ABSTRACTS

First Main Session 8:45 – 10:15am Eastern Standard Time

Henrico Langeveld, University of Pretoria – South Africa
"Novel kinases play an essential role during asexual proliferation of *Plasmodium falciparum* parasites"

Novel antimalarial drugs and drug targets are constantly need due to the emergence of malaria strains resistant to artemisinin-based combination therapies. The "in search of novel drug targets" field exploits the fact that there is still a lot that is not known about the atypical biology of the parasite. Within these unexplored biological avenues of the parasite, unique and essential proteins can be identified and studied as potential drug targets. One of these biological avenues is the extraordinary kinome, consisting of unique protein kinases. Four genes encoding uncharacterized protein kinases with unknown functions within the parasite were identified from transcriptome analyses of the parasite's atypical asexual cell cycle. This study aimed at functionally validating the essential nature of these uncharacterized kinases within the parasite's life cycle, exposing potential novel targets in the kinase inhibitory space. The four identified kinase genes were targeted for gene disruption to prove their essentiality. In addition, in the case of essentiality, genes were also cloned into other systems to enable the conditional knocksideways (FKBP system) to uncover the functions of the kinases. Transfectant lines were created for P falciparum with these genes manipulated using a selection-linked integration technique in order to evaluate gene function. Results: The gene fragments were successfully directionally, in-frame cloned into the respective plasmids, and validated by restriction enzyme digest and Sanger sequencing. Transgenic lines were successfully generated. After targeted gene deletion, each of the four kinase genes were found to be essential for parasite proliferation. The proteins were also found to localized to the cytosol. The results indicated that all of the kinase proteins are essential asexual progression. This study reveals a new set of kinases that can potentially be considered as new drug targets and this study could be vital in any further research into the kinase network governing the life cycle of *P. falciparum* parasites.

Jean Leon Mugenzi, Centre of Research in Infectious Diseases – Cameroon
"Impacts of a 4.3kb structural variant on gene expression and insecticide resistance phenotype in the malaria vector *Anopheles funestus* s.s."

Insecticides are essential in controlling the vector populations which transmit malaria. To better manage insecticide resistance and prolong the effectiveness of insecticides, an understanding of the genetic drivers of insecticide resistance is needed. Most of the genetic variants identified and characterized are mostly single-nucleotide polymorphism and small indels with little known about the contribution of structural variants (SVs) in the evolution of insecticide resistance. Here we identified and characterized

a 4.3kb SV inserted in the promoter region of an insecticide resistance gene CYP6P9b. This SV was found in *An. funestus* populations of Central (Cameroon) and East Africa (Uganda) at very high frequencies approaching fixation and absent elsewhere. Genetic crosses revealed that this SV impacted the expression of nearby genes and was associated with resistance to type II pyrethroid. Our findings highlight the underexplored role of SVs in the evolution of insecticide resistance and provide additional tools for molecular surveillance of insecticide resistance.

Maximilian Julius Lautenbach, Karolinska Institutet – Sweden
"Systems analysis shows a role of cytophilic antibodies in shaping innate tolerance to malaria"

Natural immunity to malaria develops over time with repeated malaria episodes but protection against severe disease, called tolerance, develops more rapidly. Here, we comprehensively profiled the blood immune system in patients over one year after acute symptomatic *P. falciparum* malaria, either infected for the first time, or with a previous history of malaria. Using a data driven analysis approach to describe the immune landscape over time, we could show that a dampened inflammatory response was associated with reduced gdT cell expansion, early expansion of FcγRIII + monocytes and parasite-specific antibodies of IgG1 and IgG3 isotypes. This also coincided with reduced parasitemia and hospitalization. Our data indicate that antibody mediated phagocytosis during the blood stage infection leads to lower parasitemia and less inflammatory response with reduced gdT cell expansion. This enhanced control and reduced inflammation points to a potential mechanism on how tolerance is established following repeated malaria exposure.

Anna Strampelli, Imperial College London – England
"A Male-Drive Female-Sterile (MDFS) system in *Anopheles gambiae* targeting doublesex"

We developed a male-drive female-sterile (MDFS) population suppression strategy in the main malaria vector *Anopheles gambiae*, consisting of a system that drives in males whilst simultaneously causing dominant female sterility. Specifically, we disrupted the female-specific exon 5 of the doublesex (dsx) gene using a CRISPR construct expressing cas9 under the vasa2 promoter, and a gRNA targeting the insertion site of the construct (i.e., the dsx exon 5). The MDFS allele was tested in heterozygous males, as well as in males trans-heterozygous for the MDFS and a non-driving autosomal sex distorter (X-shredder). In both cases, the MDFS allele exhibited extremely high inheritance rates, respectively of 99.5% (±0.35 s.e.m.) and 99.7% (±0.26 s.e.m.), and caused complete dominant sterility in the female progeny. The design of a cage trial is underway, the results of which will inform on the suitability of the MDFS strategy as a population suppression tool.

Himanshu Gupta, London School of Hygiene and Tropical Medicine – England
"Assessing the use of microRNAs for the diagnosis of severe *falciparum* malaria"

Cerebral malaria (CM) is a major cause of mortality in *Plasmodium falciparum* infection. Prompt diagnosis and treatment are key for a positive outcome but remain challenging. Recent reports identified age-specific magnetic resonance imaging (MRI) features on admission associated with fatality in CM. Because neuroimaging facilities are rarely accessible in malaria-endemic countries, we investigated the

potential of microRNAs (miRNAs) as biomarkers of such MRI features across different age groups in a cohort of Indian patients with and without CM. We showed that increased plasma levels of specific miRNAs were associated with CM mortality, and these levels decreased significantly at day 30 post-admission in survivors, strongly suggesting a cerebral specificity. Remarkably, we also demonstrated that some of these miRNA levels correlated independently with MRI features associated with poor outcomes in pediatric and adult CM, respectively.

Jean Erick Massamba, Congolese Foundation for Medical Research – Congo
"Prevalence of *Plasmodium falciparum* submicroscopic infection and pregnancy outcomes in Congolese women at delivery"

The present study sought to determine the prevalence of submicroscopic *P. falciparum* infection in women at delivery from southern Brazzaville, where IPT-SP has been implemented since 2004, and investigate the relationship between the submicroscopic infection and pregnancy outcomes. This cross-sectional study was carried out from March 2014 to April 2015 with 281 women randomly recruited at delivery at a Health Centre in Brazzaville. Matched peripheral, placental, and cord blood collected from each woman with malaria negative thick smears, were used for the diagnostic of *P. falciparum* submicroscopic infection by PCR. The prevalence of *P. falciparum* submicroscopic infection was 31.7%, 36.37%, and 12.9%, when using the peripheral, placental, and cord blood respectively. The maternal anemia was slightly more prevalent in submicroscopic infected than in non-infected women. These data indicate that the prevalence of submicroscopic *P. falciparum* infection is high in women at delivery in Brazzaville; and might represent a risk for maternal anemia.

KEYNOTE LECTURE 10:25 - 11:30am Eastern Standard Time

Etienne Bilgo, Institut de Recherche en Sciences de la Santé – Burkina Faso
"Using transgenic fungi to kill malaria mosquitoes"

Conservative estimates put the number of new malaria infections each year over 200 million. The ongoing rise of pesticide resistance in mosquitoes that vector these parasites has reduced our control options. Insect pathogenic fungi have potential to meet our need for new vector control agents. Mosquito-killing fungi can be genetically engineered to serve as a platform for biotechnology solutions. We engineered *Metarhizium pingshaense* (Mp) to specifically deliver an insect-specific neurotoxin (Ca++/K+ channel blocker) into the mosquito hemocoel. We reported that strains could be engineered to be lethal at a dose of one spore per mosquito. In a malaria-endemic region of Burkina Faso, we conducted the first-ever semi-field trials of fungi genetically engineered to kill mosquitoes more effectively. We have further shown that infection with a transgenic strain expressing an insect-specific toxin could interfere with the ability of mosquitoes to resist insecticides. We envision this flexible biotechnology can play an important role for integrated vector management by strengthening current and future vector control tools. For example, we have also shown that transgenic spores transferred during mosquito mating are sufficient to kill the mate. Through these means transgenic mosquito-killing fungi could be compatible with other innovative vector control strategies involving sterile insects and *Wolbachia*-based strategies. Scientific, regulatory and social questions must be resolved to convert this promising strategy into a validated public health intervention.

Second Main Session 11:30 - 12:55pm Eastern Standard Time

Audrey Brown, University of Virginia – USA
"Broad nutrient stress increases *P. falciparum* survival of artemisinin"

As many stress-responsive programs overlap, mild stressors may alter metabolism in a manner that enhances survival of subsequent, distinct stressors. We demonstrate that short-term growth under mild, physiologically relevant nutrient stress leads to superior recovery from dihydroartemisinin (DHA). In these experiments, parasites are metabolically "primed" by restriction of key nutrients for 36-72h prior to a 6h 200nM DHA pulse. Over ten days of recovery in normal nutrient conditions following DHA, primed parasite populations show 2-4-fold higher cumulative proliferation. These differences are mirrored by ≥2-fold increases in the outcome of DHA ring stage survival assays. We are currently investigating if this phenotype is dependent on known stress responsive factors, including eIF2a-phosphorylation, which is implicated in nutrient stress and DHA survival. Evidence of interplay between relevant metabolic pathways and antimalarial survival has implications for the ability of parasites to evade cure, thereby increasing the pool of drug exposed parasites for resistance selection.

Willem Laursen, Brandeis University – USA
"Up close and personal: Cellular and molecular bases of short-range attraction in *Anopheles* mosquitoes"

Female mosquitoes use multiple sensory cues to locate hosts. While more is known about the mechanisms used to detect CO2 and odors from meters away, less is known about the detection of temperature and humidity, cues that dissipate within centimeters of the host. These short-range cues influence landing and promote blood-feeding, offering potential targets for disrupting disease transmission. We recently showed that heat-seeking in *Anopheles gambiae* is driven by the Ionotropic Receptor IR21a. However, the cellular and molecular basis of humidity-seeking in mosquitoes remains unknown. Using molecular genetics to target a related receptor, IR93a, we identified distinct groups of IR-dependent thermo- and hygro-receptors in the antenna and characterized their stimulus sensitivity. At the behavioral level, loss of IR93a eliminates water-seeking, reduces the ability to maintain attraction to hosts, and dramatically decreases blood-feeding. Together, these data suggest that Ir93a-dependent detection of short-range cues has a profound influence on mosquito blood feeding.

Heather Kudyba, National Institutes of Health – USA
"Conditional knockdown of essential proteins in *P. falciparum* Midgut Stages"

While several conditional knockdown/knockout systems have been implemented to study essential genes in the asexual stages of *P. falciparum*, few systems have been applied to study the mosquito stages. Our work aims to implement the glmS ribozyme knockdown system to study genes essential for the parasite to develop in the midgut. To test the efficacy of the system in the mosquito stages, we have generated an NF54 reporter line where GFP is tagged with the glmS ribozyme. Additionally, we have created an NF54 control parasite line expressing GFP tagged with M9, a glmS ribozyme containing inactivating mutations. We have identified a gene of interest, PfERC (Endoplasmic Reticulum Calcium-binding Protein), that we have tagged and are using to validate the system in the

midgut of the mosquito. Our current data suggests that this system may be adaptable to studying essential parasite genes in the mosquito.

Diego Giraldo, Johns Hopkins University – USA
"A semi-field system for quantitative tracking of *Anopheles gambiae* olfactory preferences"

Female mosquitoes detect chemical cues emanating from humans to locate them. Differences in human odor composition can influence mosquito attraction to hosts, with important implications for malaria transmission. We have developed a large scale, semi-field system in Zambia for the quantitative tracking of mosquito olfactory preferences to human body odor and warmth. Using infrared imaging and a high-content tracking algorithm, we found that mosquitoes were attracted to heated landing platforms baited with CO2 and whole human body scent. CO2 was preferred over heat alone, and human scent was preferred over CO2. When presented with scent from two humans, mosquitoes consistently preferred one human over the other. These data indicate that this multi-choice olfactory assay has potential to accurately quantify *An. gambiae* olfactory preferences and form the basis of an innovative method to screen for humans that are highly attractive or unattractive to *An. gambiae*.

Manuela Runge, Northwestern University – USA
"Estimating the impact of RTS,S deployment in areas with seasonal malaria chemoprevention"

RTS,S recently became the first malaria vaccine to be recommended by the WHO for use in children. However, RTS,S interactions with interventions already in place, such as seasonal malaria chemoprevention (SMC), and optimal deployment modes remain unclear. We use a malaria transmission model to investigate the potential impact of RTS,S alone or with SMC across different settings, including a range of transmission intensities, case management levels, seasonality patterns, and booster timings. RTS,S averted the most cases at high transmission, when case management and SMC coverage were low. Even at high SMC coverage, adding RTS,S substantially decreased the number of cases, especially in children 1-3 years. Despite a rebound in incidence in older children, the overall number of malaria episodes experiences by vaccinated children <10 years was lower than in non-vaccinated children. These model results can inform deployment priorities for RTS,S in the context of other available interventions in resource-limited settings.

VEuPathDB Team
"Beta.clinepidb.org: A platform for sharing and exploring malaria epidemiological datasets"

Open access to data from epidemiological studies on malaria has tremendous potential to preserve data over time, increase secondary data use, and accelerate discovery and translational impact, but there are significant technical, practical, and confidentiality barriers. The clinical epidemiological database, ClinEpiDB.org, was developed four years ago to facilitate access to de-identified data from large, high-quality global health studies. An intuitive point-and-click interface allows users to search these studies, explore associations between variables, and download data for further analysis. ClinEpiDB enables investigators to not only meet, but surpass, the requirements of journals and

funders to make data publicly available by integrating study data with standardized ontologies to make data more easily reusable. Observational and experimental epidemiological data elucidating interactions between malaria parasites, mosquitos, human hosts, and public health interventions are available, linked whenever possible to -omics data at VectorBase.org and PlasmoDB.org. ClinEpiDB is expanding in 2021 with inclusion of additional datasets (including malaria studies on mass drug administration and bednet distribution), enhanced visualization tools at Beta.clinepidb.org, and significant international educational activities.

LIGHTNING TALKS | 1:00 - 1:30 PM Eastern Standard Time (EST)

SESSION A

Moderators: Andrew Hammond, Johns Hopkins University and Anna Strampelli, Imperial College London

Awosolu Oluwaseun, Universiti Sains Malaysia
"Trends in *falciparum* malaria prevalence, parasite density and associated risk factors in Akure, Southwestern Nigeria: An implication for malaria elimination"

Malaria is a serious public health challenge worldwide, particularly in tropical and sub-tropical regions. This study was designed to investigate the trends in falciparum malaria prevalence, parasite density and associated risk factors in Akure, Southwestern Nigeria. A randomized cross-sectional and hospital-based study was conducted. Blood samples were collected from volunteered participants and examined through standard parasitological techniques of microscopy, RDT and Nested-PCR. Subject's information was obtained through a pre-tested questionnaire. The gDNA was extracted from DBS using QIAamp DNA blood and tissue kit and amplified using Plasmodium genus and species-specific primers for identification. A total of 601 subjects were examined. The overall prevalence by microscopy, RDT and PCR was 64.89%, 65.7% and 67.39% respectively. The total geometric mean parasite density of participants was 1096.93 parasite/ μ L. Age group $\leq$12 years had the highest malaria prevalence of 77.0% and geometric mean parasite density of 1891.21 parasite/µL. malaria prevalence differed significantly by sex.

William Garrood, Imperial College London – England
"Assessing off-target effects of CRISPR-Cas9 based gene drives in *Anopheles gambiae*"

CRISPR-Cas9 gene drives have been developed with the goal of controlling *Anopheles gambiae*. A key risk assessment for these Anopheles mosquitoes is understanding the extent to which CRISPR-induced off-target mutations can be created across the genome. We tested four different gene drive strains, including a set-up that was deliberately promiscuous (non-germline restricted promoter for SpCas9 and a guide RNA that had many closely related sites across the mosquito genome). This setup showed that off-target mutations can be generated within the mosquito genome, observed at frequencies no greater than 1.42%. Also, there was no evidence that CRISPR-induced off-target mutations could accumulate in a mosquito population, despite exposure to the CRISPR-Cas9 construct for several generations. Moreover, sensible guide RNA design and tight temporal restriction of Cas9 expression to the germline was able to render off-target mutations undetectable, demonstrating that CRISPR-Cas9 can be highly specific when used in a mosquito gene drive.

Jacques Gnambani, Institut de Recherche en Sciences de la Santé – Burkina Faso
"Heritable cultivable bacteria associated to wild *Anopheles gambiae s.l*. in Burkina Faso for paratransgenic control perspectives of malaria"

Mosquito microbiota has recently gathered increased interest because of its potential influence on vector competence. However, the wild mosquito microbiota remains poorly studied, especially cultivable and vertically transmitted bacteria. This work was designed to isolate potential bacteria for use in the paratransgenesis approach for malaria control. Singles males, couples in swarms were used in this study. Spermathecae of females and testes of males were dissected and plated onto media. Then, bacterial colonies and species were isolated and were biochemically identified using the VITEK 2 system. Bacteria colonies was higher in testes than spermathecae. An average 40 different bacteria colonies were isolated in testes of males. Promising bacteria such as *Lactobacillus* sp, *Serratia* sp, *comamona*s sp, *Pseudomonas* sp, *Pantoea agglomerans* and other bacteria have been identified. Sequencing of bacteria genomes should improve our knowledge of the microbiota and its interactions with host the host mosquito.

Rosalia Nghitalesheni Joseph, University of Namibia
"Evaluation of neonicotinoid (Fludora fusion and SumiShield) insecticides against pyrethroid - resistant *Anopheles gambiae s.l*. in Zambezi region, Namibia"

Malaria vector control interventions, such as indoor residual spraying and long-lasting insecticidal nets heavily rely on the use of pyrethroids. Pyrethroid resistance was confirmed in all malaria-endemic regions of Namibia by entomological surveillance in 2020. Neonicotinoid insecticides have different modes of action to pyrethroids and have increased the number of insecticides available for vector control. It is imperative to establish susceptibility of local malaria vectors to the novel insecticides to evaluate their potency. This study was conducted to determine the susceptibility of *An. gambiae s.l.* in Zambezi region, to Fludora fusion and SumiShield insecticides. Three to five-day-old *An. gambiae s.l.* reared from larvae were tested against Fludora fusion and SumiShield in WHO susceptibility tube and CDC bottle bioassays, respectively. Susceptible *An. arabiensis* (KGB) mosquitoes were used as controls. Fludora fusion induced 100% mortality 24hours post-exposure, while SumiShield induced 98.75% mortality 72hours post-exposure in *An. gambiae s.l.* vectors in Zambezi are susceptible to neonicotinoids. The unique mode of action and an absence of cross-resistance to other insecticide classes renders both Fludora fusion and SumiShield potential insecticides for inclusion in indoor residual spraying in Zambezi region.

Mbama Ntabi Jacques Dollon, Fondation Congolaise pour la Recherche Médicale (FCRM) – Congo
"Prevalence of non-*falciparum* malaria in individuals living in south of Brazzaville and its surroundings in Republic of Congo"

Background: This study aimed to determine the non-*falciparum* malaria prevalence in south of Brazzaville and its surroundings.
Methods: A cross-sectional survey was conducted in 380 volunteers. Socio-demographic and Clinical parameters were recorded, and the *plasmodium* infection was detected in blood samples using microscopy and Nested PCR.

Results: From 289 participants positive to *Plasmodium spp*., 73% were from rural area, and 97.2% were afebrile. Microscopic and sub-microscopic prevalence's were 37.1% (95%CI: 32.2-42.2) and 39.0% (95%CI: 34.0–44.1) respectively. *P. malariae* (1.6%) was the only identified non-*falciparum* species in microscopy. The sub-microscopic results showed a prevalence of 17.4% (95%CI: 13.7–21.6) of *P. malariae* and 8.9% (95%CI: 6.3–12.3) of *P. ovale*. The co-infection of *P. malariae* or *P. ovale* with *P. falciparum* was at 15.8% and 7.4% respectively.
Conclusion: This study shows an importance of considering the non-*falciparum* species in the strategic plan of malaria eradication in the Republic of Congo.

Rebecca Rosenberg, Johns Hopkins University – USA
"VectorCAM: a low cost hand held tool for automated, rapid morphological identification of mosquito species"

We have developed a handheld field tool and a novel computer vision algorithm for automated morphological identification of mosquitoes species. This technology aims to enable expansion of vector surveillance efforts in countries in sub-Saharan Africa by task-shifting the process of mosquito identification to lesser trained community volunteers. The technology is enabled by our recently published convolutional neural network (CNN) algorithm, which can identify 39 species with an F1 macro score of 86.07±1.81%. The algorithm can also mark as 'unknown', any species previously not seen by it. We recently traveled to partner sites in Africa where Vector Control Officers, Community Health Workers, and entomologists in Uganda and Ghana interacted with our device, used it to capture photographs of over 1000 field caught specimens, and provided positive usability feedback. We plan to perform an implementation pilot in collaboration with the National Malaria Control Division, Uganda in early 2022

SESSION B
Moderators: Victoria Balta, Johns Hopkins University and Audrey Brown, University of Virginia

Mariko Kanai, Columbia University – USA
"Identification of a novel determinant associated with quinine resistance in a *Plasmodium falciparum* genetic cross"

To gain insight into the enigmatic mechanism of *Plasmodium falciparum* resistance to quinine (QN), we conducted a genetic cross between Cam3.II (QN-resistant) and NF54 (QN-sensitive) parasites using human liver-chimeric mice, and obtained 112 independent recombinant progeny. These parents also differ in their sensitivity to chloroquine (CQ). Drug assays, whole-genome sequencing, and quantitative trait loci (QTL) analyses were conducted for QN, CQ, and the active CQ metabolite monodesethyl-CQ. Resistance to all antimalarials mapped to a 140kb region on chromosome 12, which appears to be co-inherited with pfcrt. CQ and md-CQ resistance localized to a chromosome 7 locus (60kb) harboring pfcrt. High-grade QN resistance (IC90) mapped to a novel chromosome 7 peak (20kb) 282kb from pfcrt, including a putative drug/metabolite transporter (pfdmt1). Studies are underway to characterize newly obtained 160 progeny and validate candidates. Our results will provide new insights into mechanisms of action and resistance to QN, CQ, and related antimalarials.

Heather Colvin, Wake Forest University – USA
"An *ex vivo* model of oxidatively stressed red blood cells demonstrates a role for exogenous amino acids in enhancing bystander red blood cell function and morphology in malaria"

Malaria is a highly oxidative parasitic disease in which both infected and uninfected red blood cells (RBCs) are destroyed contributing to the development of anemia. RBCs are limited in their response to oxidative stress due to lack of a full complement of organelles and as such, rely on their endogenous antioxidant capacities to protect against cellular damage. As glutathione itself is not permeable through the RBC membrane, we hypothesized that exogenous cell-permeable amino acid precursors of glutathione (glutamine, cysteine and/or glycine) would aid the RBC's antioxidant capacity and alleviate oxidative stress in our ex vivo stressed RBC model. Here, we documented that exogenous amino acids did indeed reduce oxidative damage in RBCs and we hypothesize that this protection occurs via the glutathione antioxidant pathways. In future studies, we plan to investigate the impact of exogenous amino acids on uninfected RBCs in the context of malaria.

James McLellan, University of Texas at San Antonio – USA
"Single-cell quantitative bioimaging of *P. berghei* liver stage translation"

Antimalarial drug resistance poses a critical threat to the treatment and prevention of *Plasmodium* infections, highlighting the need for new multistage drugs and drug targets, along with assays to speed their identification. *Plasmodium* growth and maturation are causally dependent on protein synthesis, a complex cellular process essential throughout the parasite lifecycle, abundant with potential drug targets. Our approach to identifying translation inhibitors relies on a fluorophore-clickable analog of puromycin, combined with automated confocal fluorescence microscopy, to detect discrete changes in the nascent proteome of *Plasmodium berghei* liver stages in vitro. This image-based assay provides a direct readout of protein synthesis in single parasites and surrounding hepatocytes simultaneously, and allows discrimination between direct and indirect translation inhibitors, thus opening up the *Plasmodium* liver stage to quantitative studies of translation in single parasites and populations.

Frank Addae, University of Ghana
"Evaluating the effects of sorbitol synchronization of clinical isolates of *Plasmodium falciparum* on their susceptibility to antimalarial compounds"

The development of the *Plasmodium* parasite in the human host is highly synchronous, however, in in vitro culture, the parasite is asynchronous. Several synchronization methods including the use of 5% sorbitol, Percoll, and magnetic separation synchronization methods have been developed for synchronizing *P. falciparum* parasites for downstream studies. Although the effects of these synchronization methods on malaria have been demonstrated, not much is known about the effect of these synchronization methods on the response of clinical isolates of *Plasmodium falciparum* with different drug susceptibilities to different antimalarial compounds. This study evaluated the effects of 5 % sorbitol synchronization of clinical isolates on their susceptibility to chloroquine, artesunate and gossypol using in vitro SYBR green-1 fluorescence-based growth inhibitory assay. The study showed varied responses in the synchronous and asynchronous cultures of clinical isolates to chloroquine, artesunate and gossypol. The synchronous culture of the clinical isolates was found to be more

susceptible to artesunate than chloroquine. However, the asynchronous A11 was more susceptible to gossypol than the synchronous culture. A11 was also found to be resistant to chloroquine. The results from this study suggest that 5% sorbitol synchronization affects the response of clinical isolates of *Plasmodium falciparum* to antimalarial compounds. Therefore, synchronizing clinical isolates for downstream studies should consider variations in parasites' behavior arising from the synchronization step.

Kyle McLean, Massachusetts Institute of Technology (MIT) – USA
"A modular cloning system for high efficiency production of *Plasmodium falciparum* transfection vectors"

The extreme AT-bias and low complexity of the *Plasmodium falciparum* genome makes traditional cloning methods error-prone. We have created a new, minimized linear plasmid to circumvent these issues, while enabling implementation of the rapid, reliable, and scalable Modular Cloning (MoClo) strategy. MoClo uses type-IIS restriction enzymes to achieve multi-piece, single-pot assembly of biological parts with high efficiency. We are creating standardized parts libraries of promoters, terminators, epitope tags, reporters, effectors, and selectable markers, and can reliably assemble these into large, complex, AT-rich transfection-ready plasmids designed to achieve various genome manipulation outcomes in *P. falciparum*. This resource will accelerate the malaria community's ability to address important biological questions more easily.

Deepti Shrivastava, Central Drug Research Institute (CSIR) – India
"Mitochondrial multidrug resistant transporter (MDR6) and its interaction with the ISC pathway in *Plasmodium falciparum*"

PfMDR6 is a putative ABC transporter, absent in other apicomplexans. It has been associated with altered drug sensitivity with a predicted essential role in parasite development. We purified recombinant β-PfMDR6-β from E. coli. β-PfMDR6-was a homodimer, also analysed by TEM and targeted to the parasite mitochondrion. ATPase activity of β-PfMDR6-β was stimulated two-fold in the presence of GSSG with low Km value and high catalytic efficiency. UV-VIS analysis indicated the presence of [4Fe-4S]. Moreover, ATPase activity was significantly enhanced in the presence of [4Fe-4S] and GSSG compared with GSSG alone, suggesting a possible conformational role for the cluster. Pull down from Pflysate and in vitro interaction established that PfMDR6 can interact with ISC pathway scaffold and transfer proteins which could serve as [4Fe-4S] donors. PfIscU could also transfer [4Fe-4S] onto PfMDR6, thus suggesting that the latter serves as a transporter linking mitochondrial and cytosolic [Fe-S] biogenesis.

Laura M. Hagenah, Columbia University – USA
"Modeling the emergence of piperaquine-resistant *Plasmodium falciparum* malaria in Africa"

Global efforts to control *Plasmodium falciparum* malaria have been thwarted by the emergence of multidrug resistance, including high-grade piperaquine (PPQ) resistance in Asia that is mediated primarily by mutant PfCRT. Given the interest in using dihydroartemisinin-PPQ in Africa, we examined whether PfCRT mutations could mediate PPQ resistance (PPQ-R) when added to existing isoforms. We

introduced African mutant pfcrt alleles (GB4 and Cam783) into Asian Dd2 parasites and then added the PPQ resistance mutations T93S, F145I or I218F. Only parasites with T93S exhibited PPQ-R, suggesting an effect of the other PfCRT variant residues that differ between Africa and Asia. All edited lines showed a reduction in CQ susceptibility similar to Asian PPQ-R parasites. Ongoing fitness assays will reveal the impact of our set of mutant pfcrt on parasite in vitro growth rates. Our findings are relevant to global health efforts to identify region-specific treatments and to combat the spread of PPQ-R.

SESSION C
Moderators: Emma Camacho, Johns Hopkins University and Manuela Runge, Northwestern University

Abdou KDJ Fall, Université de Paris – France
"Influence of opsonizing antibodies to *Plasmodium falciparum* merozoites in the control of asymptomatic malaria infection in Beninese children"

We previously observed among Beninese infants with a parasitological and clinical follow-up from birth to 18 months, that infants able to control *P. falciparum (Pf)* asymptomatic malaria infections (CAMI) had higher IgG levels to *Pf* asexual antigens than others. Here, the IgG functionality was explored using opsonizing antibodies to *Pf* merozoites, taking into account individual IgG3 characteristics (G3m phenotypes). Opsonic phagocytosis (OP) was performed using plasma purified IgG as well as THP-1 monocyte cell line and primary neutrophils, genotyped for their Fc gamma receptors (FcgR). CAMI infants presented higher OP than others when using both THP-1 and neutrophils. High OP was associated with high IgG1 and IgG3 levels to *Pf* asexual antigens and with three FcgR-G3m combinations. This highlights the importance of i) opsonizing antibodies to *Pf* merozoites in the control of asymptomatic malaria infection and of ii) IgG3 individual characteristics on the opsonizing capacity of antibodies to *Pf* merozoites.

Victoria Shelus, University of North Carolina at Chapel Hill – USA
"Inappropriate sales of antimalarials in private-sector drug shops in rural Uganda"

In rural Uganda, private-sector drug shops are often the initial point of care for febrile illness, and clients can purchase antimalarials without a confirmed diagnosis. To document malaria diagnostic and treatment practices, we trained 46 drug shop vendors to collect data and capillary blood samples from clients requesting antimalarials. Blood was stored on ice and transported to a laboratory for RDT testing. 934 clients visited study drug shops during the period of observation, of whom only 40% were aware of their malaria status: 25% who had an RDT performed at the drug shop and 20% previously tested at a public health center. Among those with negative tests, 36% still purchased antimalarials. 65% of clients who purchased an antimalarial without an RDT subsequently tested negative. Interventions are needed to address poor malaria case management practices in drug shops, specifically low RDT use and the administration of antimalarials despite negative test results.

Hongquan Li, Stanford University – USA
"Octopi: a ready-to-deploy high-throughput imaging solution for malaria management and malaria research"

We report the latest development of Octopi, an open, configurable and high-throughput imaging solution for malaria diagnosis and research. Since the original preprint in 2019, we have significantly improved the hardware for robustness, scaling-up readiness and performance. Without further engineering for cost reduction, the current system costs less than $5k, is ready to deploy and scale up, can screen 0.5M - 2M red blood cells per minute (depending on magnification), and offers performance at the level of facility research microscopes that typically cost > $100k. At per test reagent cost <$0.05, we show how spectral shift of DAPI staining can enhance the contrast of malaria parasites for sensitive and specific detection. Before its application in clinical diagnosis and treatment monitoring, we believe Octopi can be a valuable tool in research programs in vaccine trials, drug efficacy testing, screening for new treatments, as well epidemiological research.

Lucie Jelinkova, University of New Mexico – USA
"Virus-like particle-based malaria vaccine displaying the L9 epitope of the circumsporozoite protein confers sterilizing protection in a mouse challenge model"

Pre-erythrocytic malaria vaccines that target the central repeat (CR) region of the *Plasmodium falciparum* circumsporozoite protein (PfCSP) – a protein that densely covers the surface of invading sporozoites – provide modest protection from infection. Recent identification of human mAb L9 that targets a novel CSP epitope outside of the CR and potently protects animal models from malaria infection points to new site of vulnerability in CSP. Here, we assessed the efficacy of bacteriophage Qß-based virus-like particle (VLP) vaccine that multivalently displays the highly conserved L9 mAb epitope. We show that the L9 VLP vaccine elicits long-lasting and high-titer anti-CSP antibodies, reduces liver parasite burden and confers sterilizing immunity when combined with TLR9 agonist + aluminum hydroxide-based adjuvant in a PfCSP- *P. berghei* mouse mosquito challenge model. This single epitope-displaying VLP-based vaccine is a promising candidate that highlights the utility of targeting the L9 epitope as a specific site of vulnerability for pre-erythrocytic vaccine development.

Caroline Abanto Alvarez, Universidad Peruana Cayetano Heredia – Peru
"Factors associated with asymptomatic recurrences due to *Plasmodium vivax* infection after radical cure treatment in communities in the Peruvian Amazon"

Asymptomatic *Plasmodium vivax* infections represent one of the main challenges towards malaria elimination worldwide. Hereby, we aim to provide a comprehensive longitudinal analysis of the factors associated to experience a recurrent asymptomatic infection in a 2-year, monthly follow-up longitudinal study. We analyzed the geographic, epidemiological, and parasites genetic data retrieved from a cohort of 302 participants who received the radical cure treatment, chloroquine, and primaquine. During follow-up, in multiple communities in the Peruvian Amazon, these patients recorded 609 recurrences. The parasite density, parasites' genetic variability, geographic origin, and collection year were associated with asymptomatic infections. The recurrent infections were predominantly monoclonal (ratio: 2.2: 1 versus polyclonal) and carried mostly heterologous parasites compared to the first infection (ratio: 3.7:1 versus homologous). Our findings provide relevant information about the origin of asymptomatic infections and provide relevant information to support the elimination efforts of the hidden malaria reservoir.

Sophie Bérubé, Johns Hopkins University – USA
"Distinct antibody responses to *Plasmodium* antigens reveal candidate biomarkers of the intensity and timing of past exposure to malaria"

Malaria surveillance in low transmission settings poses challenges including capturing asymptomatic or mildly symptomatic incident infections. Serological data can provide measures of past exposure without detecting incident infections. However, markers of infection history have yet to be validated. Kobayashi et al. (2019) used a protein microarray to measure antibody responses to malaria antigens across 479 samples collected from three malaria endemic regions with different historical levels of transmission: Choma District, Zambia, Nchelenge District, Zambia, and Mutasa District, Zimbabwe. Using a random forest classifier and the ranks of fluorescent intensity for 64 antigens we classified samples into their most likely region of origin with 94.0% accuracy among adults. Classifying adults, who have longer exposure histories, into locations of different historical transmission levels suggests that these antigens could be markers of the intensity and timing of past exposure. Further validation of these antigens as biomarkers should lead to improved malaria surveillance tools.

Hannah L. Markle, Johns Hopkins University – USA
"Comparison of overnight to early evening malaria vector collections in indoor setting in Nchelenge District, Zambia"

Nchelenge District, Zambia has a high malaria burden, although effective drugs and vector control measures are in place. The complexity of mosquito-to-human *Plasmodium* parasite transmission coupled with *Anopheles* vector behaviors sustain disease incidence in the region, warranting further vector foraging and feeding studies. To distinguish behaviors between the locally predominant vector *Anopheles funestus* and other understudied vectors, CDC light traps were placed in indoor sitting rooms of twenty-four Nchelenge households from 4pm-10pm and 10pm-6am over two weeks in August 2019 to capture foraging mosquitoes. Mosquitoes collected overnight were morphologically and molecularly identified with PCR assays and amplicon sequencing. Assays to identify bloodmeal source as well as *P. falciparum* within mosquitoes will be completed in coming months. These results from overnight collections will be compared with early evening mosquitoes and epidemiological data, translating to a better understanding of malaria transmission in Nchelenge and the potential for more refined vector control strategies.

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