology/Translational Medicine, University of Texas Medical Branch, Galveston, Texas, USA. [4] Department of Neurobiology, University of Texas Medical Branch, Galveston, Texas, USA. [5] Department of Neurology, University of California San Francisco, San Francisco, California, USA. [6] Department of Molecular Microbiology and Immunology, The University of Texas at San Antonio, San Antonio, Texas, USA. [7] Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA.

Severe malaria including cerebral malaria (CM) caused deaths of 627,000 people yearly with symptoms such as altered consciousness, seizures, and coma. Both post-mortem study of brains of children who died with high *Plasmodium falciparum* parasitemia and no other cause of coma, and models of experimental CM showed extensive thrombi. *Plasmodium chabaudi*-infected IL-10 deficient mice exhibit vascular congestion, fibrinogen deposition and activated microglia. We previously observed reduced mortality using low molecular weight heparin (LMWH), presumably due to reducing clotting. Here we examine the roles of fibrin deposition and CD11b activation during the pathology of eCM, by infecting fibrinogen mutant mice; including fibrinogen-alpha deficient (Fiba -/-), and fibrinogen mutated to discriminate clothing effect or to eliminate fibrinogen - CD11b interaction; with *Plasmodium berghei* ANKA. Fibrinogen Gamma-Mac mice showed an increase in disease severity, specifically hypothermia, suggesting that the interaction between clotting and CD11b is necessary for regulation of pathology. Microglia also showed activated phenotypes near the vasculature and are recruited to clotting vessels. Therefore, we blocked fibrinogen binding to microglia via CD11b using intranasal delivery of a fibrinogen-derived inhibitory peptide (gamma (377-395), and found that microglial activation, measured by immunohistology with Tmem119 and Iba1, is significantly reduced in eCM. These results suggest that microglia are activated by release of fibrinogen, potentially linked to clotting events during eCM.

Funding Acknowledgment: R01 NS106597 (NDD, AC, GV, RS)

9. Loss of Siglec-7 correlates with enhanced Natural Killer cell function and protection from malaria symptoms

Jenna K. Dick[1], Jules A. Sangala[1], Benjamin T. Zandstra[1], Peter D. Crompton[2], and Geoffrey T. Hart[1]

[1] Department of Medicine, Division of Infectious Diseases, and International Medicine, University of Minnesota, Minneapolis, Minnesota, USA. [2] Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland, USA.

An effective malaria vaccine is urgently needed, but progress towards this goal has been hampered by our limited understanding of effective malaria immunity. Natural killer (NK) cells inhibit the growth of *Plasmodium falciparum in vitro* through antibody dependent cellular cytotoxicity (ADCC). We went on to show that a subset of NK cells in malaria endemic subjects, known as adaptive NK cells, lack the Fc receptor γ chain ($FcRg^{neg}$) have enhanced ADCC function. However, it was unclear if the lack of Fc γ chain was the reason for increased functionality or if it served as a marker of adaptive NK cells. Using CRISPR/Cas9, we found that the absence of FcRg does not enhance ADCC. We then searched for other alterations in adaptive NK cells in malaria subjects and found that a decrease in the expression of the inhibitory receptor Siglec-7 (Sialic Acid Binding Ig-like lectin 7) correlated with increased NK ADCC function. Importantly, Sigelc-7 negative NK cells correlated with $FcRg^{neg}$ NK cells in malaria. Because Siglec-7 is an inhibitory receptor, we hypothesized that when the NK cells lack this receptor, this could be cause for adaptive NK cell increased function. Using CRISPR/Cas9, we then ablated Siglec-7 and found enhanced ADCC functionality. Therefore, we predict Siglec-7 is an important protein of interest to study for the protective effects of NK cells in malaria. Ultimately, the goal is to use this data and ongoing work to leverage insights from NK cell protective mechanisms to create better therapeutics and vaccines for malaria.

10. Identification of immunodominant T cell epitopes within *Plasmodium falciparum* merozoite surface protein-11 (PFMSP-11)

Georgina Agyekum[1,2], Abena Fremaah Frempong[1], Abigail Pobee[1], Ebenezer Addo Ofori[1,3], Augustina Frimpong[1], Sutaya Galevo[1], Kwadwo Akyea-Mensah[1], Oscar Darko[1,2], Yaw Aniweh[4], and Kwadwo Asamoah Kusi[1,2]

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Malaria remains a major menace to developing countries. A lot of preventive and treatment strategies are hampered by the emergence of *Plasmodium falciparum* resistant strains, even to the new anti-malarial drugs. An effective malaria vaccine is needed as an additional intervention to drive malaria elimination. One characteristic of a successful malaria vaccine is its ability to elicit immune responses against different variants of the parasite in a genetically heterogeneous population, hence the need for multi-epitope malaria vaccine which can incorporate conserved HLA restricted peptides from multiple antigens. This study, therefore, sought to identify immunodominant T cell epitopes within *Plasmodium falciparum* Merozoite Surface Protein -11 (PfMSP-11), a new malaria vaccine candidate antigen. The HLA A and B type binding specificity of MSP-11 synthetic peptides were predicted using the *in-silico* algorithm known as NetMHC and cryopreserved PBMCs from 5 HLA-typed subjects were stimulated with 22 synthetic PfMSP-11 peptides in Fluorospot Assays. The data were expressed as spot-forming cells per million (sfc/million) PBMCs and a set of criteria was used to determine the positivity of the peptides. Out of the 22 peptides tested against the five participant PBMCs, four responded positively to four peptides that bound to HLA A02, A03, B07, B27, B44 and B58 supertypes. In conclusion this study has identified immunodominant regions within PfMSP-11 antigen in this limited number of HLA-typed subjects, with potential to identify more MSP-11 epitopes within a larger population.

11. Hit-to-lead optimization of SQ109 analogues towards improved transmission-selective activity of *Plasmodium falciparum*

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The search for new anti-malarial compounds has generally been biased towards compounds targeting biology important to the ABS, with screening campaigns prioritizing hits based on ABS activity, and LG activity merely adding dual-active value to these compounds. Alternative approaches that included *de novo,* parallel screening against multiple stages has identified new chemical matter that are able to target specific life cycle stages of *P. falciparum*. Interestingly, several compounds were identified with preferential activity against *P. falciparum* mature gametocytes, providing new chemical starting points for further development. One such compound was the well characterized *N*-geranyl-*N*'-(2-adamantyl)ethane-1,2-diamine, SQ109, which displayed activity across all transmission-blocking platforms. SQ109 is a second-generation ethylenediamine and has completed phase II clinical trials against *Mycobacterium tuberculosis* infections. The target of SQ109 was identified as mycobacterial membrane protein large 3 (MmpL3) where it participates in cell wall biosynthesis. Additionally, SQ109 indirectly inhibits MmpL3 activity by proton motor force (PMF) disruption. Previously, a series of SQ109 derivatives were synthesised and tested for their anti-TB activity, by introducing changes to the geranyl tail, proving that the alkene moiety is important for anti-TB activity. Inhibitory activity of SQ109 is not limited to *Mycobacterium* as is also has activity against a range of other non-MmpL3 containing intracellular parasites, where is displayed uncoupling activity as a main mode of action. Here, we perform an extensive structure-activity analysis around SQ109, with a particular focus on the ability of these compounds to retain selective activity towards gametocyte stages of *P. falciparum.* These data provide a basis for performing hit-to-lead optimization of highly active compounds with the goal of establishing structure-activity relationships for antimalarial activity.

12. Characterization of extracellular vesicles from *Plasmodium falciparum* infected red blood cells reveals distinct proteomic and transcriptomic profiles

Tosin Opadokun[1] and Petra Rohrbach[1]

[1] Institute of Parasitology, McGill University, Canada.

P. falciparum-infected red blood cells (RBCs) release extracellular vesicles (EVs), which are small membrane bound particles that contain biomolecular cargo and serve as a means of communication between and within cell populations. Despite the important roles these EVs play in malaria, such as immunomodulation and the promotion of gametocytogenesis, EVs released by the different blood stages of *P. falciparum* are yet to be fully characterized. Our lab is investigating the biomolecular cargo of EV subtypes released by *P. falciparum* stage-specific infected RBCs to delineate their unique profiles, identify shared and unique biomolecules, as well as understand malaria EV biology and biogenesis. Using nanoparticle tracking analysis, flow cytometry, western blot analysis, transmission electron microscopy, mass spectrometry and RNA sequencing to study malaria derived EVs isolated by differential centrifugation, we have found that EVs derived from RBCs infected with the ring, trophozoite and schizont stages of *P. falciparum* are quantitatively and qualitatively distinct, with implications for their specific roles in malaria pathogenesis and parasite survival. Proteins and genes that are crucial for parasite survival and pathology in the human host, as well as for understanding EV biogenesis in the parasite have been identified.

13. M-O-M mediated denaturation resistant P2 tetramer on the infected erythrocyte surface of malaria parasite imports serum fatty acids

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Plasmodium falciparum (Pf) undergoes repeated rounds of asynchronous nuclear division during intraerythrocytic schizogony. In Pf, repeated nuclear divisions precedes cytokinesis and cell body formation. Regulation of nuclear division through the import of serum components was largely unknown in Apicomplexan parasites. At the trophozoite stage, Pf ribosomal protein P2 is trafficked to the infected erythrocyte (IE) cytosol and to the surface as a denaturation-resistant tetramer. The blockage of the IE surface exposed PfP2 tetramer to its bona fide ligand led to the arrest of nuclear division. Here we show that at the onset of nuclear division, denaturation-resistant PfP2 tetramer on the IE surface trap serum fatty acids for its import into the parasites for subsequent membrane biogenesis during schizogony and progeny formation. Blockage of fatty acids import through PfP2 tetramer reversibly halted nuclear division at the onset of schizogony. In ^{11}Met-O-Met^{11} mediated denaturation resistant PfP2 tetramer, the $^{12/53}$Cys-Cys$^{12/53}$ redox switch regulates the binding and subsequent release of fatty acids on the IE surface based on oxidized/reduced state of disulfide linkages. This mechanistic insight of fatty acids import through PfP2 tetramer reveals a unique regulatory mechanism operational at the onset of parasite schizogony.

LIGHTNING TALK | Session B: Vectors and epidemiology
Live streamed in Anna Baetjer room (w1030)
Moderator: **Stephanie Rankin-Turner,** Johns Hopkins Bloomberg School of Public Health

14. *Convergent-parallel approach to investigate malaria in indigenous communities under the COVID-19 pandemic*

Susan Paredes Fernandez[1], Luis Rojas Muro[3], Paloma Diab García[1], Hilde Bastiaens[2], Sybil Athierens[2], Steven Abrams[1], Jean-Pierre Van geertruyden[1], Stella Chenet[3], and Christopher Delgado-Ratto[1]

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In Peru, the interruption of the malaria control program due to the COVID-19 pandemic led to increased cases in urban areas and has significantly affected also other health programs. This scenario was the same in other low and middle-income countries. Still, the actual burden and care situation in remote, indigenous areas remained unknown. Hereby, through a mixed-method study, we explored the prevalence and factors associated with malaria infections in four Amazonian indigenous communities from the Amazonas region in Peru (Nueva Esperanza, Alianza Progreso, Caterpiza and Chapiza), their populations' and health workers' perceptions about the COVID-19 impact on malaria burden and care. By conducting surveys, we identified factors associated with malaria infections that were the quantitative components of the research; as for the qualitative component, 10 to 30 minutes interviews were conducted to the population and health workers. Surveys and interviews findings were integrated through analytic assessment, resulting in a joint display.
Population and health workers' perceptions pointed out the prioritization of COVID-19 interventions, leaving malaria control activities aside, which was reflected in the fluctuation of malaria prevalence (17.6% for 2021 and 25.5% for 2022). Also, the population highlighted the limited access to healthcare facilities during the pandemic, the lack of medication for malaria treatment, and the absence of health posts in two communities played a role in the attendance of malaria. The latter one explains how communities with health posts were associated with lower odds of having an infection (OR=0.19 and 0.47; $p<0.05$).

15. Population genetic analysis of *Plasmodium falciparum* erythrocyte binding antigen-175 (*Pf*EBA-175) gene in Ghana

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Malaria still causes around 600,000 annual deaths, primarily in Africa. *Plasmodium falciparum* (*Pf*) merozoites, crucial for malaria development, invade erythrocytes through ligand-receptor interactions. Erythrocyte Binding Antigen (EBA)-175 serves as a significant ligand, facilitating invasion via glycoprotein A (GPA) receptors. The *Pf*EBA-175 exists in two dimorphic alleles (F and C) and mixed allelic haplotype (F/C). However, the prevalence of these alleles and their correlation with age, parasitemia and season in the Ghanaian population remain elusive. Archived Dried Blood Spots (DBS) from 210 malaria-infected individuals in Ghana were analyzed. With parasitemia levels pre-determined via microscopy, DNA was extracted and quantified using Qubit Fluorometer. Selective *Pf*EBA-175 gene amplification and characterization revealed F, C and F/C alleles through Agarose Gel Electrophoresis. Using R programming, Ghana's allelic distribution was linked to parasitemia, age, and season. Results showed 67.2% F, 18.82% C, and 13.98% F/C alleles. F allele dominance persisted across seasons, while C and F/C expression leveled during the dry season. Parasitemia correlated with F then C allele expression. F/C allele prevailed at moderate parasitemia. Consequently, T test yielded a p value of 0.3887 at 95% CI; F dominated hyper-parasitemia. F allele dominated all age groups, C followed, and F/C was higher in ≥ 60 age group, hinting at a possible immunocompromised targeting; however, T test yielded a p value of 0.5617 at 95% CI, indicating no statistical significance. Slightly high C allele expression in 'high' parasitemia suggests its possible role in merozoite invasion.

16. Merozoites surface proteins based genetic variations in *Plasmodium falciparum* and *Plasmodium vivax* field isolates from Nowshera district of Pakistan

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Background: The genetic diversity of malaria parasites contributes in their ability to adapt to environmental changes, development of drug resistance and escape from the host immune system, hence very important for control measures of malaria. This study aimed at analyzing the genetic diversity of *pfmsp1*and *pfmsp2* genes in *P. falciparum* and *Pvmsp-3α* gene in *P. vivax* isolates from District Nowshera in Pakistan.
Methods: Blood samples from 124 consented patients with uncomplicated malaria presenting to different hospitals of district Nowshera, were collected during March-August 2019, representing 28*P. falciparum* and 96*P. vivax* isolates. DNA of all samples was subjected to nested-PCR based allele specific markers analysis. *Pvmsp-3α* amplified fragments were further treated with Restriction fragment length polymorphism (RFLP) based *Hha1* restriction enzyme.
Results: In *P. falciparum* 21 alleles were detected, including 14 alleles for *Pfmsp-1* and 7 alleles for *Pfmsp-2*. Sub-allelic families MAD20 (50%) in *Pfmsp-1* and FC27 (75%) in *Pfmsp-2* family were predominant. Multiplicity of infection (MOI) was calculated as 1.4 and 1.2 for *Pfmsp-1* and *Pfmsp-2* respectively, with overall mean MOI of 1.34. In *P. vivax*, 4 allelic variants as Type A-D were detected for *Pvmsp-3α*through nested PCR while after RFLP digestion of amplicons, 9 sub-allelic variants (A1-A4, B1, B2, C1, C2 and D1) were observed at *Pvmsp-3α* locus.
Conclusion: This is the first ever report of molecular characterization of *P. falciparum* and *P. vivax* genotypes from District Nowshera Pakistan. Both *P. falciparum* and *P. vivax* field isolates exhibited moderate to high allelic diversity in district Nowshera, Pakistan.

17. Spatiotemporal correlation of malaria intensity and vector abundance in a pre-elimination setting of Choma district, Southern Zambia

Mukuma Lubinda[1], Anne Martin[2], Japhet Matoba[1], Caison Sing'anga[1], Harry Hamapumbu[1], Ben Katowa[1], Michael Musonda[1], Limonty Simubali[1], Twig Mudenda[1], Monicah Mburu[1], Mary Gebhardt[3], Edgar Simulundu[1], Timothy Shields[2], Douglas E. Norris[3], and William J. Moss[2] for the Southern Africa ICEMR

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Despite elimination efforts, low-level malaria prevalence of about 2% with seasonal outbreaks has persisted in Choma District, Southern Zambia. Previous geospatial risk maps found that parasitemic individuals tended to be clustered in areas near streams at lower elevations. In this way, spatiotemporal analysis of low-level parasite prevalence has been critical in informing strategies targeting elimination. This study aimed to produce malaria risk maps by assessing the spatiotemporal correlation of malaria parasitemia by quantitative polymerase chain reaction (qPCR) and vector breeding sites within rural health centre (RHC) catchment areas. Malaria case and entomological data were collected in 2022 and 2023 in Macha, Mapanza, and Simaubi RHCs. Passively-reported index case and neighbouring households within 250 meters were surveyed from April 2022 to April 2023. Residents were tested for parasitemia using qPCR from dried blood spots. In October 2022, entomological surveillance began at 38 sentinel households visited bimonthly. CDC light traps were set indoors and outdoors at each household, and larval breeding sites within 500 meters were sampled and geo-located. Intensity maps were created of the households of qPCR positive residents and breeding sites using kernel estimation with optimized bandwidths, and clustering by the K-function. The intensity and clustering maps were further compared by the cross-K function. Analysis was done in R and ArcGIS. We will present the results of the malaria intensity mapping, and clustering. We expect the results will inform further malaria risk mapping and targeted interventions in this pre-elimination setting.

18. Impact of volatile pyrethroid spatial repellant (VPSR) on the abundance of outdoor biting anophelines in a low malaria transmission setting, Southern Zambia

Limonty Simubali[1], Timothy Burton[1,2], Lewis Kabinga[1], Pebble Moono[1], Justin Moono[1,] Alpha Simudoombe[1], Charlton Munsanje[1], Jennifer Stevenson[1,2], Edgar Simulundu[1,2], Monicah Mirai Mburu,[1] and Neil F. Lobo[1,2]

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Introduction: Residual transmission in Southern Province, Zambia, is exacerbated by outdoor exposure to malaria vectors. This study aimed at evaluating the entomological impact of volatile pyrethroid spatial repellant (VPSR) product for peri-domestic use (outdoor kitchens) in semi-field-system (SFS) and village structures.

Methodology: The transfluthrin-based VPSR tool enables spatial protection against host-seeking mosquitoes. The two phases of this study included 1) The SFS study utilized replica outdoor kitchens with VPSR devices to evaluate their impact on laboratory *Anopheles gambiae*, mosquitoes assessing mortality and reproduction. 2) During the transmission season, a study was conducted on forty households in two villages, examining the impact of VPSR treated and untreated kitchens on human landing.

Results: SFS and field results demonstrated a significant reduction (40-60%) of mosquito numbers entering VPSR-treated structures. In addition to a reduction in landing, there were also mortality and reduction of fitness impacts with mosquitoes exposed to the VPSR.

Conclusion: This study demonstrates that VPSR can reduce the exposure of humans to vectors in both (SFS) and field (village) structures, with additional evidence for community impact through mortality and other impacts seen in transfluthrin-exposed mosquitoes. VPSRs are potential gap fillers as an alternative outdoor malaria intervention.

19. Spatial population genetics of *Anopheles coluzzii* in oceanic islands

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Anopheles coluzzii is a significant malaria vector in Africa and is the sole known vector present in two African oceanic islands São Tomé and Príncipe. Deployment of current and new control strategies against this disease vector is reliant on accurate assessment of mosquito dispersal patterns, and in this study, we characterized local gene flow patterns of *A. coluzzii* on São Tomé and Príncipe, using the spatial population genetics approach. Due to their geographic isolation, the two islands offer a specific field setting for assessing the local gene flow that is not affected by the immigration of mosquitoes beyond the islands' borders. We focused on the individual-based analyses of whole-genome sequencing data from the field specimens collected from 37 and 39 breeding sites in São Tomé and Príncipe, respectively, covering a significant portion of their inhabited areas. We found significant isolation-by-distance in São Tomé but not in Príncipe, and the spatial autocorrelation analysis revealed high genetic similarity between individuals within 7 km, indicating the likely range of mosquito's effective dispersal. The Estimating Effective Migration Surfaces (EEMS) analysis showed diverse migration rates within each island, identifying distinct regions of restricted migration on both São Tomé and Príncipe, that provide critical baseline information on mosquito dispersal for the future mosquito control activities within São Tomé and Príncipe.

20. Investigating the efficacy of native strains of *Metarhizium* from Burkina Faso on *Anopheles coluzzii* mosquitoes in both larval and adult stages for integrated vector management

Issiaka Sare[1,2,3], Mafada Viana[4], Abel Millogo[1], Lucien Lamy[1], Abdoulaye Diabate[1,2], Francesco Baldini[4], and Etienne Bilgo[1,2]

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Background: Entomopathogenic fungi can infect a wide range of insect hosts and have been used as an environmentally friendly alternative to chemical insecticides for pest control. This study aims to evaluate the effect of wild fungal strains from Burkina Faso on malaria? mosquito larvae and their reproduction behavior.

Methods: Wild isolated *Metarhizium* conidia (strains s10 and s26) were first tested against *An. coluzzii* adults by spraying the mosquitoes with spore suspensions. The survival of their larval and adult progeny was then measured. The *An. coluzzii* larvae were subsequently put in a solution with *Metarhizium* spores and the survival of the larvae and adults that emerged, the wing size, oviposition, blood meal rate, and reproductive behaviour (swarm formation and size, presence of mating pair) were measured. Survival was analyzed using Cox proportional hazards model, and the other history traits using generalized linear mixed models.

Results: Fungal infected adult mosquitoes laid more eggs than untreated mosquitoes ($p<2.2^{-16}$) but fewer larvae become adults compared to controls ($p<2.2^{-16}$). Once the fungal solution was applied to the water where larvae were reared, fewer larvae become pupae and adults compared to controls ($p= 2.2^{-16}$). However, adult body size was similar between the groups ($p= 0.1773$) and no difference in the blood meal rate was observed ($p= 0.379$).

Conclusion: This study shows the ability of the fungus to control mosquitoes, and these results show that conidia have potential for use on mosquitoes in both the larval and adult stages.

21. First report of *Anopheles stephensi* from Southern Ethiopia

Dawit Hawaria Logita[1], Solomon Kibret[2], Daibin Zhong[3], Ming-Chieh Lee[3], Delenesaw Yewhalaw[4], and Guiyun Yan[3]

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Anopheles stephensi is an emerging exotic invasive urban malaria vector in East Africa. The study sought to determine the geographic distribution of *An. stephensi* in southern Ethiopia. A targeted entomological survey, both larvae and adult, was conducted in Hawassa City, Southern Ethiopia between November 2022 and February 2023. *Anopheles* Larvae were reared to adults for species identification. CDC light traps and BG Pro traps were used to collect adult mosquitoes. Prokopack Aspirator was employed to sample indoor resting mosquitoes in the morning. Adults of *An. stephensi* was identified using morphological keys and then confirmed by PCR. Larvae of *An. stephensi* were found in 28 (16.6%) of the 169 potential mosquito breeding sites surveyed. Out of 548 adult female *Anopheles* mosquitoes reared from larvae, 234 (42.7%) were identified as *An*. *stephensi* morphologically. A total of 449 female anophelines were caught, of which 53 (12.0%) were *An. stephensi*. Other anopheline species collected in the study area included *An. gambiae* (s.l.), *An. pharoensis, An. coustani,* and *An. demeilloni.* The study, for the first time, confirmed the presence of *An. stephensi* in southern Ethiopia. The presence of both larval and adult stages of this mosquito attests that this species established sympatric colonization with native vector species such as *An. gambiae* (s.l.) in Southern Ethiopia. The findings warrant further investigation on the ecology, behavior, population genetics, and role of *An. stephensi* in malaria transmission in Ethiopia.

ORAL TALKS | SESSION 3
Sheldon Hall (w1214)
Moderator: **Emily Stucke,** University of Maryland School of Medicine

22. Gene expression analyses reveal the mode of action of artesunate on *P. vivax* parasites

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Plasmodium vivax is the second leading cause of malaria worldwide. In Cambodia, artemisinin combination therapies (ACTs) are the standard-of-care treatment for *P. falciparum* and *P. vivax.* While the *P. falciparum* response to ACTs has been well-studied, the molecular consequences of these drugs on *P. vivax* has been largely unexplored. Here, we used RNA sequencing of blood from *P. vivax*-infected Cambodian patients collected before artesunate treatment and at one-, two- and four-hours post-treatment to investigate the transcriptional response of the parasites to treatment. In contrast to the dramatic reduction in parasitemia, we observed few differences in gene expression one hour after treatment and no changes in stage composition. However, 1,373 parasite genes changed their expression between one- and two hours post treatment. Among these, we observed increased expression of genes involved in the endoplasmic reticulum stress response (e.g., eIK1, PK4), in DNA repair (e.g., DnaJ, RAD14), and in ubiquitin modification (e.g., ubiquitin-conjugating and -activating enzymes) suggesting that some of the mechanisms of artesunate killing are shared between *P. vivax* and *P. falciparum*. Additionally, we found increased expression of apoptosis-related genes (e.g., metacaspases), consistent with parasite death at this time point. These analyses provide insights into the mechanisms and timing of *P. vivax* killing by artesunate, which has been difficult to examine previously due to the lack of *in vitro* culture systems for this parasite. Our findings will improve our understanding of the mode of action of artesunate in *P. vivax* and help to prevent the emergence of drug resistance.

23. Implementation of a Human Cell-Based Malaria-on-a-Chip Phenotypic Disease Model for Drug Efficacy Evaluation

Michael J. Rupar[1], Stephanie Rogers[1], Hannah Hanson[1], Narasimhan Sriram[1], Brianna Botlick[1], Justin Zuniga[1], Zhanhe Liu[1], Steven Trimmer[1], Russell Emmons[1], Christopher J Long[1], Christopher W McAleer[1], Stephan Schmidt[2], Paola Favuzza[3], Phil Lowe[3], Nathalie Gobeau[3], and James J Hickman[1]*

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Of all the *Plasmodium* spp. found in humans, the infectious protozoans responsible for malaria, the *falciparum* species is the largest contributor to malaria mortality rates. A human-based platform to model disease pathophysiology and monitor drug efficacy is much needed for preclinical drug development. This Malaria-on-a-Chip model has demonstrated the capability of culturing all stages of the intraerythrocytic parasite life cycle and was utilized to evaluate drug efficacy and off-target toxicity of common antimalarial therapeutics. The optimum efficacious doses for chloroquine, artesunate, and lumefantrine were determined by analyzing the max tolerable dose and no observed adverse effects level in this human, multiorgan, serum-free platform. These doses were then implemented to study the clearance times of *P. falciparum* and any potential recrudescence of the chloroquine sensitive and resistant strains, 3D7 and W2. The viability of all organ constructs, as well as hepatic metabolic activity and splenic immune response, were monitored over the course of infection and treatment. Compounds were delivered as a monotherapy in a single, bolus dose immediately after infection with the parasite. A dose dependent clearance of the parasite was observed in both strains for all compounds. Recrudescence of the 3D7 strain was observed by day 7 for all chloroquine and lumefantrine treatments, but not with artesunate treatment. Recrudescence was not observed in the W2 strain. However, W2 infected systems exhibited a stabilization of parasitemia levels by day 7 when treated with chloroquine and lumefantrine, while those treated with artesunate continued to diminish. A significant dose dependent decrease in infected organ viability resulted from chloroquine treatment but no significant decrease in infected organ construct viability was observed when treated with lumefantrine or artesunate.

24. Immune responses in mice immunized with *Plasmodium falciparum* mRNA liver stage antigens: antibody, CD4 Tfh, CD8 T_{RM} and CD8 T_{EM} cells.

Steven Tan[1], Mohamad-Gabriel Alameh[3], Jonathan P Renn[2], Joselyn Allen[1], Alexander V Pichugin[1], Patrick E Duffy[2], Drew Weissman[3], and Urszula Krzych[1]

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Malaria remains one of the most threatening parasitic infections in the world, with *Plasmodium falciparum* (Pf) as the deadliest of the Plasmodium spp. The highly complex parasite lifecycle combined with a large genome present a challenge for developing efficacious vaccines with lasting protection. The most developed vaccine, RTS,S, based on Pf circumsporozoite protein (CSP) provides incomplete protection. Exploration of other pre-erythrocytic stage targets such as liver stage (LS) has provided new candidates for vaccine development. This strategy promises a broader repertoire of Ag-specific antibodies (Ab), CD4 and CD8 T cells. We have shown that mice immunized with a combination of *P. berghei* (Pb) CSP and LS Ags expressed as DNA-Adenovirus generate higher protection than CSP alone. Protection is associated with Ag-specific Abs and liver CD8 T_{RM} cells. We hypothesized that spz that escaped CSP-specific Abs were targeted by LS-Ag-specific responses. Our current efforts are directed towards utilizing mRNA template as it can accommodate many Ags. We designed mRNA-LNP expressing single Pf-LS Ags, TBP and SHMT, based on protective rodent parasite LS Ag orthologues, for immunization of mice. Analyses of mRNA-Pf-TBP- and Pf-SHMT-induced immune responses showed a divergence of reactivities. Whereas Pf-TBP induced high Ab titers, Pf-SHMT induced liver CD8 T_{RM} and CD8 T_{EM} cells that exceeded Pf-TBP-induced CD8 T cells. Peptides corresponding to Pf-SHMT and Pf-TBP-recalled high IFN-g responses, a signature of protective immunity. We continue to characterize and evaluate contributions of T cell and B cell responses induced with combinations of Pf-LS Ags including Pf-CSP to protective immunity against transgenic parasites. mRNA vaccines provide a rapid, flexible platform to explore malaria vaccine improvements where liver CD8 T_{RM} and CD8 T_{EM} cells, as well as CD4 Tfh, cells, B cells and Abs may be crucial for highly efficacious and protracted protection.

Support: MIDRP, Grant NIH NIAID, NIH Intramural, BioNTech SE

POSTERS

25. *Metarhizium* uses odors to attract hosts providing a biotechnological means of duping mosquitoes

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Metarhizium-based mycoinsecticides are promising tools for controlling insecticide-resistant mosquitoes, and their efficacy depends on the percentage of mosquitoes infected. We show that mycosed insect cadavers specifically produce volatile insect attractants, including the floral odor longifolene, that can increase pathogen spread through multi-host communities. We identified the responsible olfactory receptors in *Drosophila melanogaster* and showed that flies without these receptors are less frequently infected. Unlike the parental wild-type, transgenic *M. pingshaense* expressing pine longifolene synthase also produced longifolene on a medium for mass-producing fungi. The spores efficiently lured and killed male and female *Aedes albopictus, Anopheles sinensis*, and *Culex pipiens*. This attraction lasted over eight weeks, was not blocked by host odors, and was effective over an area 18-fold larger than World Health Organization experimental huts.

26. Decreased levels of PDGF-bb and VEGF are associated with increased risk of readmission or death in children with severe malarial anemia

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Background: Children with severe malarial anemia (SMA) typically have low mortality during acute episodes but have a high risk of post-discharge readmission or death. We hypothesized that the dysregulation of hematopoiesis, vascular growth factors, and endothelial function that occurs in SMA might affect risk of readmission or death.

Methods: Plasma was obtained from children (18 months to 12 years old) with SMA (N=145) in Kampala, Uganda on admission, and outcomes were assessed over 12 months of follow-up. Admission plasma levels of Angpt-1, Angpt-2, bFGF, EPO, G-CSF, PDGF-bb, sICAM-1, sVCAM-1, VEGF, and vWF were compared to risk of readmission or death over a 12-month follow-up period.

Results: Over 12-month follow-up, 19 of the 145 children with SMA were either readmitted or died: 15 children were readmitted (13 with malaria), and 4 children died. Children who were readmitted or died had lower levels of platelet-derived growth factor (PDGF-bb), vascular endothelial growth factor (VEGF), and angiopoietin-1 (Angpt-1). After adjustment for age and sex, decreased PDGF-bb and VEGF levels on admission were each associated with an elevated risk of readmission or death over the 12-month follow-up (adjusted hazard ratios [95% confidence intervals], 3.33 [1.79-6.25] and 5.88 [2.01-16.67], respectively).

Conclusion: In children with severe malarial anemia, decreased plasma levels of PDGF-bb and VEGF are associated with an elevated risk of readmission or death in the year following admission.

27. Application of machine learning in a rodent malaria model for rapid and accurate parasite counts

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Rodent malaria models (*P. yoelli* & *P. berghei)* serve as important preclinical antimalarial and vaccine testing tools. Efficacy measurements of these models often require manually counting parasite-infected red blood cells (RBCs), a time-consuming, repetitive process. We have developed machine learning (ML) software to expedite these studies by automating the counting of *Plasmodium*-infected RBCs in rodents. Previous ML methods created by our group, designed to count *P. falciparum*-infected RBCs in humans, accurately measure parasitemia in humans but need to be optimized to measure parasitemia in rodents. Using transfer learning, we retrained our ML model to target *P. yoelii* and *P. berghei*-infected RBCs, instead of *P. falciparum*. Our improved algorithm reliably measured *P. yoelii* and *P. berghei*-infected RBCs at a wide parasitemia range (0.13-74.12%). Automated parasitemia measurements strongly correlated with manual results (r = 0.996 for *P. yoelii*). The program was highly accurate for parasitemia >1%, with a mean error rate of 10.09%. Very low parasitemia (<1%) affected accuracy. However, our new software was designed to allow users to optionally verify and correct mislabeled infected RBCs, a quick process at parasitemia <1%. Testing on 3 different microscope setups produced results meeting WHO malaria microscopy standards. The software has been developed as an application for Windows and MacOS, outputting an excel file with graphical representations of the results. The dataset is currently being trained to stage parasites and identify reticulocytes. Automation of blood-stage parasitemia counts will help in the rapid evaluation of novel vaccines and antimalarials using an easily accessible *in vivo* model.

28. Determination of glycophorin C genotypes prevalence and association with *Plasmodium falciparum* density and diversity in malaria patients in Ghana

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Genetic diversity poses a barrier to success of vaccine development targeting *Plasmodium* species as evolutionarily favourable genes that have been chosen have significantly been impacted by it. Several malaria-protective polymorphisms have been linked to genes in the red blood cells that modify or impair their structure or activity. Identifying the genes involved and their impact on malaria risk is a potentially useful technique of examining the host-parasite relationship. The study investigated the genotypes of Glycophorin C (GYPC) protein and their effect on *Plasmodium falciparum* density and diversity in symptomatic malaria patients across Ghana. A total of 214 dry blood spot archived samples collected in 2021 from ten randomly chosen health facilities across Ghana's sixteen regions were used to characterize GYPC genotypes in the Ghanaian population into GYPC homozygous wild type, heterozygous and homozygous GYPC exon-3 deletion using PCR and agarose gel electrophoresis and determine its association with *P. falciparum* density, diversity using PET-PCR and nested PCR respectively. Out of the 214 samples with a history of febrile illness, 201(94%) had the GYPC heterozygote with 13 (6%) of these being with the homozygous GYPC wild type and no record of the homozygous GYPC exon-3 deletion wild type in the Ghanaian population. There was however no significant association between the GYPC genotypes and *P falciparum* density (p=0.285) and diversity (p=0.805).This study serves as a baseline study to provide functional data on the impact of GYPC exon-3 deletion on *Plasmodium falciparum* infection in Ghana thus adding up to our current understanding of vector-host interactions from which further studies may bring bare the relationship between functional and structural diversity of RBC invasion concerning GYPC exon-3 deletion and *P falciparum* as well as knowledge on the possible development of transmission intervention tools and vaccines.

29. Comprehensive characterization of *Plasmodium vivax* antigens using high-density peptide array

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Plasmodium vivax is the second most prevalent *Plasmodium* parasite species and has the widest global distribution. *P. vivax* malaria has long been thought to cause milder disease than *Plasmodium falciparum,* but recent studies have shown an increase in both severe disease and drug resistance associated with vivax malaria. Additionally, *P. vivax* has a distinct life cycle with the notable formation of dormant liver-stage hypnozoites that are capable of reactivating and establishing relapse infections weeks to months after the initial infection has been cleared. Identification of serological markers of exposure to and protection against *P. vivax*, as well as biomarkers for hypnozoites could significantly improve vivax malaria control and elimination efforts. Here we describe a high-density peptide array containing 5.7 million peptides covering the entire coding sequences of all known and putative *P. vivax* proteins. We probed this array with 30 serum samples from Cambodian adults with vivax malaria who either went on to relapse or clear the infection. Our preliminary analyses reveal novel *P. vivax* antigens with very high reactivity in Cambodian patients and highlight the high variability among patients in the epitopes recognized. The results of this project will provide a comprehensive profile of the serological response to *P. vivax* antigens in the context of clearance and relapse, which will be invaluable to therapeutic development.

30. Leveraging structural homology for characterization of *P. falciparum* PUFs — proteins of unknown function

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The current annotation of the *Plasmodium falciparum* genome contains 5318 predicted protein coding genes. Of these, 1537 genes contain the words “unknown function” in the gene product description, while many genes lack informative GO/InterPro/Pfam annotations. Such *P. falciparum* **p**roteins of **u**nknown **f**unction(**s**) (PUFs) could provide novel rapid diagnostic and therapeutic targets. *In silico* prediction of GO/InterPro/Pfam annotations is commonly undertaken using sequence similarity approaches such as BLAST where high sequence similarity between query and annotated proteins are used to transfer annotations. However, low sequence similarity between annotated proteins and *P. falciparum* PUFs poses a challenge for annotation. To circumvent this, we have employed a “structural homology” approach *i.e.,* searching for similarities between tertiary protein structures. We used TM-Vec (Hamamsy *et al.*, 2023 Nature Biotechnology *in press*), a deep learning framework which utilizes protein language models and neural networks for computing structural similarity scores and alignments between protein sequences, to generate novel annotations for poorly annotated proteins and PUFs. We utilized a tailored weighted k-nearest neighbors strategy in the embedded space to transfer functional annotations from a curated database of proteins from species in phylum Apicomplexa to *P. falciparum* proteins, while paying particular attention to proteins with intrinsically disordered regions. Here we present putative InterPro and Pfam annotations for PUFs predicted to contain a singular globular domain. This is a promising approach for predicting novel annotations in *Plasmodium* proteins and PUFs using structural homology instead of traditional sequence similarity approaches.

31. Artemisia afra will save Africa

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A team of medical doctors in RDCongo, Jerome Munyangi and Michel Idumbo, have run randomized clinical trials on a large scale in the Maniema province with the participation of some 1000 malaria infected patients. The trials were run in conformity with the WHO procedures and compared Artemisia annua and Artemisia afra with ACTs (Coartem and ASAQ). For all the parameters tested herbal treatment was significantly better than ACTs: faster clearance for fever and parasitemia, absence of parasites on day 28 for 99.5% of the Artemisia treatments and 79.5% only for the ACT treatments. A total absence of side effects was evident for the treatments with the plants, but for the 498 patients treated with ACTs, 210 suffered from diarrhea, and/or nausea, pruritus, hypoglycemia etc. The large-scale trials confirm those of Constant Kansango in Katanga who had found in a trial with 44 Plasmodium falciparum infected patients that after 7 days of treatment with 20 gr of capsules containing A afra powder the gametocytes had completely disappeared, except for one patient. Artemisia afra does not contain artemisinin. The best explanation available is the high arginine content of Artemisia plants.

32. Novel lipoic acid salvage inhibitors prevent growth in intraerythrocytic *Plasmodium falciparum*

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Lipoic acid (LA) is an essential cofactor that *Plasmodium falciparum* parasites salvage from human hosts during the intraerythrocytic development cycle (IDC) for mitochondrial function and survival. The salvage pathway consists of two essential enzymes, LipL1 and LipL2 that attach LA to the pyruvate dehydrogenase complex (mPDH), the alpha-ketoglutarate dehydrogenase complex (KDH), and the H-protein. Human hosts lack a homologous salvage pathway, making this pathway an attractive target in *P. falciparum* for anti-malarials. Previously, 8-Bromooctanoic acid (BrO) was shown to inhibit lipoate protein ligases (LPLs), including LipL1 with killing activity in blood-stage *P. falciparum*. Here, we tested two novel inhibitors, C3 and LAMe, that were designed using a competitive proteomics screen with a lipoyl probe against homologous LPLs in bacteria. We then screened their activity against BrO in *P. falciparum* and obtained EC_{50} values for BrO, C3, and LAMe as 19uM, 27uM, and 15uM respectively. Notably, C3 and LAMe achieved effective parasite clearing at lower concentrations and bound LipL1 more tightly compared to BrO. Thus, C3 and LAMe represent the most potent LA salvage inhibitors reported to date and further validate LA salvage as a target for putative anti-malarials.

33. Investigating mitochondrial gene essentiality in blood stage *Plasmodium falciparum* parasites via inducible and efficient *piggyBac* transposon-driven mutagenesis

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The mitochondrion is critical for the growth and survival of blood-stage *Plasmodium falciparum* parasites. Currently, the two known essential mitochondrial processes in blood-stage malaria parasites are pyrimidine biosynthesis and acetyl-CoA synthesis; our lab has identified a collection of genes essential for these two processes via reverse genetics techniques and metabolic bypass mechanisms. However, we were unable to identify all the factors and transporters required for either pyrimidine biosynthesis or acetyl-CoA production through reverse genetics approaches alone. To identify the complete complement of mitochondrial genes essential for pyrimidine biosynthesis or acetyl-CoA synthesis, we will use a forward genetic tool in conjunction with two metabolic bypass conditions. The forward genetic tool *piggyBac* transposon-driven mutagenesis has previously been used to screen blood-stage *P. falciparum* parasites for essential genes, but the screen took many years to complete and resulted in a library of only 22,622 mutants. We have modified the *piggyBac* platform to create an inducible system with high transposon efficiency that has allowed us to generate a library of over 150,000 mutants in a single experiment. Now, following optimization of our quantitative insertion-site sequencing (QIseq) method for *Plasmodium* mutant libraries, we will combine our modified *piggyBac* transposon-driven mutagenesis system with the two metabolic bypass mechanisms to identify the full cohort of essential mitochondrial genes responsible for pyrimidine biosynthesis or acetyl-CoA synthesis.

34. Beyond the barcode: Unlocking the potential of the mitogenome for mosquito identification

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Globally, mosquitoes implicated in the spread of vector-borne diseases are expanding in geographic range. Likewise, understudied anopheline mosquitoes; with many belonging to cryptic species complexes may be associated with driving malaria transmission in regions where primary and well-recognized vectors are close to elimination. *Anopheles species 6*, recently identified as *Anopheles gibbinsi* have shown to be associated with malaria transmission in Kenya. Similar in morphology to other well-established malarial vectors; *An. gibbinsi* was previously reported to be present in east Africa and has now been reported from southern Africa. Recent collections in Zambia have shown this mosquito species to generally exhibit zoophilic and exophilic behavioral patterns, with occasional contact with humans. With continued monitoring of *An. gibbins*i as a potential malaria vector, tools for accurate mosquito identification are necessary; with current molecular tools proving to be problematic for resolving members belonging to species complexes due to the paucity and limitations of current genetic data. Sequencing strategies have revealed that the full mitochondrial genome is useful in rectifying species classification in comparison to commonly targeted molecular reference barcodes. Here we describe a genome skimming strategy used to capture the mitogenome of *An. gibbinsi*, with phylogenetic analyses with other well-established vectors of human malaria demonstrating that this mosquito species separates into its own clade. This study demonstrates skimming as relatively inexpensive and efficient approach for generating reference genomics and taxonomic rectification for mosquito species, which contribute valuable information for the development of vector mitigation strategies.

35. Assessing the management and outcomes of pediatric severe malaria in rural western Uganda

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Severe malaria is a leading cause of pediatric morbidity and mortality in Uganda. Most information on quality and effectiveness of care is sourced from urban referral centers, while less is known about rural settings. Herein we present interim results from an ongoing prospective, observational cohort study of severe malaria among children admitted to a level IV health center in Kasese, Uganda. We collect demographic, clinical, and laboratory results. We contact participants 14 days post-discharge to assess vital status and interim care seeking. We enrolled 52 children (median age: 4 years, IQR: 1.5-10) in summer 2023. 92.3% of participants had a parasitologically confirmed diagnosis of malaria. The majority (n=43, 82.7%) had previously sought care elsewhere including at drug shops (34.9%) or government health facilities (32.6%). Almost all (96.2%) patients received ≥1 dose of intravenous artesunate and 80.8% also received an antibiotic. More than a quarter (n=13, 27.7%) had evidence of severe malarial anemia (hemoglobin <5 g/dl), of whom 92.3% received a blood transfusion. Most patients (67.3%) were transitioned to oral artemisinin-based combination treatments prior to discharge. Of the 34 patients that had reached the follow-up point, 38.2% of patients reported still experiencing symptoms related to their hospitalization, with nausea/not feeding being the most common symptom (61.5%). One death of a seven-month-old was documented. The management of severe malaria among children at the first tier of care with full services appears to generally align with guidelines. The high proportion of participants seeking care at lower-level facilities prior to admission warrants further investigation.

36. A silicon rhodamine-fused glibenclamide to label and detect malaria-infected red blood cells

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The malaria parasite *Plasmodium falciparum* affects the life of millions of people worldwide every year. Recently, naturally acquired cases of malaria occurred in the USA for the first time in decades, which underlines the danger of this neglected tropical disease to this day. Therefore, the examination, as well as the detection, of the parasites replicating within the red blood cells of their human host is of paramount importance. For that matter, appropriate tools are needed to simplify the investigation of parasitized red blood cells. Herein, we design and apply a silicon rhodamine-fused glibenclamide (**SiR-glib**). We showcase this far-red fluorescent, fluorogenic and organelle-targeting sulfonylurea in living mammalian cells and tissues, before characterizing its labeling performance in red blood cells infected with the asexual developmental stages of *Plasmodium falciparum* by confocal microscopy. We further successfully tested **SiR-glib**-labeled parasitized red blood cells with a portable smartphone-based microscope, a first step towards a potential usage of this probe in diagnostic procedures in malaria endemic areas.

37. Identifying Jhum (shifting) cultivation as a risk factor by ecological, epidemiological, and entomological studies: Targeted and Tailor-made Intervention cocktail for accelerated malaria control in Jhum Cultivator Tribes of Dhalai District, Tripura State, India

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With India aiming to eliminate malaria by 2030, several unique strategies are required to reach the hard-to-reach population, serving as the Elimination bottleneck. Tripura, a small State in North-Eastern India, faces some specific and unique challenges, with a malaria epidemic in 2014, outbreaks and some consistent high malaria-endemic pockets. However, the vast majority of the cases are persistently and disproportionately concentrated in a few areas of Tribal pockets of some Districts with lots of micro-heterogeneity between the PHCs, Subcentres and even between villages. Using high-resolution satellite imaging, ecological features like the Jhum cultivation (where villagers slash and burn the forests in one hillock deep inside the forest area and cultivate for a year), other agriculture, plantation, etc. Land-Use-Land-Covers were mapped for Tripura, and the relationship to malaria cases was explored, leading to a strong correlation between malaria and Jhum cultivation. The final model, after rigorous model selection protocol, demonstrated a significant positive correlation effect of the relative proportions of block area under jhum cultivation and negative for urban settlements, while none of the other LULC types or climatic variables were found to be associated. We have identified some specific and unique features of parasites, vectors and the host leading to the gaps responsible for the persistently high incidence in these pockets, which led us to design a targeted additional intervention package and evaluate its impact and operational feasibility. Time series analysis showed slide-positivity-rate reduction and good acceptance and compliance to some additional interventions and provided several insights into the existing vectors and vector control measures. me Based on these observations, several recommendations are communicated to State and National Health Program authorities, and a scale-up quasi-experimental study is lined up.

38. Unearthing mitogenomic variation in the *Anopheles coustani* species group

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Cryptic anopheline species complexes pose significant obstacles to malaria eradication because they potentially perpetuate residual transmission. Members of cryptic species complexes are morphologically indistinguishable but genetically and possibly phenotypically distinct. They also occupy variable ecological niches, some of which are not accessible by traditional indoor vector control methods. For example, members of the *Anopheles coustani* species complex typically feed outdoors on non-human hosts but have demonstrated human and indoor foraging behaviors and associations with *Plasmodium falciparum* transmission. The primary method of cryptic species identification is DNA barcoding via sequencing the mitochondrial *COI* and nuclear *ITS2* regions. This approach has yielded variable and inconsistent results on species identity when compared to GenBank using BLAST and conflicting phylogenies, making it insufficient for taxonomic resolution and discrimination. Both regions' short sequence length is enough to identify species but not for constructing phylogenetic trees with high statistical support and inferring deeper evolutionary relationships and groupings. We will address this challenge by sequencing the entire mitochondrial genomes of four Zambian species of the *An. coustani* species complex; *An. coustani* s.s., *An. ziemanni, An, paludis*, and *An. tenebrosus,* on an Illumina platform, using NOVOPlasty for assembly, and annotating on MITOS. These mitogenomes will provide genetic references for developing improved DNA barcodes or molecular tools that can distinguish these taxa, reducing misidentification and the potential for improper deployment of control measures. These data will also allow additional insights into the evolutionary history of this species group and their relationship to other African anophelines.

39. Differential *fikk* gene expression in severe clinical cases of malaria (*P. falciparum*) in Malian children under the age of 5

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Background: Plasmodium falciparum is responsible for the most severe form of human malaria. Unlike other *Plasmodium* species, *P. falciparum* actively exports approximately 18-22 *fikk* kinases into its membrane and host cell via Maurer's clefts. These novel *fikk* kinases appear to carry out the activation and subsequent trafficking of molecules in an infected erythrocyte (IE). Hence, they may play a critical role in the stage-dependent remodeling of the IE membrane and PfEMP1 expression. In this paper, we investigated the differential expression of 22 *fikk* kinases in severe clinical syndromes of *P. falciparum*—specifically cerebral malaria (CM), severe malaria anemia (SMA), and concurrent syndromes (SMACM)—in Malian children under the age of 5.

Methods: Blood sample data was sourced from a 2018 matched case-control study of cerebral malaria in young children in Bandigara and Bamako, Mali. A differential expression analysis pipeline was employed to generate expression data, incorporating genomic/protein library preparation, read quality control, mapping and quantification, transformation and RNAseq bias removal, and subsequent functional analysis via R programming language. In addition, unsupervised machine learning algorithms, hidden Markov models, and domain prediction algorithms were used to predict *fikk* functionality and cellular location. Our next step is to test for the statistical significance of *fikk* gene read counts and expression levels through the Wilcoxon signed-rank test. Library data was sourced from Plasmodb, the official *Plasmodium* genome database.

Preliminary Results: Preliminary findings on an initial 13 clinical samples suggest possible differential expression of *fikk8, fikk9.2,* and *fikk4.2,* with increased expression levels in more severe cases. Additionally, we found that one *fikk* type did not classify well into any of the annotated reference genome *3D7* genes, suggesting the existence of a new fikk subgroup located on chromosome seven. We found that *fikk* subgroup expression is consistently diverse throughout both severe clinical syndromes and matched controls. This may suggest that *fikk*s undergo synchronous concurrent expression as opposed to individual asynchronous expression. Upcoming studies will lend more conclusive insight into the differential expression of *fikk* genes in severe cases versus uncomplicated controls.

40. Challenges of malaria elimination in Botswana: A decade perspective from 2009-2019

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Background: In 2008 the Ministry of Health was inspired by an overall decline of malaria prevalence from 4.2% in 2000 to less than 1.0% in 2008 of the total population, these results motivated Botswana to embark on Malaria Elimination with a target to eliminate malaria by 2015. Epidemic of 2017 after heavy rainfalls, gave the Government of Botswana a huge setback towards elimination of malaria due to that cases of malaria were reported in the South-East District, a region not classified as endemic in Botswana. Insufficient funding, shortage of manpower, and better understanding of the behavior of malaria vector species in Botswana are associated with the challenges of elimination of malaria in Botswana.

Objective: To establish challenges associated with elimination of malaria in Botswana.

Methodology: Retrospective data from Ministry of Health was used exp the total expenditure allocated to the national malaria program in Botswana from 2009 to 2019, as well as the number of trained personnel and knowledge about the vector species affecting the endemic regions. The national strategic plans outlined their expenditure budgets which the national funding from the government never allocated the required funds and donor funding could not fill the gap.

Results: Funding of the national malaria program reduced by 20% from 2009 and the number of trained skilled personnel reduced although the program has insufficient vector species information.

Conclusions: Challenges of malaria elimination in Botswana are associated with lack of funding and shortage of skilled manpower.

41. What's for dinner?: Development of a multiplexed PCR blood meal identification assay

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Blood meals taken by hematophagous arthropods can provide insight into feeding behaviors and the role of vectors that transmit vector-borne pathogens including the malaria parasite. Through the development of a multiplexed PCR assay, blood meals taken by anopheline mosquitoes may be identified to specific vertebrate hosts and provide insight into the efficacy of vector control methods currently applied in malaria endemic regions. Additionally, integral knowledge can be gained on the foraging behaviors of understudied vectors or those that may be exhibiting alternative foraging behaviors to drive residual malaria transmission. In this study, a multiplexed PCR assay was developed to identify host blood meals on animals of interest and humans. DNA was extracted from human, goat, cow, pig, dog, chicken and goat blood or tissue controls. Preliminary results reveal the reliable detection of DNA at 5 ng/µL for all hosts used, with varying limits of detection for each respective animal host. Furthermore, experiments have demonstrated that this assay can identify blood meals taken by both field-collected *Anopheles gambiae* s.s. and *An. funestus* s.s., both major vectors of malaria in study sites, and has the potential to identify contributing hosts in mixed blood meals. Future work includes assay efficacy on digested blood meals using time points post feeding as well as its application on other anopheline and mosquito species of medical importance.

42. Bioinformatic approach to design a *Plasmodium falciparum* PfRipr multi-epitope vaccine construct

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Transmitted via mosquito, *Plasmodium falciparum* is the most lethal parasite of its genus but has evaded many treatment and vaccine efforts due to its complex life cycle and redundant invasion mechanisms. Epitope-based vaccines hold significant promise for malaria vaccine development due to their ease of development and ability to target dominant regions in antigenically variable pathogens. The recently characterized protein *P. falciparum* merozoite Rh5 interacting protein (PfRipr) is nonredundant, highly conserved, and essential for erythrocyte invasion, making it an ideal target for a bloodstage malaria vaccine. Using *P. falciparum* sequences collected from Burkina Faso and Uganda, we assessed the immunogenic potential of PfRipr epitopes regarding T-cell receptor binding and B-cell recognition. T-cell receptor binding was predicted using NetMHCpan searching against MHC I and II alleles with high regional frequencies. Using an in-silico 3D model of PfRipr predicted via AlphaFold, tertiary structures of all PfRipr sample sequences were predicted via SWISS-MODEL then analyzed by ElliPro to identify linear and discontinuous B-cell epitopes. Putative epitopes were filtered using allele coverage, conservation, antigenicity, and allergenicity. Between the two datasets, there were 19 matching epitopes with 7 MHC I, 9 MHC II, 1 linear, and 2 discontinuous. These epitopes were used to design a multi-epitope-based blood stage vaccine construct against *P. falciparum*. To validate their predicted immunogenicity, epitopes can be further investigated using *in silico* protein stabilization and docking simulations, *in vitro* methods such as HLA stabilization or T-cell activation assays, and *in vivo* methods using transgenic mouse models.

43. Revealing functions of hemocytes affecting *Plasmodium falciparum* infection in *Anopheles gambiae*

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Hemocytes are the professional immune cells of mosquitoes since they are the only cells that mediate both cellular and humoral immune responses. Despite many mechanisms of hemocytes' immune responses being well known, their functions against human pathogens such as *Plasmodium* are still unknown. Here we present results showing hemocytes depletion in the mosquito *Anopheles gambiae* decrease the number of *P. falciparum* oocyst. This effect is intensity-dependent and only occurs in mosquitoes infected in high doses. On the other hand, hemocytes depleted mosquitoes show high mortality after blood feeding, which is related to the loss of midgut epithelium integrity. These results suggest, that hemocytes play an agonist role against *P. falciparum* and not an antagonist as occurs against *P. berghei* and help to keep the integrity of the midgut epithelium.

44. Two CLIP serine proteases regulate *Plasmodium* oocyst melanization in *Anopheles gambiae* mosquitoes

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Anopheles gambiae mosquitoes rely on their innate immune responses to fight against *Plasmodium* infection. Among these responses is the melanization immune response, which is characterized by the deposition of melanin on the surface of the parasite preventing it from gaining nutrients and thus triggering its death. Here, we attempt to compare the immune factors known to be involved in the melanization of *Plasmodium* ookinete to those that regulate oocyst melanization. We found that two CLIP serine proteases, *CLIPA2* and *CLIPA14*, are involved in regulating both *Plasmodium* ookinete and oocyst melanization. Cosilencing *CLIPA2* and *CLIPA14* triggered both *Plasmodium falciparum* ookinete and oocyst melanization. Our future aim is to generate a CLIPA2/A14-knockout mutant *Anopheles gambiae* mosquito capable of melanizing early and late *Plasmodium* developmental stages and thereby blocking the parasite transmission.

45. The impact of transmission intensity and human mobility on detecting patterns of genetic relatedness across *Plasmodium falciparum* populations.

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Background and aims: Malaria parasite genomic data are increasingly used to identify highly related infections which can reveal spatial corridors of parasite transmission including routes of parasite importation. 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 different levels of parasite relatedness and connectivity across geographic regions. Further investigation is required to determine which patterns of connectivity we expect to be able to reliably detect with high quality, densely sampled genomic data under different human mobility and transmission patterns.
Methods and Results: To this end, we constructed an individual human and mosquito model of malaria transmission calibrated to entomological, epidemiological, and parasite genomic data from a densely sampled longitudinal cohort in a moderate transmission region of Western Kenya. We incorporated human movement in this model using gravity models that account for trip length and explored a wide range of mobility patterns. We evaluated two identity-by-state (IBS) measures of parasite relatedness and used hypothesis testing to assess whether these measures accurately identified the expected spatial structure generated by various mobility and transmission patterns across sampled regions. We further investigated how our hypothesis testing behaves under different sampling schemes, levels and mechanisms of missingness, and gradients of transmission intensity. In settings of moderate transmission and connectivity across locations, we found that IBS measures of relatedness, even using frequently sampled longitudinal infection data, were unable to reliably detect any spatial structure. We found that as mobility and transmission increase there is a sharp drop off in the ability to reliably detect spatial structure using parasite genetic data and IBS measures.
Implications: This finding suggests that in many settings, identity-by-state measures of relatedness will be underpowered to identify spatial corridors of parasite transmission. This has important implications for the use of parasite genomic data in illuminating patterns of transmission.

46. A naturalistic system to decode the chemosensory neurobiology of mosquito attraction to human scent

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Mosquitoes exhibit strong innate sensory drives to seek out humans. Human body odor is a crucial cue used by mosquitoes for hunting humans over a range of spatial scales. We engineered a naturalistic mosquito behavioral assay that tracks landing preferences of *Anopheles gambiae* towards heated targets mimicking human skin temperature that are baited with whole body odor from humans or other host-related olfactory stimuli. We determined that *An. gambiae* prefers to land on heated targets baited with body odor from one human over CO_2, and the scent of one human over another. When simultaneously presented with a choice between the scent of six humans, we found individuals at both ends of the attractiveness spectrum who are innately more or less attractive relative to other humans over replicate nightly trials. We identified a panel of airborne compounds including specific volatile carboxylic acids putatively associated with modulating human attractiveness to *An. gambiae*. Additionally, we applied CRISPR-Cas9 T2A-In Frame Fusions and the QF2-QUAS system to gain genetic access to the mosquito olfactory sensory system. We targeted olfactory sensory neurons expressing chemoreceptors ORCO, Gr22, Ir25a and Ir76b. Using these reagents in combination with calcium imaging we visualized responses of different classes of olfactory sensory neurons to components of human odor. These optimized methods to quantify mosquito olfactory preferences in naturalistic conditions, in combination with imaging of mosquito neural activity have the potential to be applied to yield fundamental insights into the molecular and cellular basis of mosquito attraction to humans and olfactory preferences.

47. Leveraging community health workers to sustain universal bed net coverage in rural Uganda: interim results of an ongoing pilot feasibility study

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Background: Long-lasting insecticidal nets (LLIN) are a cornerstone of malaria control programs but sustaining target coverage levels (1 LLIN per 2 household members) between mass distribution campaigns is challenging. We are evaluating the feasibility of a novel distribution strategy leveraging community health workers (CHW) under an integrated community case management (iCCM) program.
Methods: We conducted a cross-sectional household survey in two villages in Kasese District, Western Uganda to assess baseline LLIN coverage and parasitemia, followed by an open-label, pilot feasibility trial. CHWs in both villages were trained to measure household LLIN coverage when a child is RDT positive. CHWs in the intervention village distributed LLINs to households below target levels.
Results: At baseline 83, 27% of households had at least 1 LLIN. Parasitemia $PfPR_{2-10}$ was 10% in Nyarukungu (control) and 38% in Katebe 1 (intervention) with percentages of 0% vs 10% and 20% vs 39% when stratified by LLIN coverage comparing homes meeting and not meeting target levels, respectively. In August 2023, CHWs saw 44 children in Nyarukungu and 56 children <5 years of age in Katebe 1 for malaria with 59% testing positive in each village. In the intervention village, a total of 29 ITN were distributed to 8 households. The study is ongoing.
Discussion: Our results demonstrate low proportions of universal LLIN coverage near the end of the 3-year distribution cycle with higher proportions of parasitemia in households reporting below target coverage levels. While preliminary, LLIN distribution through iCCM appears feasible and may complement mass distribution campaigns.

48. Conserved subgroups of *Plasmodium falciparum* RIFIN antigens predominate in cerebral malaria cases from Mali and Malawi

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Cerebral malaria is the deadliest manifestation of *Plasmodium falciparum* infection. Central to its pathogenesis are parasite antigens displayed on infected erythrocytes that enable cytoadherence in the microvasculature. These antigens belong to diverse multi-gene families, the largest of which are the RIFINs. We investigated *rif* mRNA transcript diversity in cerebral malaria cases from two distinct African regions. We hypothesized that predominant *rif* transcripts in infections from West Africa (Mali) and East Africa (Malawi) share high sequence identity, suggesting important roles in pathogenesis. We collected blood from Malian (n=22) and Malawian (n=10) children with cerebral malaria. For each sample, we conducted RNA sequencing, assembled *de novo* parasite transcriptomes, and identified mRNA transcripts encoding RIFINs. We performed multiple sequence alignment on the translated protein sequences and visualized sequence similarity with principal component analysis. The average amino acid identity shared between RIFIN transcripts was 44%. We identified two large transcript clusters corresponding to the A-RIFIN and B-RIFIN subgroups, each with similar distributions of Malian and Malawian cases. Although parasites expressed a breadth of *rif* variants, conserved subgroups predominated. One cluster of *rif* transcripts shared ~93% sequence identity and was highly expressed by Malian parasites. The other cluster shared ~99% sequence identity and diverged significantly from the majority of transcripts; these transcripts predominated *rif* expression in both Malian and Malawian infections. These two conserved RIFINs are interesting candidates for further investigation as vaccine targets. Next steps include using unsupervised approaches to define additional RIFIN subgroups and comparing their relative expression in cerebral vs. uncomplicated malaria.

49. Testing CD1 mice with VAR2CSA peptides for immunogenicity

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VAR2CSA expressed by *Plasmodium falciparum* has been isolated from the placenta and codes for Pfemp1, which is found on the membrane surface of RBCs that are infected by the parasite. Women naturally acquire resistance to *P. falciparum* over successive pregnancies as they acquire antibodies against 7 parasitized red cells that bind chondroitin sulfate A (CSA; serves as receptor for adhesion in infected blood cells in the placenta), which proves that a vaccine is possible. As VAR2CSA has been proven to be a very effective target for placental malaria, its size of 350 kDa is too big, which would cause difficulties in creating a whole protein vaccine. Therefore, creating a vaccine with subunits of VAR2CSA with the most immunogenic peptides is the next best approach. A series of ELISAs were performed in this study to characterize the individual peptides' derived from VAR2CSA. Peptides with the highest correlation between antibody reactivity and BIA were identified and narrowed down from a number of 1,807 unique VAR2CSA peptides to only 33. 5 peptides of these are used in this animal study; P6-8, P12-13, P14, P21, P25. P6-8, P12-13, and P21 have been conjugated to the carrier protein EcoRm, and P14 and P25 have been conjugated to BSA. Full length VAR2CSA peptides were then used for binding inhibition assays to test the ability of the peptide-specific antibody to block the parasite binding to CSA. Next, the capacity of these antibodies were assessed to recognize native VAR2CSA expressed by the parasite in Flow cytometry.

50. Investigating the role of non-selective stress in structural rearmaments of the *Plasmodium falciparum* genome

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Changes in the copy number of large genomic regions, termed copy number variations (CNVs), contribute directly to drug resistance and impact fitness in the malaria parasite. We hypothesize that the uniquely AT-rich *Plasmodium falciparum* genome facilitates the generation of CNVs, particularly in response to selective pressures. However, we do not know if *P. falciparum* is spontaneously generating CNVs randomly across the genome in the absence of stress, or if they are stimulated in response to low level stress. Detection of these 'rare' CNVs requires techniques capable of single cell resolution. Here we use our single cell genomics pipeline to assess the abundance of rare CNVs in ~40 stressed and unstressed 2-cell parasite samples. In combination with our own approach to CNV calling, our collaborators have co-developed a bioinformatics pipeline specialized for detecting rare CNVs in small, haploid genomes. By using these tools with the inclusion of 10-cell samples to control for sequencing noise, we are able to generate high quality low-genome data (mean coverage = 37.5, norm. dev = 3.0). This enabled us to identify a low basal rate of rare CNV generation that is exacerbated by the addition of stress. Further, we validate that we do not artificially select for the generation of CNVs through carful stage isolation and comparative genomic analysis, particularly at known resistance loci. Ultimately, these results begin to help us better understand CNV evolution in the malaria parasite, and emphasizes the roll of stress in the mechanism. In future work, we will apply these techniques to parasites of different origin to better understand how these important structural rearrangements contribute to genetic heterogeneity globally.

51. In silico data mining reveals impressive diversity of antimicrobial peptide-coding genes in malaria vector *Anopheles gambiae*

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Malaria remains a major concern in global public health. The extent of malaria worldwide and the urgent need for new chemoprophylactic and chemotherapeutic agents foster the investigation of new drug pipelines that improve or replace current treatment strategies. Antimicrobial peptides (AMPs) have been shown to be promising candidates to target malaria parasites. Additionally, AMPs can be envisioned to target parasites within mosquito hosts. Although a few classes of AMPs have been described inhibiting sporogonic stages of Plasmodium in mosquitoes, our understanding of the molecular diversity and function of AMPs in the major malaria vector An. gambiae is largely lacking. We have initiated a study on the discovery of novel mosquito AMPs through a comprehensive in silico survey. Data mining on publicly available An. gambiae genome revealed an impressive diversity of over 30 potential novel gene-encoded AMPs. Spatial-temporal transcript distribution was analyzed in distinct mosquito tissues and developmental stages, along with their response to bacterial stimuli and P. falciparum infection. Additionally, the participation of AMPs during parasite infection was studied in vivo. To reveal their antimicrobial, or other, functions, 12 peptides were selected for in vitro activity testing against a panel of bacteria, fungi, and Plasmodium. The discovery of new classes of AMPs was further supported by the identification of ortholog genes in other mosquito species. Our study provides the first step towards the expansion of AMP repertoire in medically relevant mosquito species and paves the way for the development of novel strategies to mitigate the impact of vector-borne diseases.

52. Developing a mosquito transmission model of placental malaria in mice

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Placental malaria significantly contributes to the increased morbidity and mortality experienced by pregnant people compared to non-pregnant people. Current models of placental malaria fail to recapitulate maternal-fetal tolerance and the natural infection route. To address these limitations, outbred CD1 mice of different gestational statuses (non-pregnant, embryonic day (E)6, or E10) were infected with Plasmodium *berghei* Diego-Luciferase or mock-inoculated by mosquito bite (5-7 mosquitoes, for 5 minutes). Survival of infected E10 dams was significantly reduced following delivery compared to infected E6 and non-pregnant mice. Parasitemia onset is delayed in infected pregnant dams compared to infected non-pregnant dams. Perinatal outcomes differ by gestational age at infection. E6-infected dams experienced significant pregnancy loss, while infected E10 dams delivered stillborn pups. Progesterone is responsible for the normal progression of pregnancy and is reduced in human placental malaria. Progesterone concentrations are significantly reduced in malaria-infected dams compared to mock-infected dams at 8 days post-inoculation, which did not correlate with same-day parasitemia. Experimental infection by mosquito bite induces severe perinatal outcomes. Future directions include utilizing this model to interrogate the role of progesterone in mediating adverse perinatal outcomes in the context of placental malaria.

53. Adherence to malaria diagnosis and treatment guidelines among healthcare workers in Gambella, Ethiopia

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Introduction: Adherence to National malaria diagnosis and treatment guideline (NMDTG) improves the quality of care and patient outcomes. Non-adherence to guidelines exposes the patient to the abuse of anti-malarial medications, higher expenditures resources, patient discontent and increases non-malaria morbidity and mortality. The level of adherence to the NMDTG is not known, particularly in Gambella, Ethiopia.

Objective: The aim of this study was to assess the levels of adherence to the NMDTGs and explore perceived facilitators and barriers for health care provider's adherence to the NMDTG in Gambella, Ethiopia.

Methods: A mixed method design was conducted in the Gambella town of Ethiopia. Quantitative method was used to extract 402 malaria patient records from the medical records of each patients and using qualitative methods 29 in-depth interviews were conducted with healthcare workers. The collected data were then cleaned and entered into SPSS for analysis. Descriptive statistics were used to summarize the characteristics of the study participants and to determine the level of adherence to the NMDTG. The qualitative data were analyzed using thematic analysis.

Results: In this study the overall adherence level to the NMDTG among health care workers were 61%. The perceived facilitators and barriers identified with adherence to the guideline were endemicity, presence of typical sign and symptom, lack of supportive supervision and mentorship, lack of training, and absence of job aids/guideline at the consultation room.

Conclusion: The study showed adherence to the NMDTG among health care workers were sub-optimal, indicating considerable interventions to improve adherence to the guidelines by provision of job aides/guideline, providing malaria training, and conducting regular supervision and mentorship. There is also a need for orientation for health workers, especially those at higher-level facilities and higher-level professionals, on adherence to NMDTG to avoid the health consequences of malaria.

54. Validation of the human protective efficacy of a *Plasmodium vivax* CS synthetic vaccine candidate, in a rodent model

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Development of malaria vaccines has shown to be a challenging and lengthy process due to the parasite complexity, funding constraints, and logistic limitations, including suitable *in vitro* systems and animal models. This study was focused on developing an experimental model that could accelerate the development of a *Plasmodium vivax* malaria vaccine. With significantly greater constraints than *P. falciparum*, the *P. vivax* CS (*Pv*CS), has been investigated and progressed to clinical development. A phase II clinical trial conducted in 42 volunteers using a vaccine formulation based on long synthetic peptides (LSP) encompassing relevant fragments of the *Pv*CS (i.e., N, R, C) has successfully confirmed high safety, tolerability, and immunogenicity, and more importantly, protective efficacy against *P. vivax* sporozoite-controlled human malaria infection (CHMI). Sterile protection was observed in 42% and malaria semi-immune and 36% of naive volunteers, and significant parasitemia reduction was observed in several other volunteers, leading to an overall protection of 54% and 64%, respectively. Despite these encouraging results, the time elapsed (~30 years) since the initiation (1992) of *Pv*CS characterization and this clinical trial (2022) is, at the same time, discouraging. Here, we describe a rodent model that has rapidly validated the immunogenicity and protection achieved in humans with the same vaccine formulation. C57Bl/6 mice were immunized with the same *Pv*CS LSP formulation and similar vaccination strategy and were further subjected to an infectious challenge using *P.berhei*/*P.vivax Pv*CS transgenic sporozoites. Protective efficacy, as determined by the liver parasite burden using *in vivo* imaging system (IVIS), indicated overall protection of 56%, equivalent to that obtained in humans. We propose this rapid and inexpensive model as an alternative to the costly and scarce non-human primate model for *P. vivax* vaccine development.

55. *P. falciparum* survival in a pulse-drug model indicates reversibility of hemozoin-binding by 4-aminoquinoline drugs and a heme-artemisinin adduct in drug-resistant parasites

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4-aminoquinoline drugs target *P. falciparum* erythrocytic stages through the primary mechanism of binding and inhibiting hemozoin formation in the digestive vacuole of the parasite. Previous work *in vitro* indicates different heme-binding patterns within the class of 4-aminoquinoline drugs. We used a pulse-drug model to measure stage-specific survival of drug sensitive and resistant parasite cultures after short (1.5hr-6hr) exposures to hemozoin-binding drugs such as chloroquine, pyronaridine, and amodiaquine. Survival recovery after removing drug and the resulting shift in IC50 curves reflects the reversibility of drug inhibition. The effect of 4-aminoquinoline binding patterns was also explored through heme-extension assays using synthetic heme. Additionally, comparing survival between parasites harboring the *PfKelch13* C580Y mutation and their isogenic *PfCrt* K76T mutant parasites (which are resistant to chloroquine), we see how the presence of these mutations affect stage-specific activity of each drug differently, which may inform on drug-resistant mechanisms in these parasites. Finally, we explored the heme-binding mechanism of the heme-artemisinin adduct both through heme-extension and in our pulse-drug model to understand binding reversibility in drug-resistant parasites.

56. Antigens reversibly conjugated to a polymeric glyco-adjuvant induce protective humoral and cellular immunity

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Fully effective vaccines for malaria should induce a robust humoral immune response, as well as $CD4^+$, and $CD8^+$ T cell responses to protect against infection and respond to the liver-stage and blood-stage of *Plasmodium* sp. Infection. Here, we present a synthetic glyco-adjuvant named p(Man-TLR7), which, when conjugated to antigens, elicits robust humoral and cellular immunity. p(Man-TLR7) is a random copolymer composed of monomers that either target dendritic cells (DCs) via mannose-binding receptors or activate DCs via Toll-like receptor 7 (TLR7). Protein antigens are conjugated to p(Man-TLR7) via a self-immolative linkage that releases chemically unmodified antigen after endocytosis, thus amplifying antigen presentation to T cells. Studies with ovalbumin (OVA)-p(Man-TLR7) conjugates demonstrate that OVA-p(Man-TLR7) generates greater humoral and cellular immunity than OVA conjugated to polymers lacking either mannose targeting or TLR7 ligand. We show significant enhancement of Plasmodium falciparum-derived circumsporozoite protein (CSP)-specific Tfh cell, $CD4^+$, $CD8^+$T cell responses, expansion in the breadth of the αCSP IgG response and increased inhibition of sporozoite invasion into hepatocytes with CSP-p(Man-TLR7) when compared with CSP formulated with MPLA/QS-21-loaded liposomes-the adjuvant used in the most clinically advanced malaria vaccine. Given the plug-and-play design of our antigen-p(Man-TLR7) conjugates, our platform has the potential to improve subunit vaccines for a variety of infections.

57. Boosting *Anopheles gambiae* melanization, a potential strategy for malaria control

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In insects, the synthesis of melanin plays critical roles in immunity, wound healing, cuticular hardening, and egg chorion tanning. The melanin-based immune response is one of the most effective defense mechanisms; however, in *Anopheles gambiae* melanization-mediated killing of the human malaria parasite *Plasmodium falciparum* is rare in the field or laboratory conditions. In this study, we set out to investigate whether adult *Anopheles* mosquitoes could modulate melanin-related physiological processes in response to a sugar diet supplemented with L-DOPA, a natural melanin precursor. Our results demonstrate that dietary L-DOPA in adult mosquitoes influenced their cuticle and eggshell pigmentation, heat absorption, life span, and susceptibility to the human malaria parasite. Forthcoming global warming and current obstacles to eradicating malaria, this study provides valuable insight into specific adaptations in the *Anopheles* mosquito vector with potential translational applications for the development of malaria control strategies.

58. Determinants of uptake of 4th dose of malaria vaccine among children less than 59 months in Machinga District, Malawai

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Background: In 2021 WHO recommended widespread use of RTS, S/AS01 (RT, S) in sub-Saharan region in a schedule of 4 doses from 5months to 36 months of age. At an interval of 4 weeks between first three RTS, S/AS (RT, S) doses then a fourth dose ~12 -18 months after the third dose. The evaluation of 4th dose is still ongoing but there is 19-39% loss to follow. According to WHO a dropout rate of >10% is undesirable.

Objective: This study aimed to assess determinants to the uptake of malaria vaccine 4th dose in Machinga district, Malawi.

Methods: This was a community based cross sectional study that included 541 caregivers to children between 22 - 59 months old from Nthorowa and Mlomba clusters, Machinga.

Results: Uptake of RTS, S/AS, 4 was at 72.5%. Factors that were found to be associated with RTS, S/AS, 4 drop out were: Ownership of child's health card, Community trust on the effectiveness of Malaria vaccine, number of the children caregiver had and socioeconomic status. Children with good immunization history and from catchment area under Christian Health Association of Malawi facilities had lower odds of drop out.

Conclusion: Uptake of 72.5% was below WHO target. The finding suggested the need to strengthen awareness about immunization among caregivers beyond a year in order to improve uptake of 4th dose and reduce the risk of malaria in Machinga. If the trends of RTS, S/AS, 4 will be unaddressed, it will be hard to achieve sustainable development goal 3.

59. How environmental, geographic, socio-demographic, and epidemiological indicators influence malaria prevalence

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Background: The interactions of environmental, geographic, socio-demographic, and epidemiological factors in shaping mosquito-borne disease transmission dynamics are complex and changeable, influencing the abundance and distribution of vectors and the pathogens they transmit. In this study, 27 years of prevalence data (1990-2017) were used to examine the effects of environmental (temperature, precipitation, NDVI, isothermality), geographic (elevation), socio-economic (population density, GDPPC, human development index), and epidemiological (basic reproductive number) factors on *Plasmodium falciparum* and *Plasmodium vivax* malaria in Africa and Asia.

Methods: Monthly long-term, open-source data for each factor were compiled and analyzed using two approaches: generalized linear models (GLM) and classification and regression trees (CART). Both approaches were used to identify associations between environmental, geographic, socio-economic, and epidemiological factors with malaria prevalence.

Results: Both CART and GLMs provided robust predictions of malaria prevalence. An optimum temperature for malaria prevalence around 25 ◦C for *P. falciparum* prevalence was observed in Africa but not for *P. falciparum* and *P. vivax* in Asia (below 25 ◦C). The decline of malaria prevalence from 2000 to 2012 was well captured by the models as well as the resurgence of *P. falciparum* prevalence in Africa and Asia. Higher predicted malaria prevalence in regions with lower GDPPC and lower HDI.

Conclusion: This study showed that there was a combination of environmental, geographic, socioeconomic, and epidemiological indicators driving malaria prevalence. Identifying these, and describing their associations provides key information to inform planning strategies and interventions to reduce malaria burden in Africa and Asia.

60. External quality assessment of malaria diagnosis and human African trypanosomiasis in the democratic republic of Congo: Lessons learned and perspectives

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We reviewed five previously published external quality assessment (EQA) sessions assessing diagnosis of malaria and Human African Trypanosomiasis (HAT) performed in Congo-Kinshasa (2010- 2014) and compiled the main results. Main objective was to assess the reading/interpretation of the microscopy of blood parasites (Trypanosoma spp. and Plasmodium spp.) and the malaria rapid diagnostic tests (RDT) through EQA sessions.

The EQA slides for HAT and malaria were validated by expert's team and were distributed to the participating laboratories under optimum conservation and transport conditions. EQA of malaria RDT were based on photographs and SMS.

These studies demonstrated the feasibility of EQA in a resource-poor settings context when there is collaboration between vertical disease control programs. Participation increased from 174 to 400 and 1,849 to 2,344 for laboratories and individual malaria RDT end-users respectively. Poor performance was reported of microscopic diagnosis for *Plasmodium falciparum* gametocytes, Plasmodium spp., Trypanosoma spp. and estimate parasite density as well as quality of thick blood film collected from participating laboratories. SMS photographs-based EQA of reading and interpretation of malaria RDT first time used allowed direct assessment of end-user rather than the laboratory. Errors in reading/interpreting results as overlooking faint or weak test lines, failure to distinguish the correct Plasmodium species and to recognize invalid and negative malaria RDT, have been confirmed among participants. Feedback returned at the end of each EQA session encouraged participants and contributed to a relative improvement in performance.

Experience with the disease, participation in the training and regular participation in the EQA session were related to the best performance.

61. Genotype distribution of Thioester-containing protein 1 (Tep1) and its impact on development of *Plasmodium* Oocyst in *Anopheles gambiae* sensu lato mosquitos Southwest Ethiopia

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*Background***:** Environmental factors have a role on the evolutionary selection of malaria vectors that are either susceptible or resistant to *Plasmodium* parasites. Due to these factors, adaptable species genotypes may be introduced more frequently, which may have an effect on malaria transmission. Thioester-containing protein 1 (TEP1) is a crucial component of mosquitoes' natural resistance to parasites. To effectively combat malaria, studies need to understand how TEP1 polymorphism affects phenotypic traits during infections. Therefore, the purpose of this research was to identify the Tep1 genotype frequency in south-western Ethiopia and investigate its effect on *Plasmodium* oocyst development in *Anopheles gambiae* s.l. mosquitoes.

*Method***:** Using plastic dippers, *Anopheles* spp. larvae were collected from several aquatic settings in Asendabo, Arjio Dedessa, and Gambella in 2019 and 2020. Collected larvae reared to adult female mosquitoes were first identified morphologically. Then *An. gambiae* s.l. was identified as a sibling species by PCR. TEP1 alleles were determined in 330 Anopheles mosquitoes using RFLP-PCR, and to validate the TEP1 genotyping results, a representative sample of the alleles was sequenced.

*Result***:** Three distinct tep1 gene haplotypes (TEP1*S1 and TEP1*R1) have been identified in south-west Ethiopia. Haplotype diversity ranged from 0.48871 to 0.63161, whereas nucleotide diversity ranged from 0.36554 to 0.46751 across all loci. All sample locations had positive Tajima's D values, which were statistically significant. Nonetheless, across all research sites, positive and statistically significant Fu's Fs values were detected. All inter-population comparison chi-squares values were significant except for Asendabo vs. Gambella (P>0.05). In addition, the TEP1 *RR genotype is susceptible and produces fewer *Plasmodium* oocysts than the TEP1 *SR and TEP1 *SS genotypes.

*Conclusion***:** TEP1*R allele frequencies are greater in high malaria transmission environments compared to low transmission situations. Furthermore, *An. arabiensis* carrying the TEP1*R gene was susceptible to *Plasmodium* infection.

62. *Plasmodium*-infected mosquito bite elicits an immune response at the bite site

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The first encounter between the host's immune system and a pathogen is critical for inducing long-lasting immunity. *Plasmodium* sporozoites are the infective stage of the malaria parasite, inoculated into the skin by infected mosquitoes as they probe for blood. While ~20% reach the liver, a substantial number (~50%) remain in the infected area for at least 6 hours, while shedding an abundant amount of their major surface protein, the circumsporozoite protein (CSP). Approximately 15% of the inoculum is removed via the lymphatic system and goes to the draining lymph node (dLN) where immunity should be generated. Nonetheless, despite repeated exposures and the presence of foreign antigens in the skin and the dLN, sterile immunity against sporozoites or CSP does not occur, suggesting that *Plasmodium* sporozoites may modulate the host immune response. Here we compare the immune response elicited in response to *P. berghei*-infected and uninfected mosquito bites in the skin and the dLN. We find differences between the gene expression patterns of pro- and anti-inflammatory cytokines. In an ongoing study we investigate the host's early and local anti-inflammatory response induced by sporozoites infection over time. We observe an elevated expression of inhibitory mediators of inflammation, suggesting a mechanism by which *Plasmodium* sporozoites suppress the immune response to their presence.

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Future of Malaria Research Symposium keynote addresses

Year	*Title*	*Keynote speaker*
2022	The challenges of malaria control in a high burden setting	**Jane Namuganga** Infectious Disease Research Collaboration, Uganda
	Dissecting the antibody response to *Plasmodium falciparum*	**Joshua Tan** National Institute of Allergy and Infectious Diseases
2021	Using transgenic fungi to kill malaria mosquitoes	**Etienne Bilgo** Institut de Recherche en Sciences de la Santé / Centre Muraz
2020	Malaria parasite communities, evolution, and transmission across Africa	**Alfred Amambua Ngwa** Medical Research Council Unit, Gambia
2019	*Plasmodium* is a planner – molecular preparations for efficient malaria transmission	**Scott Lindner** Pennsylvania State University
	Translational research to improve surveillance in an era of malaria elimination	**Caroline Buckee** Harvard University
2018	1+1=1: targeting endosymbiosis for antimalaria drug discovery	**Ellen Yeh** Stanford University
	Spatial epidemiology of *P. knowlesi* in Sabah, Malaysia: identifying environmental risk factors for an emerging zoonotic disease	**Kimberly Fornace** The London School of Hygiene and Tropical Medicine
2017	Unraveling human and parasite factors that determine the transmission of *P. falciparum* to mosquitoes	**Teun Bousema** Radboud University Medical Center, Netherlands
	Beyond hemoglobin: Discovering erythrocyte host factors for malaria	**Elizabeth S. Egan** Standford University School of Medicine
2016	Discovering molecules to probe and treat malaria	**Emily Derbyshire** Duke University
	Tracking malaria by its shadows	**Bryan Greenhouse** University California, San Francisco
2015	Wolbachia-microbe interactions in *Anopheles* mosquitoes	**Grant Hughes** University of Texas Medical Branch

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Emma Rowley, Emily Stucke, Stephanie Rankin-Turner, and Rubayet Elahi
Miriam Laufer and David Sullivan (Faculty advisors)

2022
Adriano Franco, Annie Martin, and Mary Gebhardt
Abhai Tripathi (Faculty advisor)

2021
Victoria Balta, Emma Camacho, and Andrew Hammond
Rahul Bakshi (Faculty advisor)

2020
Diego Giraldo Sanchez, Jessica Schue, and Cecilia Springer Engdahl
Amy Wesolowski (Faculty advisor)

2019
Rachel West, Emma Camacho, and Chinmay Tikhe
Matthew Ippolito (Faculty advisor)

2018
Gibbs Nasir, Julia Pringle, and Genevieve Tauxe
Clive Shiff (Faculty advisor)

2017
Melanie Shears, Matthew Ippolito, and Joel Vega-Rodriguez
Prakash Srinivasan (Faculty advisor)

2016
Giovanna Carpi, Hugo Jhun, and Krithika Rajaram
Conor McMeniman (Faculty advisor)

2015
Amanda Balaban, Raul Saraiva, and Leah Walker
Sean Prigge (Faculty advisor)