RESOURCE HUB *Correspondents*

1st Women in Malaria (WiM) Conference 2021

Complete series

MESA Correspondents bring you cutting-edge coverage from the 1st Women in Malaria (WiM) Conference

22 - 24 March 2021

Virtual Conference

The MESA Alliance would like to thank Joanne Power (Pennsylvania State University, USA) and the organizers Elena Gómez Díaz (Institute of Parasitology and Biomedicine López-Neyra, Spain) and Sarah Reece (The University of Edinburgh, UK) for providing senior editorial support.

The MESA Alliance would also like to acknowledge the MESA Correspondents Sushma Ambekar (Iowa State University, USA), Christine Markwalter (Duke Global Health Institute, USA), Rosheen Mthawanji (Malawi Liverpool Wellcome Trust, Malawi) and Nkahe Diane Leslie (University of Yaoundé I, Cameroon) for their crucial role in the reporting of the sessions.

Table of contents

Day 1: 22nd March 2021

Welcome and introduction

The first Women in Malaria (WiM) Conference (22 - 24 March, 2021) began virtually, with over 500 registrants attending from all over the world. The organizers, **Elena Gómez-Díaz** (Institute of Parasitology and Biomedicine Lopez-Neyra - IPBLN, Spain) and **Sarah Reece** (The University of Edinburgh, UK) began by welcoming everyone and providing a brief overview of the history, inspirations, and goals of the WiM community. WiM was created in 2018 to address inequities that women in Global Health and Parasitology (with a focus on malaria) face in their daily lives and within the scientific community. The goal of this conference is to showcase the contributions of women to the malaria field, to guarantee diversity and inclusion of all individuals, and provide training, mentoring and networking opportunities for women in the malaria research community. The entirely virtual format enables women from all around the world to participate in the conference.

Session 1 - New frontiers in "omics" approaches

The chairs, **Angela Early** (Harvard T.H. Chan School of Global Health, USA) and **Victoria Ingham** (Heidelberg University Hospital, Germany), welcomed participants to one of the two first parallel sessions of the conference, "New frontiers in "omics" approaches", in which researchers use genomics, proteomics, and metabolomics screening techniques to get insights into the biology of the parasite and further the fight against malaria.

Silvia Kariuki (KEMRI-Wellcome Trust Research Programme, Kenya) discussed her novel approach to functional validation of human host variants that confer protection against clinical malaria. Focusing on the Dantu blood group variant, she performed *in vitro* invasion studies in red blood cells (RBCs) from homozygous, heterozygous, and non-Dantu individuals. Flow cytometry experiments identified a significant reduction in invasion in the Dantu homozygote RBCs across multiple *P. falciparum* strains, and live-video microscopy observation confirmed that merozoites attempted to invade Dantu RBCs, deforming the cells, but were unable to invade at the same rates as non-Dantu variants. Further biophysical characterization of RBCs across all blood groups identified RBC membrane tension as the primary mechanism by which merozoites were prevented from invading RBCs, and the median tension of Dantu-variant cells was significantly higher than that of non-Dantu cells. Kariuki's work established a protective mechanism for Dantu variants whereby the inhibition of *P. falciparum* invasion is mediated primarily by its impact on RBC membrane tension. She noted that recent whole-genome sequencing of underrepresented groups has enabled improved host genome-wide association studies for novel host genes that confer malaria protection in African populations, and in the future, she plans to probe whether tension-mediated invasion inhibition is generalizable across multiple host variants associated with malaria protection.

Franziska Hentzschel's (Wellcome Centre for Integrative Parasitology, UK and Heidelberg University, Germany) presentation was a comprehensive characterization of *Plasmodium berghei* across erythropoietic niches. Using mouse infection models, flow cytometry, and single-cell RNA sequencing (scRNA-seq), Hentzschel explored the distribution and transcriptomes of *P. berghei* parasites in different host cell types and host organ environments. Overall, parasitemia was higher in the spleen and blood compared to bone marrow, particularly for reticulocytes. Parasite scRNA-seq did not show any transcriptional differences between parasites in distinct erythropoietic organs. Despite an enrichment of infected splenic cells relative to blood, the vast majority of merozoite invasion occurred in circulating blood. Parasites demonstrated a bimodal invasion pattern with respect to host cell age, invading either early CD71+ reticulocytes or mature RBCs. Parasite transcriptomes differed depending on whether they invaded early reticulocytes or mature RBCs. Three genes associated with the purine salvage pathway were upregulated in parasites that invaded mature RBCs, which may be a response to decreased metabolite availability in mature cells compared to reticulocytes. A transcriptional factor for gametocyte commitment, AP2-G, was upregulated in parasites that invaded CD71+ reticulocytes, and *in vivo* studies confirmed a higher gametocyte rate in reticulocytes independent of erythropoietic organ. Overall, these data suggest that host cell age, rather than the host organ, determines transcriptional changes in the *Plasmodium berghei* rodent malaria model.

Lisl Esherick (Massachusetts Institute of Technology - MIT, USA) presented her work on developing a scalable pipeline for functional genetic analysis in *Plasmodium falciparum*. The CRISPR-based platform enables gene disruption via knockout and/or the TetR-DOZI system, which allows for conditional gene knockdown. In the latter approach, the sequence for a TetR-DOZI-binding aptamer is integrated into the genome upstream of a gene of interest. When TetR-DOZI is bound to the transcription product, translation is inhibited, resulting in gene knockdown. Adding anhydrotetracycline (aTc), which binds TetR-DOZI with high affinity, disrupts the complex and enables gene expression. Importantly, such an approach opens the door for conditional knockdown of essential genes. Using this platform, Esherick performed functional genetic analysis of a set of 60 pilot target genes with unknown function to determine their essentiality and potential as drug targets. Growth assays after conditional knockdown enabled the classification of targets as essential, slowed growth, or dispensable. Esherick applied this conditional knockdown system to drug target identification, demonstrating proof-of-concept with aminoacyl tRNA synthetases (aaRSs). 275 potential phenylalanyl-tRNA synthetase (FRS) inhibitors were screened and 13 hits were identified. In total, three inhibitors that targeted aaRSs were identified using this scalable CRISPR-based platform. In future, Escherik aims to use this new scalable pipeline for chemogenetic screening of pooled transgenic parasites (~250 genes) against small molecules of unknown function.

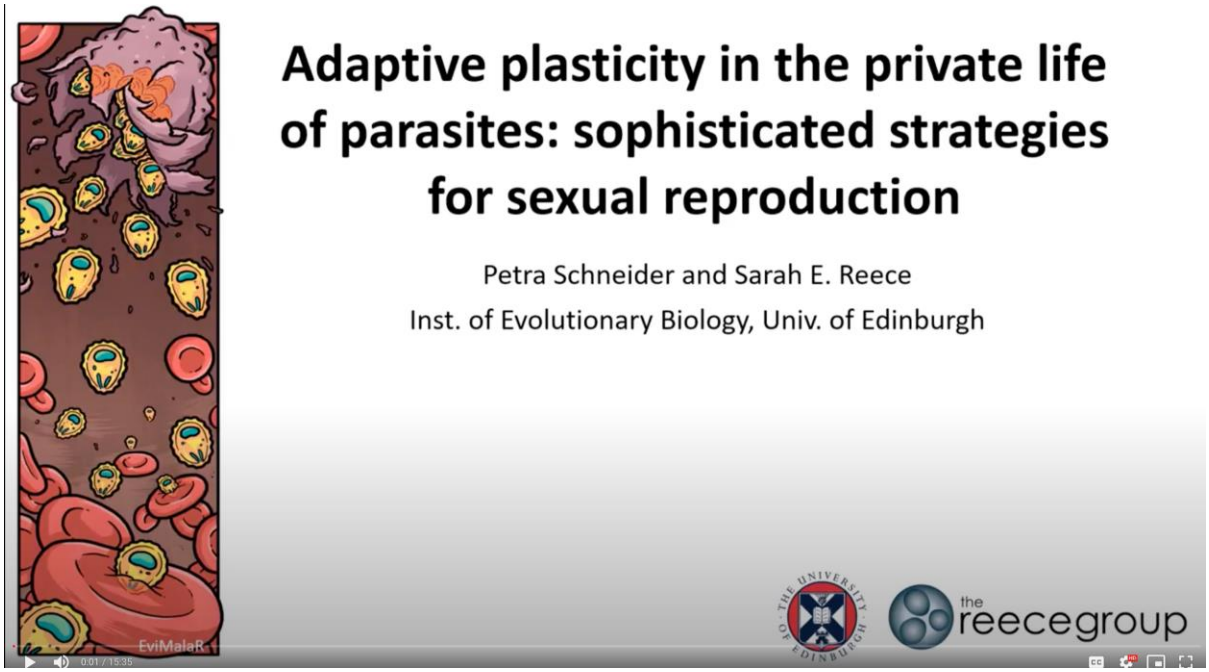
Carla Proietti (Australian Institute of Tropical Health & Medicine, James Cook University, Australia) discussed a systems-based approach for identifying novel antibody and T-cell targets for rational malaria vaccine and immunodiagnostic design. Protein microarrays populated with 2320 antigen fragments (representing ~25% of the proteome) were used to screen *Plasmodium falciparum*-specific antibody responses in serum samples. A highly multiplexed interferon gamma (IFN-γ) Elispot assay was used to probe T-cell responses to 29000 peptide epitopes from 1450 pre-erythrocytic antigens. Immune responses in a cohort of malaria-exposed individuals in Papua New Guinea demonstrated that only 30% of the parasite proteome is targeted by host immune responses, and both antibody and T-cell targets are dispersed throughout the genome. Additionally, T-cells and antibodies recognized distinct antigens, which could be accurately predicted (100% sensitivity and 70% specificity) based on 8 genomic attributes. These findings suggest that different vaccination approaches and antigen targets may be necessary, depending on the desired immune response. Building on this, a 1-year longitudinal study in Ghanian children showed an inverse relationship between the maximum intensity and breadth of IgG antibody responses, suggesting that next-generation malaria vaccines will need to target a combination of a large number of antigens to elicit a high-breadth response. Machine learning and regression techniques revealed a signature of IgG responses to 15 antigens that were predictive of clinical malaria immunity, and these signatures were validated in a separate Malian cohort of children. These findings could be useful in identifying novel vaccine or immunodiagnostic targets.

Mara Lawniczack (Wellcome Sanger Institute, UK) discussed two exciting projects in her group: (1) a novel targeted amplicon sequencing panel for *Anopheles* vectors and (2) the Malaria Cell Atlas. For the *Anopheles* project, the group developed a multilocus DNA barcode panel targeting 62 mosquito primer sites and 2 *Plasmodium* loci that enables a global view on *Anopheles* species present, their population structure, and if each contributes to malaria transmission. After a simple, cost-effective, and minimally-destructive DNA extraction, targets are amplified and sequenced. This approach is useful because it does not require prior knowledge of vector populations and can provide insight into changes in population structure after vector control interventions. It is applicable to any *Anopheles* sample, including adults, larvae, bulk traps, or environmental DNA in breeding sites. An *in silico* analysis showed within-species geographic population structure based on SNPs on 62 loci, and a 2-stage hierarchical classifier accurately (98%) assigned species to 133 wild-caught and 28 mosquitoes *in silico*. The second part of the talk focused on the Malaria Cell Atlas project which consisted in profilling single-cell transcriptomes of parasites in all stages in human and mosquito hosts for *Plasmodium berghei* and *P. falciparum*. This platform has been applied to answer a variety of research questions, and interactive data are available at malariacellatlas.org. Future work will look at the relationship between sexual/asexual parasite stages within single symptomatic and asymptomatic hosts in Mali, as well as building the Malaria Cell Atlas database for *P. ovale* and *P. malariae*.

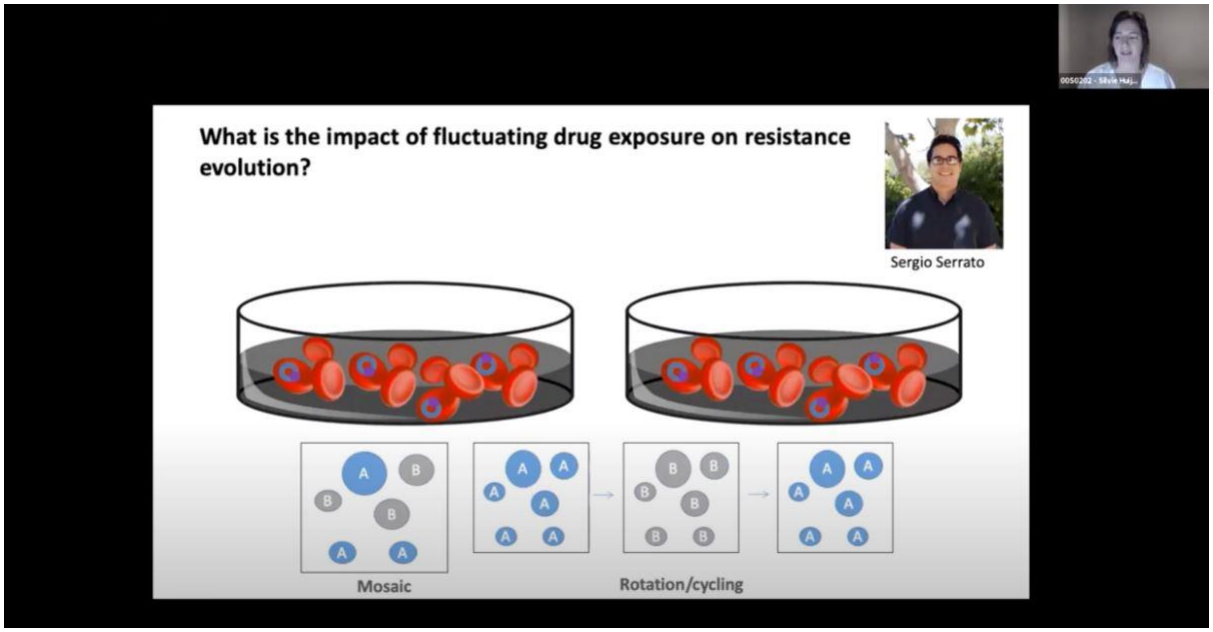
Session 2 - Evolutionary ecology of malaria parasites

Farah Ishtiaq (Tata Institute for Genetics and Society, India) and **Lindsey Plenderleith** (The University of Edinburgh, UK) welcomed everyone to the second Women in Malaria conference session of the day, a session that focused on studying malaria parasites in the light of genetic and environmental factors.

Petra Schneider (The University of Edinburgh, UK) started her talk by highlighting the importance of understanding the evolutionary ecology of malaria parasites. Her work focused on the proportion of asexual malaria parasites that commit to producing thee gametocyte stage, i.e. the "conversion rate" of parasites into sexual forms. Schneider and colleagues collected data from *Plasmodium chabaudi*-infected mice that were exposed to different dosages of the antimalarial drug, Pyrimethamine, over time. It was observed that the parasites altered conversion rate (using phenotypic plasticity) according to the proportion of asexuals stages killed by each dose of pyrimethamine. Further, it appeared that *Plasmodium* parasites detected changes in their environment within the host by measuring changes in asexual parasite population density, as well as detecting changes in the number of host red blood cells. The results of this study demonstrated the predicted non-linear, fitness maximising (adaptive) reproductive strategy of *Plasmodium* spp. parasites, i.e. that smaller losses in asexual parasite density within the host blood resulted in a reduced conversion rate (reproductive restraint), with loss of an irrecoverable proportion of asexually-replicating parasites inducing an increased conversion rate (terminal investment). Schneider ended her talk by highlighting that an understanding of the mechanisms that underlie this adaptive plasticity in gametocyte conversion rate could inform future malaria control strategies as knowledge of these parasite behaviours can be used to manipulate the parasite into responding in ways that are detrimental to parasite fitness.



Silvie Huijben (Arizona State University, USA) presented her work on the evolution of both insecticide resistance and drug resistance and emphasized the global challenges of these issues in malaria control. The main questions that the Huijben group are trying to answer are if there are better ways to manage resistance and if resistance can be predicted. The evolution of resistance is sparked by two processes: mutations and the natural selection of the mutated entities. The current method used in treating resistance dates back to Alexander Fleming and is known as an aggressive treatment, where the dosage of the drug is increased to kill resistant populations of parasite or mosquito. In susceptible populations, natural fluctuations in the density will occur, however, the introduction of drugs leads to a population decline. Huijben used a rodent malaria model to show that if a resistant genotype exists in the population at the point when aggressive drug treatment is administered, then the resistant genotype will thrive and increase due to a release from competition as the susceptible strain reduces. Huijben suggests (1) minimizing chemical use, (2) evolutionary refugia, (3) fluctuating environments and (4) harnessing immunity as ways to reduce the spread of resistance. She ended her talk by introducing a mathematical model to determine which of the 4 previous suggestions is more efficient for drug/insecticide resistance management by exposing mosquitoes to different doses of an insecticide and determining selection.



Anne Vardo-Zalik (The Pennsylvania State University, USA) presented her findings on the effect of drought-like conditions on parasite ecology in the lizard malaria parasite, *Plasmodium mexicanum,* and the infection prevalence on its host, the western fence lizard, *Sceloporus occidentalis.* There is a good amount of research on how long-term drought conditions and vector-borne diseases intersect in terms of their factors like prevalence, transmission foci, and host concentration, but not on how such conditions affect competition or cooperation in multi-clonal infections. The Vardo-Zalik group collected data from California's 'Thousand-year drought' in 2013-2014 and other droughts that occurred between 1978 - 2016 to look at parasite prevalence and genetic diversity. Accounting for variation in sex, size, and site of capture for each lizard, their analysis revealed that there was a significant decrease in clonality with drought-like conditions. Infection prevalence, however, showed contrasting results for short-term and long term data-sets. Regardless of the drought measure, there was always a positive association for the former and a negative association for the latter. Vardo-Zalik concluded her talk by saying that the disconnect between prevalence and infection complexity could be due to the transmission of multiple clones, and competition caused due to the drought or transmission foci.

Kathryn E Tiedje (University of Melbourne, Australia) started her presentation by throwing light on the importance of malaria disease surveillance using molecular and genetic approaches. Specifically, the work presented was focused on markers under immune selection such as variant surface antigen encoding genes. PfEMP1, encoded by the *var* multigene family, is the major variant surface antigen present on infected erythrocytes which frequently undergoes clonal antigenic variation allowing the parasite to successfully evade the host immune system. *Var* genes have previously been disregarded as biomarkers for malaria surveillance due to their diversity, but Tiedje and colleagues show that a 450 bp DBLα domain can be used to study *Plasmodium* global population structure at different spatial scales. They utilized the Jumping Hidden Markov Model (JHMM), taking consideration of recombination within *var* genes and investigated the relationship between DBLα types from *Plasmodium* laboratory strains and those isolated from chimpanzees and gorillas, showing the effectiveness of such an approach. They then applied the same approach to 1248 *P. falciparum* isolates from 10 countries. While the global population structure of DBLα types was clustered, indicating a distinct division across continents, the group noted the occurrence of the 100 most frequent DBLα types occurring globally, thus stressing the importance of elucidating their biological functions.

Ana Rivero (French National Centre for Scientific Research, France) began her talk by posing a question to the audience: "Are there any general laws in parasite ecology to explain recurring patterns?" This question was discussed within the context of how parasites in diverse hosts occur in quantifiable distributions, i.e. the majority of hosts have few parasites, but a small fraction of them have a high number of parasites. Aggregation/overdispersion of parasites has very important ecological and epidemiological consequences, but the underlying mechanism remains to be dissected. To this end, Rivero discussed the hypothesis that adaptive parasite plasticity leads to temporal heterogeneity. To test this, Rivero and colleagues explored whether the aggregated distribution of three strains of the avian malaria parasite, *P. relictum*, within *Culex pipiens* mosquitoes was explained by temporal heterogeneity in parasite burden and/or infectivity triggered by the bites of mosquitoes (with the Atlantic canary (*Serinus canaria*) as the avian host). An increase in parasitemia was observed in birds that were stimulated by mosquito bites for early and late chronic infections. When the group also observed a proportional increase in oocyst numbers for these infection stages in the mosquito, they set out to compare the number of oocysts in early and late biting mosquitoes (individual and batch) over a three-hour time period. The results of these experiments echoed some of their previous observations, showing higher oocyst numbers in later biting mosquitoes, despite no changes in the density of parasites within the host, suggesting that parasites become more infective in response to the biting of mosquitoes.

Workshop I

In between sessions, there was the first Workshop of the conference for pre-selected participants on Leadership.

Session 3 - Host-Parasite Interactions

The chairs, **Maria Bernabeu** (European Molecular Biology Laboratory - EMBL, Spain) and **Camila Coelho** (National Institutes of Health - NIH, USA), welcomed participants to the third session of the Women in Malaria Conference "Host-Parasite Interactions", a session in which presenters explored the roles of human host factors on malaria infection.

Elizabeth Egan (Stanford University School of Medicine, USA) presented her work on establishing the identities and functions of red blood cel (RBC) factors in malaria parasite invasion. This is a particularly challenging line of work, because RBCs lack nuclei, making genetic studies less straightforward. Egan's solution relies on genetically modifying human hematopoietic stem cells using CRISPR/Cas9 and subsequent *ex vivo* erythropoiesis. Cultured and genetically modified RBCs can be then infected with *Plasmodium falciparum* to systematically screen for novel host factors. Using this methodology, Egan's group identified CD44 as a candidate critical for *P. falciparum* invasion. Subsequent assays showed that CD44 binds the erythrocyte-binding antigens (EBAs) 140 and 175, known targets involved in RBC invasion. Although the CD44-null RBCs did not show decreased EBA-175 binding, they did demonstrate lower surface phosphorylation relative to the wild-type, suggesting that EBA-175 induced phosphorylation of erythrocyte cytoskeleton proteins could be dependent on CD44. These findings on CD44 and future implementation of this workflow on other potential RBC factors could lead to novel strategies for anti-malarial interventions.

Ekta Saini (International Centre for Genetic Engineering and Biotechnology - ICEGB, India) shared with the audience a recent study on *P. falciparum* Photosensitized INA-Labelled protein 1 (PhIL-1) and the

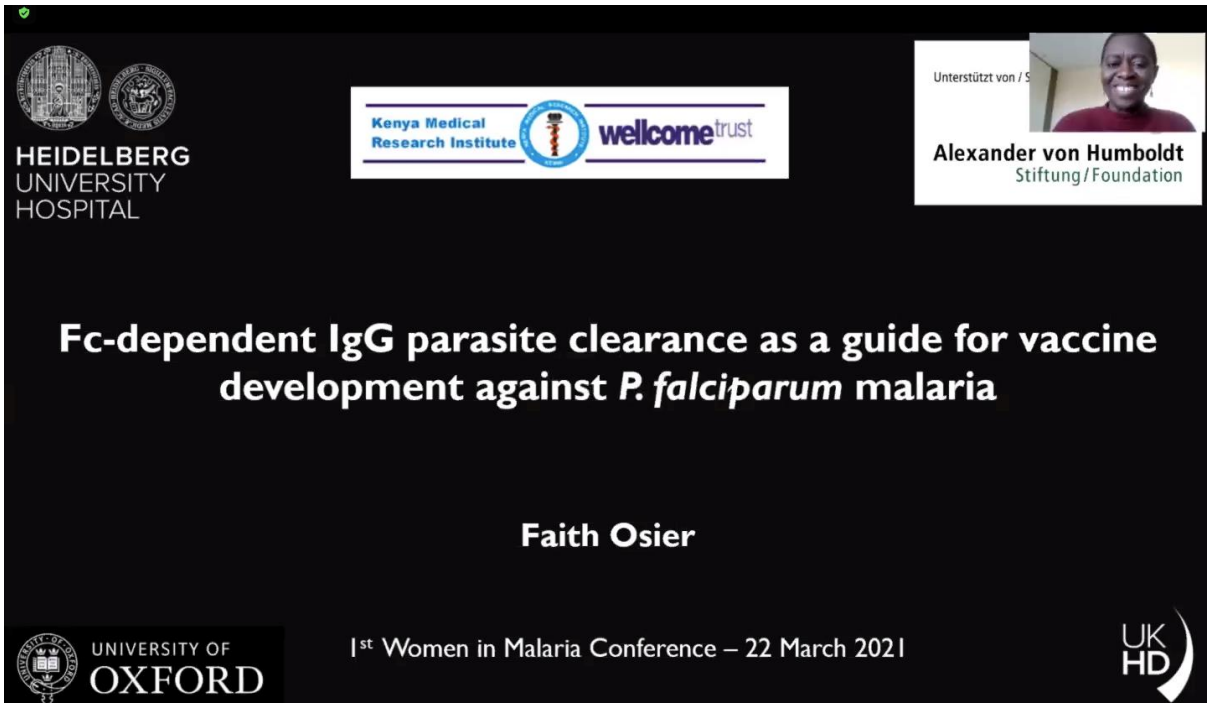
discovery of a novel PhIL-1 associated protein complex followed by its functional characterization. PhIL-1 is a part of the inner membrane complex (IMC) in the parasite and is associated with the parasite's glideosome machinery. The proteins of this complex are required for propelling merozoites into the RBC during the invasion process. Microscopy studies conducted in collaboration with Rita Tewari (The University of Nottingham, UK) showed localization of PhIL-1 to the parasite IMC. Knockdown studies for PhIL-1 showed a reduction in various parasite life stages, but invasion remained unaffected. PhIL-1 was also seen to extensively interact with a glideosome associated protein (PfGAPM2), an IMC structural protein, Alveolin 5 (PfALV5), and a previously uncharacterized protein, referred to in this study as PhIL-1-interacting protein (PfPhIP). Co-localization of these proteins with PhIL-1 was observed using immunofluorescence assays and the formation of a second, PhIL-1-associated complex at the IMC that co-existed with the glideosome motor complex was identified. Finally, GlmS ribozyme knockdown of PfPhIP showed an 80% reduction in invasion and defective segmentation of daughter parasites during schizogony, demonstrated by Saini using electron microscopy. In conclusion, the research team identified a novel protein complex associated with the *P. falciparum* glideosome motor complex and showed that it is crucial in host cell invasion.

Maya Aleshnick (Oregon Health and Science University, USA) and her group have developed an alternative non-human primate relapsing malaria model that can be used for studying *P. vivax,* overcoming limitations of current P. vivax's laboratory model. They used this model in *P. cynamolgi*, a *Plasmodium* spp. parasite that is genetically similar to *P. vivax* and develops a dormant hypnozoite stage in liver cells, a characteristic of *P. vivax* that is responsible for relapsing malaria infections. As a part of their experiments, Aleshnick and colleagues infected *Anopheles* mosquitoes with *P. cyonomolgi* parasites from rhesus macaques and have successfully observed exflagellation, oocyst formation and generation of salivary gland sporozoites. Further infection of rhesus macaques with these sporozoites was carried out and the course of infection in these non-human primates could be studied over time. Aleshnick also observed that drug treatment only cleared *P, cynomolgi* blood stages while the dormant hypnozoite stages persisted, resulting in the desired relapsing malaria model. This *in vivo,* non-human primate model allows for assessment of liver immunology and examination of hypnozoite and liver-stage parasites by minimally-invasive small liver biopsies. This group has also teamed up with other collaborators to perform single-cell sequencing of extracted cells

to identify markers of hypnozoite formation. They are also working on developing *P. cynomolgi* strains that express *P. vivax* circumsporozoite protein (CSP) as a model for vaccine development.

Martha Cooper (Australian Institute of Tropical Health and Medicine, James Cook University, Australia) addressed the heterogeneity of human immune responses to *P. falciparum* infections and how these host differences might affect malaria parasites. In particular, Cooper's research aims to identify baseline immune determinants of heterogenous infection outcomes. Using whole transcriptome and small RNA sequencing to quantify coding and noncoding RNAs following controlled human malaria infections (CHMI), Cooper identified a large number of differentially expressed RNA molecules, including coding-, long non-coding-, and micro- RNAs. Least Absolute Shrinkage and Selection Operator (LASSO) regression performed with re-sampled splitting, found a set of RNA molecules predictive of parasitemia and parasite multiplication rate after infection that accounted for about 50% of the variation in host immune responses. This work is a step toward understanding how particular immune responses control parasitemia and parasite multiplication rate after infection, which could ultimately enable rational vaccine design to elicit those specific responses.

Faith Osier (KEMRI - Wellcome Trust, Kenya and Heidelberg University, Germany) presented her research in identifying and robustly characterizing vaccine candidates, correlates of protection, and their mechanisms. Osier presented results from a controlled human malaria infection (CHMI) study in which volunteers were selected based on their previous malaria exposure as measured by seroreactivity against *P. falciparum* schizont extracts. Parasitemia was tracked daily using qPCR from day 7 onward after injection of *P. falciparum* (NF54) salivary gland sporozoites. It was found that individual infections separated into four groups: (1) those with exponential parasite growth, (2) individuals who were infected but eventually cleared parasites without treatment, (3) those who maintained consistent, low-level parasitemia, and (4), individuals who remained uninfected. Antibody levels against a panel of merozoite proteins were differential between these groups, with breadth of Fc activity against merozoite antigens positively associated with protection. These results suggest that focusing on a wide breadth of antigens that induce Fc-effector function could be beneficial for rational vaccine design.



Session 4 - Vector-Parasite interactions

This session was chaired by **Damaris Matoke** (Kenya Medical Research Institute (KEMRI), Kenya) who welcomed participants to the fourth session of the Women in Malaria conference on "Vector-parasite interactions", where researchers explore interactions between the *Plasmodium*-infected mosquito and its microbiota for efficient control of malaria.

Nsa Dada (University of Abomey-Calavi, Benin) presented her research, which focuses on the mosquito microbiome and insecticide resistance. Dada began by highlighting that the previously declining rate of malaria morbidity and mortality worldwide is stagnating, and she attributed this to the global challenge of insecticide resistance and a stall in producing adequate vector control tools. Dada pointed to four main mechanisms of insecticide resistance: (1) mosquito cuticle modification, (2) increased metabolism, (3) target site alterations, and (4) behaviour change. Dada then introduced a lesser known mechanism: (5) microbial metabolism. Mosquitoes have microbes in them that regulate host physiology. Microbiota-mediated insecticide resistance is increasing in agricultural insect pests. Dada's group questioned if the mosquito microbiota contributes to resistance or if it is affected by it. To unravel this mystery, they investigated if microbial composition differs between insecticide-resistant and insecticide-susceptible mosquitoes? Wild-caught *Anopheles albimanus* was exposed to fenitrothion and results showed that microbial compositions do differ between resistant and susceptible mosquitoes. They then aimed to investigate if these results would be similar in different locations of vectors and insecticides, and similar results were found; that insect pathogenic bacteria and bacterial antagonists were enriched in insecticide-susceptible mosquitoes. Altogether, Dada and colleagues showed that pyrethroid exposure alters mosquito microbiota; that mosquito microbiota differed by insecticide resistance phenotype, and that insecticide-metabolizing bacteria are enriched in the insecticide-resistant mosquito.

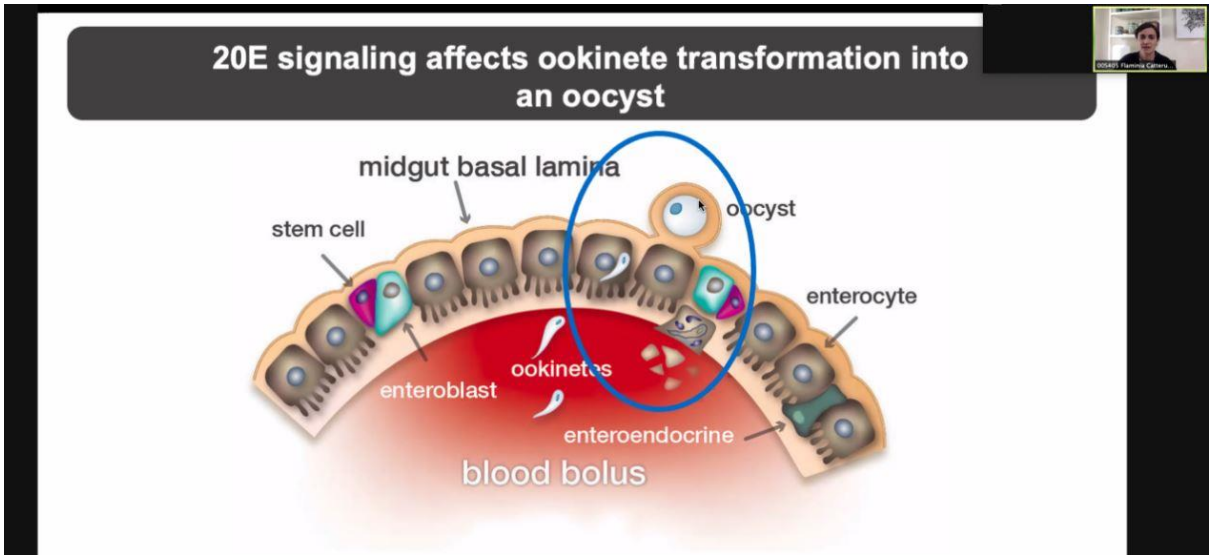
Bárbara Díaz Terenti (Institute of Parasitology and Biomedicine "López-Neyra" - IPBLN, Spain) began by mentioning that the success of the malaria transmission cycle relies on the interactions between the vector and the parasite. As such, Terenti and colleagues analyzed differential gene isoforms and alternative splicing mechanisms in the midguts and salivary glands of *P. falciparum*-infected and non-infected mosquitoes. Alternative splicing is a form of gene expression regulation that allows a single gene to code for more than one protein. It is known that *P. falciparum* infection of *Anopheles* mosquitoes causes changes in the metabolism of some nutrients in the mosquito, in the frequency of bites, in lower egg production, and in modulation of mosquito immunity. These changes are studied as changes in gene expression. However, the potential role of alternative splicing in mosquito phenotypes, such as vector competence, physiology and reproductive capacity, remained to be investigated until now. Through the work of Terenti and colleagues, hundreds of differentially-expressed gene isoforms were identified between *P. falciparum*-infected and non-infected *Anopheles gambiae* mosquitoes. Results also demonstrated that some isoforms are differentially expressed between infection of the midgut and salivary glands of mosquitoes. Most differentially-expressed isoforms were associated with vector-host interactions, including in the synthesis and metabolism of fatty acid and non-ribosomal peptides as well as immune responses. The most common alternative splicing mechanism observed in these experiments were exon skipping (ES) and the use of alternative transcriptional start sites (ATSSs). These results contribute to a better understanding of the importance of alternative splicing to the transcriptional response of mosquitoes when infected with the *P. falciparum* malaria parasite, and open the door to the design of new vector control strategies.

Sunita Swain (Tata Institute for Genetics and Society, India) defined *An. stephensi* as the most important urban malaria vector in India because of its high adaptation to its setting. Swain's group aims to develop mosquitoes' refractory to disease transmission. For this, they have assessed *Anopheles* population biology and genetics by bringing larvae and adult mosquitoes from

different parts of India and maintaining them in insectary conditions with the purpose of evaluating the vectorial capacity of different mosquito eco-types. This study also compared the susceptibility of *An. stephensi L.* populations to *Plasmodium* infection. Of 4 mosquito colonies, termed Types 1-4 (T1-4), one population (T4) has already been cultivated for more than 120 generations. The other 3 populations have surpassed 50 generations since their collection. Wild mosquitoes from T1 and T4 locations were resampled and their DNA was extracted for molecular studies in 2019, with no difference in fitness observed. Microbiota was observed to be influenced by the habitat and food intake of the mosquitoes. The ecological adaptation of *An. stephensi* complexifies its spatial distribution. Despite the high differential susceptibility of these vectors for *Plasmodium falciparum*, the susceptibility for *Plasmodium berghei* remains low among colonized populations. At present, Swain and colleagues have observed that the conditions of larval rearing, age of mosquito during feeding with *Plasmodium* spp.-infected blood, and genetic diversity all contribute to alterations in vectorial capacity of the mosquito.

Paola Carrillo-Bustamante (Max Planck Institute for Infection Biology, Germany) discussed the creation of an individual-based mathematical model for *Plasmodium* transmission that takes into account human, mosquito, and parasite factors. Simulated malaria infection shows infected mosquitoes demonstrate slow growth, reaching 20% in 300 days. The model predicts transmission events are rare and caused by long-lived mosquitoes. Recently emerged mosquitoes have a low metabolism and blood meal increases the mosquito metabolism by increasing reserves, egg laying capacity and parasite development. These parameters are positively correlated to the number of blood meals. Long sporogony is beneficial under multiple feedings. The model suggests that transmission events are extremely rare and caused by long-lived mosquitoes that feed multiple times. Plasmodium successfully competes for resources accumulated during multiple blood meals. In their next steps, they will check if malaria parasites exploit mosquitoes that feed several times to optimise transmission and if in those cases the long EIP is advantageous.

Flaminia Catteruccia (Harvard T.H. Chan School of Public Health, USA) and her group focuses on studying the interaction between mosquito reproduction and parasite development. Catteruccia's talk started with some information on the vector-parasite cycle, with emphasis on infected mosquito blood-feeding and on the location of parasite development (midgut) and mosquito egg development (ovaries). In this study, Catteruccia and colleagues aimed to investigate if parasite development affected egg development in *Anopheles gambiae.* Results showed the number of mosquito eggs was not affected by the presence of the parasite. A positive correlation between egg development and the number of oocysts was also drawn when comparing both lab and wild-caught mosquitoes. The hydrosteroid synthesised 20E by female mosquitoes after blood feeding was found to be interfering with egg development. In fact, interfering with steroid hormones reduces the number of eggs and the number of parasites, resulting in the death of the parasite when egg development is interrupted. Females in which the 20E cycle is interrupted have bigger oocysts due to faster parasite growth and also a higher number of sporozoites in salivary glands. In addition, it was found that impairing egg development increases the speed of *Plasmodium falciparum* oocyst development in the mosquito. The parasite is capable of utilising lipids transported by lipophorin proteins and can export nutrients from mosquitoes. Subsequent blood meals increase the speed of parasite growth and the extrinsic incubation period (EIP). Catteruccia ended the talk mentioning the antimalarial atovaquone. They observed that mosquitoes exposed to atovaquone had no parasites, showing that antimalarials can kill parasites within mosquitoes. This strategy was tested against insecticide resistant *An gambiae*, and it was found to be 100% efficient against insecticide resistant mosquitoes and parasites in that area.



This report is brought to you by the MESA Correspondents Sushma Ambekar, Christine Markwalter, Rosheen Mthawanji and Nkahe Diane Leslie with mentoring and editorial support from Joanne Power.

Day 2: 23rd March 2021

Session 5 - Transmission biology

Virginia Howick (University of Glasgow, UK) and **Anne Vardo-Zalik** (Penn State York, USA) chaired this session which focussed on malaria transmission biology, a known bottleneck in the parasite life cycle. Speakers presented their data on the study of host and parasite genetic and ecological factors that influence transmission and how these factors can be used in designing novel antimalarial therapeutics.

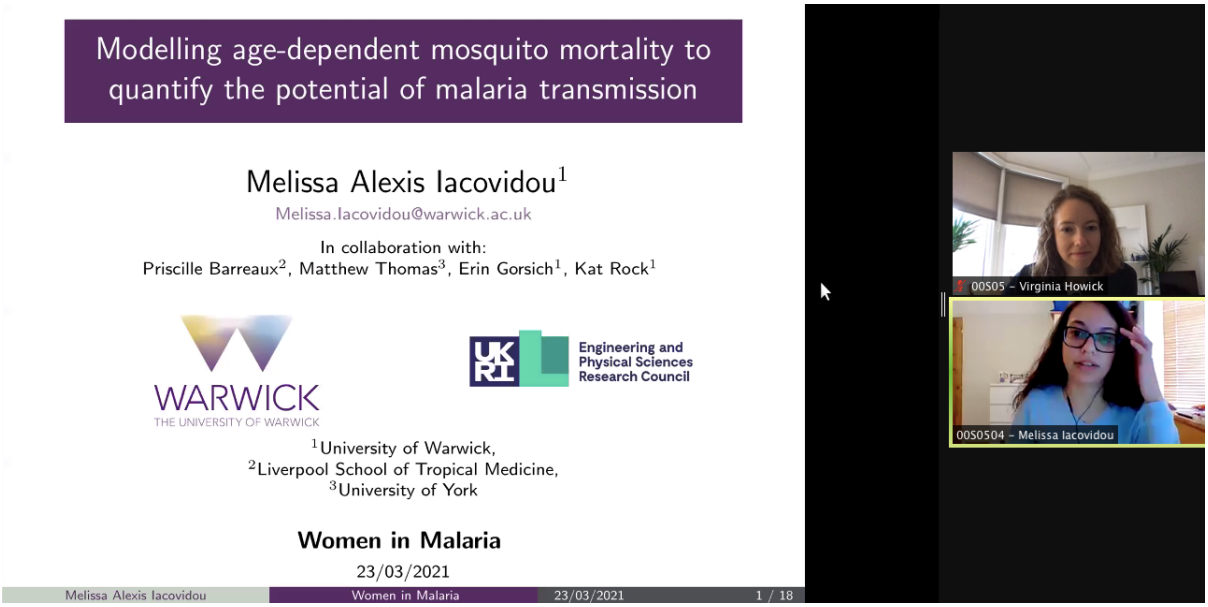
Claire Sayers (Umeå University, Sweden) presented her recent findings using sex-specific genetic screens aimed at identifying *Plasmodium* fertility genes with sex-specific roles. Obtaining such data will allow the malaria community to better grasp the underlying mechanisms of the transmission stage itself. Transfecting barcoded PlasmoGEM vectors into male-only and female-only *P. berghei* parasites, followed by transcriptomic analysis provided the research group with hundreds of genes with sex-specific roles. They observed enrichment of 74% male and 16% female-specific fertility genes in their respective clusters. To better understand male fertility, Sayers and colleagues designed a density gradient-based assay to separate male gametes after exflagellation in order to understand gene expression profiles before, during and after exflagellation. This yielded a repertoire of previously-identified genes associated with pre-motility (AP2-G), motility (DLC1) and post-motility (UIS4) along with multiple novel, unannotated gene candidates. Looking deeper into sexual male stages, they tried to identify new components of the flagellum, which is formed exclusively during the formation of male gametes in the mosquito midgut. Their results contained known and unknown flagellum components, including 12 essential male fertility genes that displayed non-motile phenotype in mutants. Sayers concluded by highlighting that these essential male fertility genes could serve as potential transmission-blocking targets.

Juliana Cudini (Wellcome Sanger Institute, UK) and her team set out to explore the sexual development of the malaria parasite by trying to find evidence of predetermined factors for male and female gametocytes. They followed 62,000 *P. falciparum* NF54 and 7G8 parasites as they exited the asexual stages into gametocyte development and carried out single-cell RNA sequencing at 8 different time points. They focused on two main branch points of the process: cyclical departure and sexual differentiation, which indeed showed varying gene expression patterns. Cudini pointed out that cyclical departure was marked by an increased expression of genes such as AP2-G, ETRAMP4, GEXP02 and G27/25 which drop rapidly during sexual differentiation. During sexual differentiation, however, genes such as Pfs16 showed increased expression. Looking further into the transcriptomic data of late male and female gametocyte markers, a distinct set of sex-specific genes were identified. However, early male and female stages exhibited the same transcriptomic profile, indicating that gene expression during early sexual commitment in immature gametocytogenesis is identical prior to male and female differentiation. This work allowed high-resolution single-cell transcriptomic analysis of sexual differentiation in the *Plasmodium* parasite, thus capturing the heterogeneity of the cells can be explored further at the Malaria Cell Atlas website.

Megan Greischar (Cornell University, USA) presented her research on how parasites balance the trade-off between making asexual stages versus producing gametocytes for transmission. Greischar attempted to answer this with a data-driven model. While within-host ecology selects for parasite proliferation to gain competitive advantage against other strains, ecology outside the host favours transmission investment. At this scale, the parasite's investment could be affected by infected hosts who recover, waxing and waning of vectors, and finally, the growth and decline of epidemics. The fitness strategy included transmission investment variation according to age of host infection. Simulated malaria dynamics show that parasites that invest in transmission stages take longer to reach

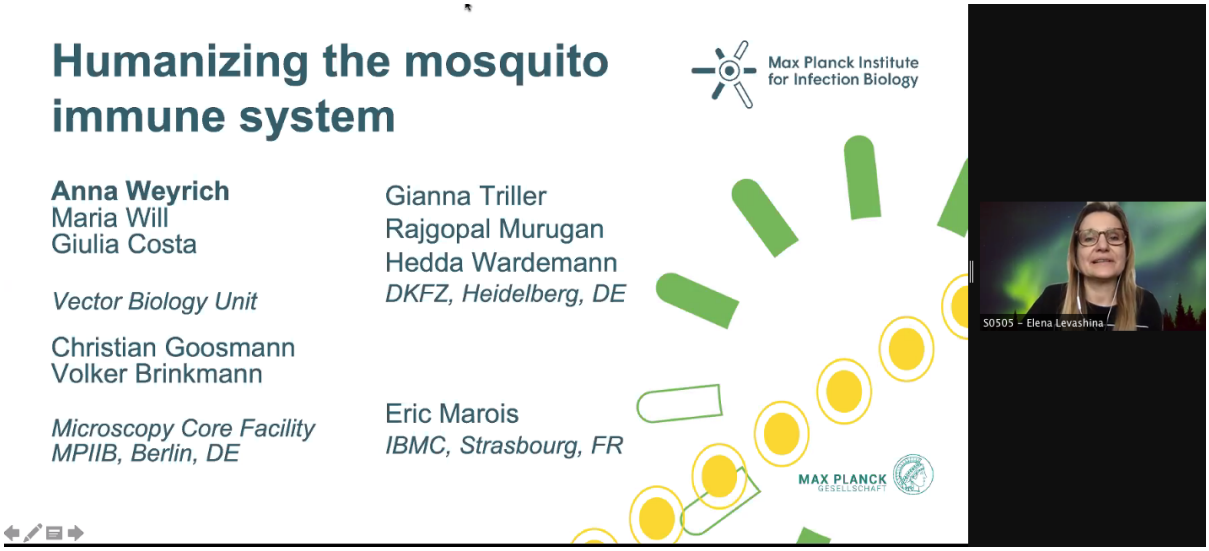
the peak of infectiousness when compared to parasites that favour proliferation. While vector dynamics, such as a shrinking mosquito larval population, forced the parasites to select for aggressive proliferation at expenses of lower transmission rate, a growing mosquito population resulted in higher transmission. Lastly, Greischar talked about epidemics altering the mean age of infection. In the early stages of an epidemic, transmission stages are lower as parasites are trying to proliferate rapidly. In this case, within-host ecology (co-infection) is favored. In conclusion, the ecology outside the host imposes selection on transmission investment, and that is where humans have the most leverage against the parasite.

Melissa Alexis Iacovidou (University of Warwick, UK) discussed efforts to study mosquitoes to quantify the potential of malaria transmission in an age-dependent manner. Her group has checked if mortality rates change due to multiple insecticide exposure and its impact on mosquitoes' vectorial capacity. They also investigated vectorial capacity age-dependency. Investigating this hypothesis involved mosquito-biting experiments in which volunteers placed their feet underneath long-lasting insecticidal nets (LLINs) and nets without insecticides. 200 mosquitoes were tested every 4 days. Age-dependent mortality, survival function, and relationship between survival and mortality function were used to assess the life trait parameters. Results showed that the higher the extrinsic incubation period (EIP), the greater the age of the mosquito, and the lower the number of bites it takes. The probability of the number of bites is lower at a younger age. Age dependency was also lower in treatment compared to control. They concluded that despite mosquitoes being insecticide resistant, the vectorial capacity is reduced in higher age mosquitoes. Therefore, anti-vectorial interventions have been underestimated when age is considered. In the future, this group will explore different distributions for the EIP, including realistic mosquito bite rate to match mosquito behaviour, constructing a disease model to see the effect of age-dependency in mosquitoes on transmission, and matching human malaria cases to disease models to evaluate control programmes.



Elena A Levashina (Max Planck Institute for Infection Biology, Germany) spoke about humanizing the mosquito immune system. The *Plasmodium* circumsporozoite protein (CSP) is the major surface protein of sporozoites and the CSP repeat region provides the highly immunogenic basis of the vaccine: RTS,S/ASO1. In this study, natural human monoclonal anti-CSP antibodies (mAbs) were studied with a traversal inhibition assay of *P. falciparum*. The team generated a new transgenic mosquito line, lipophorin:125 (Lp:125), and observed that expression of mAb125 reduced prevalence of salivary gland infection and limited the sporozoite loads. The number of oocysts was similar between wild-type and mutant, but sporozoite numbers were very low in mutants at 14 dpi and 18

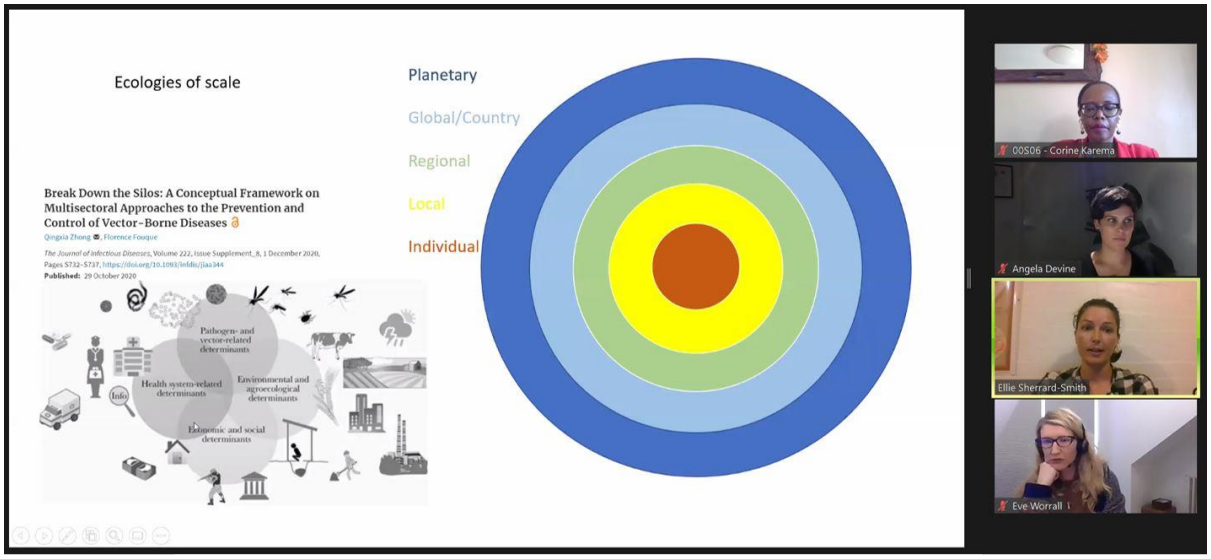
dpi, thus, they focused on oocyst development. The majority of the parasites in the mutant mosquito were not sporulating due to membrane retraction and thus showed sporogenic defects. When oocyst numbers were low, the proportion of sporulating oocysts was 0, and as oocyst numbers increased, the mAb was not sufficient to stop sporulation. Similar results were observed with the number of mean salivary gland sporozoites for low and high number of oocysts. The immune factor, thioester-containing protein 1 (TEP1) was found to limit *P. berghei* development by binding and killing ookinetes. This group will further study the titration effect of mAb125 on *P. falciparum* sporogony and in conditions of low oocyst density found in salivary glands of mosquitoes expressing scFv-125.



Session 6 - Social and economic aspects of malaria

Angela Devine (Menzies School of Health Research, Australia) and **Corine Karema** (African Leaders Malaria Alliance - ALMA and Swiss Tropical and Public Health Institute, Switzerland) welcomed participants to the session addressing social and economic aspects of malaria.

Ellie Sherrard Smith (Imperial College London, UK) shared her work on the global challenges of controlling COVID-19 and malaria. She pointed out that even though most studies focussed on the individual, local or regional scale, we all fall on the planetary scale and also that most publications now look at this holistic way of solving problems. As an example of this, she talked about integrated vector management (IVM), a decision-making process. From a global perspective, the landscape has been changing gradually, and this resulted in global challenges also changing with time. To identify the potential effect of COVID19 on malaria interventions, the group performed a series of simulations of malaria service interruption (IRS, LLINs, SMC, treatment) depending on COVID-19 control measures. They also investigated which age groups would be most likely to be infected by COVID-19. Results showed that younger people are less likely to have fever from COVID, so the malaria:COVID fever ratio depends on seasonality and age. They hypothesised that COVID-19 patients may have asymptomatic malaria which may have increased malaria deaths. However, WHO estimated additional 40k-50k malaria deaths in 2020 at the lowest end of the predictions. The African continent did well at controlling COVID-19 due to their timely response, younger population, and lower travel. She concluded that management of malaria control is sensitive to challenges from other risks including COVID-19, and understanding these risks can help protect communities and focus interventions.



Eve Worrall (Liverpool School of Tropical Medicine, UK) presented the cost and cost-effectiveness of screening and installing Eave Tubes (SET) in a cluster randomised control trial. Eave Tubes, which contain a net charged with insecticide, are a novel way to deliver insecticides plus screening to make the house more "mosquito proof". It is well known that malaria control tools are cost effective, with insecticide treated nets (ITNs) being the best value for money. Currently, the introduction of a new tool is cumbersome because it has to fall in the cost effective category, whilst the budget for malaria control tools shrinks. Thisstudy evaluated a combination of two tools, screening houses and the use of eave tubes (SET) (which involved installing window screens), and closing gaps in eaves and gaps on doors, walls etc. The study design was set to compare impact of screening + eave tubes (SET) vs ITNs alone on clinical malaria in children under 10 years old. Worall estimated the incremental cost-effectiveness of adding the tubes. The trial was a 2 arm randomized control trial (RCT) in Bouake town in Gbeke region, Ivory Coast. At the end of the trial, 3021 houses were repaired with SET followed by 6 rounds of re-treatment with fresh insecticide and 6 rounds of housing repairs to maintain mosquito proofing. Children living with a SET intervention were 38% less likely to have malaria than those with ITNs alone. Overall, this intervention had 74% chance of being cost-effective relative to current thresholds.

Trizah Koyi Milugo (International Centre of Insect Physiology and Ecology - ICIPE, Kenya) shared her work on identifying the preferred model for malaria education in high school students in rural western Kenya. It is well known that community participation and engagement can accelerate progress toward malaria elimination goals. Currently, most existing social engagement activities regarding malaria control primarily target adults, even though youth represent the majority of the population and the next generation of decision-makers. To address this, Milugo's group aimed to assess existing malaria knowledge, identify preferred sources of information, and determine preferred models of education among young people. To do this, Milugo performed a cross-sectional survey in 3 secondary schools, enrolling a total of 399 participants. Students completed self-administered questionnaires on malaria causes, treatment, prevention strategies, and information sources. The survey found that students had a strong knowledge of malaria transmission (over 90% correctly answered questions about mosquito vectors and breeding sites), but were weaker in their knowledge of malaria symptoms and treatment. The students also identified social media and digital sources as their preferred resources for malaria information. Additionally, students participated in a variety of arts, science, and writing-based malaria education activities. Follow-up surveys indicated that a majority of students preferred performance arts as the preferred model for malaria education. As such, these models would likely be most useful for promoting awareness and education on malaria to youth in western Kenya.

Angela Devine (Menzies School of Health Research, Australia) shared an online set of tools useful for evaluating the cost-effectiveness of *Plasmodium vivax* (*Pv*) management strategies. Radical cure of *Pv* infections requires drugs that kill both blood-stage and liver-stage parasites. Primaquine and tafenoquine are the only such treatments, and they pose a serious risk of hemolysis to individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Thus, the risks and benefits of implementing radical cures need to be weighed against these potential adverse outcomes. The heterogeneity in the epidemiology of *Pv* relapses and the severity of G6PD deficiency pose a challenge for weighing these risks. As such, Devine has developed cost and cost-effectiveness models that are available online in user-friendly applications that allow parameter adjustment. The first model (link) allows decision-makers to determine the cost and cost-effectiveness of four G6PD deficiency screening and malaria treatment strategies. The second model (link) compares the cost and cost-effectiveness of a country's baseline national policy scenario with a radical cure scenario in which radical cure is administered in a fully supervised manner, ensuring full adherence to all eligible individuals. Devine remarked that these applications balance simplicity for users and complexity of the decision models, and that they are user-friendly, provide detailed instructions and dictionaries, and restrict inputs and ranges. Although additional robust economic evaluation is recommended before implementing policy changes, Devine hopes these tools can serve as starting points for informing treatment policy for *Pv* malaria.

Rima Shretta (University of Oxford, UK) - This speaker was unable to attend due to unforeseen circumstances

Worksop II and Roundtable

In between sessions, there was two very interesting activities, the second Workshop of the Conference on Peer Mentoring.

And a Round table discussion on "Increasing women's leadership across all aspects of the malaria elimination fight - research, program implementation and policymaking" with Damaris Matoke-Muhia, Ghislaine Ouedraogo-Ametchie and Thu Do.

Session7 - Malaria functional genomics and epigenetics

The chairs for this session on "Malaria functional genomics and epigenetics", **Ana Rita Batista Gomes** (University of Montpellier, France) and **Joanne Power** (Pennsylvania State University, USA), extended a warm welcome to the audience and introduced the speakers for this session.

Katarzyna Modrzynska (University of Glasgow, UK) talked about gene expression control during *Plasmodium berghei* transmission from host to vector, which is one of the most critical phases of the parasite life cycle. The team questioned how it is possible for an organism to have such a precise and tight regulation of cell cycle and yet depend on a repertoire of less than 30 AP2 transcription factors (TFs). To answer this question, her group tagged seven apiAP2 TFs for study during ookinete development. Two TFs were found to be present in asexual stages (AP2-O, AP2-OY), four were expressed in female gametocytes or zygotes, and four in ookinetes. Chromatin immunoprecipitation and sequencing (ChIP-seq) revealed the occurrence of these TFs in developing ookinetes. The shared binding preference of these TFs for certain DNA motifs suggested AP2-TF cooperation during ookinete development. They observed that AP2-O3/FG and AP2-O/O4 co-bind to many gene promoters, which suggests that these TFs can bind as heterodimers. Additionally, comparative ATAC-seq and TF ChIP-seq enabled examination of possible transcriptionally activating roles of TF pairs. The AP2-O ChIP-seq profile in asexual parasite stages differs from those shown during ookinete development. When looking at the CHIP-seq data of the homologous AP2-O in the human malaria parasite *P. falciparum,* the team found distinctive genome occupancy compared with another factor being also expressed at this cycle stage, AP2-I. Modrzynska ended her talk by suggesting that transcription factors may change their occupancy between *Plasmodium* life cycle stages to regulate different gene sets.

Anat Florentin (The Hebrew University of Jerusalem, Israel) discussed the mystery of the malaria plastid that ended up in the parasite as a result of endosymbiosis. The apicoplast is a unique organelle and has its own genome, though most apicoplast proteins are encoded by the nuclear genome. Florentin began by posing the question "How does an ancient endosymbiont maintain some autonomy?". It turns out that in the absence of a mechanism within the apicoplast genome to regulate its own protein synthesis an apicoplast-localized caseinolytic protease (Clp) complex acts as a master regulator of apicoplast biogenesis through protein degradation. To understand this mechanism better, Florentin's group studied the Clp system and the role it plays in organelle proteostasis in *P. falciparum*. The Clp protease subunit from *Plasmodium falciparum* (PfClpP) is required for apicoplast biogenesis and null mutant parasites were unable to survive. Conditional mutants using the TetR-DOZI system were generated and knockdown was confirmed in the absence of anhydrotetracycline (aTc) by western blot. However, this knockdown had no effect on parasite growth, indicating the presence of a post-translational regulation pathway. Florentin and colleagues then utilized CRISPR/Cas9 to express catalytically dead PfClpP and were able to demonstrate that PfClpP oligomerizes as a zymogen. They expressed wild-type and mutant Clp chaperone (PfClpC) variants that indicated a functional chaperone–protease interaction. Additionally, conditional mutants of the substrate-adaptor (PfClpS) indicated its essential function in plastid biogenesis. In conclusion, the apicoplast, although not able to synthesize its own proteins, regulates its protein content and maintains homeostasis by degrading proteins using the Clp system.

Megan Gliozzi (Food and Drug Administration, USA) began her talk by addressing the huge role that post-transcriptional regulation via RNA binding proteins (RBPs) plays in stage-specific gene regulation of *Plasmodium* parasites. This suggests that RBPs have the potential to be therapeutic targets. However, the RNA-protein interactome remains mostly unknown due to the absence of effective methodologies. Gliozzi then introduced a new and improved crosslinking method, photoactivatable ribonucleotide-enhanced crosslinking (PAR-CL) which offers higher sensitivity and specificity over conventional experimental methods to identify RBP proteins and their cognate RNA binding sites. This method depends on pyrimidine salvage and generates a signature mutation in RNA at crosslinking sites. They first generated *P. falciparum* mutants that exogenously express *S. cerevisiae* genes required for pyrimidine salvage. Following pre-incubation with 4-thiouracil (4TU) and photo-activated crosslinking to capture only proximal RBPs, the RNA-RBP complexes are purified and analysed via mass spectrometry and novel sequencing techniques. Their pilot proteomic studies have identified previously known Alba proteins among others, along with other unannotated RBPs. This study, therefore, establishes optimized conditions for PAR-CL in *P. falciparum* to study RNA-protein interactions throughout the parasite life cycle.

Michaela Petter (University Hospital Erlangen, Germany) and her laboratory are interested in chromatin regulation of the malaria parasite. Eukaryotic organisms demonstrate phenotypic plasticity and are capable of forming complex life forms. This is largely due to differential gene expression made possible by two chromatin states. Euchromatin (marked by H3K27 acetylation) is permissive of gene expression and heterochromatin (marked by H3K9 trimethylation) acts inversely. In this study, with the goal of studying sex-associated differences in chromatin-associated gene expression regulation in the malaria parasite, Petter and colleagues separated male and female *P. falciparum* gametocytes by FACS sorting of a *P. falciparum* transgenic line containing a female-specific ABCG2:GFP fusion protein. They then performed RNA-seq on days 1, 4 and 6 of gametocyte development, and performed ChiP-seq of various histone modification marks, to study the gametocyte epigenome, observing global remodelling of chromatin during sexual differentiation. Comparison of the heterochromatic H3K9me3 mark shows differential enrichment in male and female gametocytes. Euchromatic H3K27ac is enriched in strongly transcribed genes. These chromatin states act as codes for certain proteins such as bromodomain proteins, which bind to acetylated lysine residues at promoter sequences. This study suggests that *P. falciparum* bromodomain protein 1 (PfBDP1) is transcribed in both male and female

gametocytes and localized to parasite nuclei. The group conditionally knocked down PfBDP1 with Shield-1 and showed abnormal parasite morphology during gametocytogenesis. Additionally, PfBDP1 was found to regulate IMC genes like GAP50, GAP45, and GAPM1, which showed lower expression when PfBDP1 was knocked down.

Karine Gaëlle Le Roch (University of California, USA) kicked off her keynote talk by reminding the audience that the first *Plasmodium* genome was published nearly 19 years ago and since then, a lot of genomic and transcriptomic data has been published. Nevertheless, we are still left puzzled by the gene regulation mechanism in the *Plasmodium* parasite. To decipher these mysteries, Le Roch's lab is looking at additional transcriptional regulation mechanisms to fully understand the *Plasmodium* parasite's complex life cycle. To identify molecular factors that determine chromatin structure, they decided to study the regulatory roles of long non-coding RNA (lncRNA) in *P. falciparum.* Extensive studies on lncRNA in other organisms have demonstrated their ability to carry out a myriad of functions including recruitment of transcription factors, chromatin-modifying complexes, and direct regulation of transcription and post-transcriptional pathways. Le Roch and her lab were able to demonstrate the localization and stage-specific expression of several lncRNAs in *P. falciparum*. To study the genome-wide occupancy of these lncRNAs, they carried out chromatin isolation by RNA isolation (with sequencing) (ChIRP-seq), which showed enrichment of genes involved in pathogenesis, erythrocyte remodelling, and regulation of sexual differentiation. They then focused on a particular lncRNA, lncRNA-14, that when was knocked out, resulted in parasites with a defective growth phenotype in gametocyte stages, indicating its involvement in sexual differentiation. All of these results suggest that in future, lncRNAs could be considered as therapeutic targets in combating malaria.

Session 8 - Epidemiology and modelling of malaria infections

The session chairs **Fola Augusto** (University of Kansas, USA) and **Lauren Childs** (Virginia Tech, USA) welcomed participants to the 8th session of the Women in Malaria Conference, which addressed epidemiology and modelling of malaria infections.

Hsiao-Han Chang (National Tsing Hua University, Taiwan) opened the session with a presentation about accounting for human mobility in malaria elimination settings using human travel and parasite genetic data. This is particularly applicable in settings where transmission is low and/or heterogeneous, as identifying sources and sinks of malaria parasites can help efficiently manage interventions and allocate resources. Chang and colleagues inferred parasite flow between populations in Chittagong, Bangladesh and estimated the proportions of imported cases using epidemiological and mobility data (travel surveys, and mobile phone calling data). Parasite genetic barcode data revealed 101 single nucleotide polymorphisms (SNPs) and identified genetic mixing, highlighting regions with high connectivity. These approaches showed high malaria incidence in eastern Chittagong and high importation in western Chittagong. Next, she parameterized a multi-patch model to observe incidence data. The model revealed significant heterogeneity in transmission intensity after incorporation of mobility data, and it was used to estimate the impact of interventions, such as vector control. Finally, Chang used both empirical and simulated genetic data to determine SNP numbers and sample sizes needed to reliably distinguish levels of gene flow between populations. She found that the ability to rank highly and less-highly connected population pairs improved with increased sample size and SNPs; however, the ability to rank with a small sample size was not substantially improved by using more SNPs. Taken together, Chang's work provides insight into the effect of human movement on transmission, which is critical for malaria control and elimination.

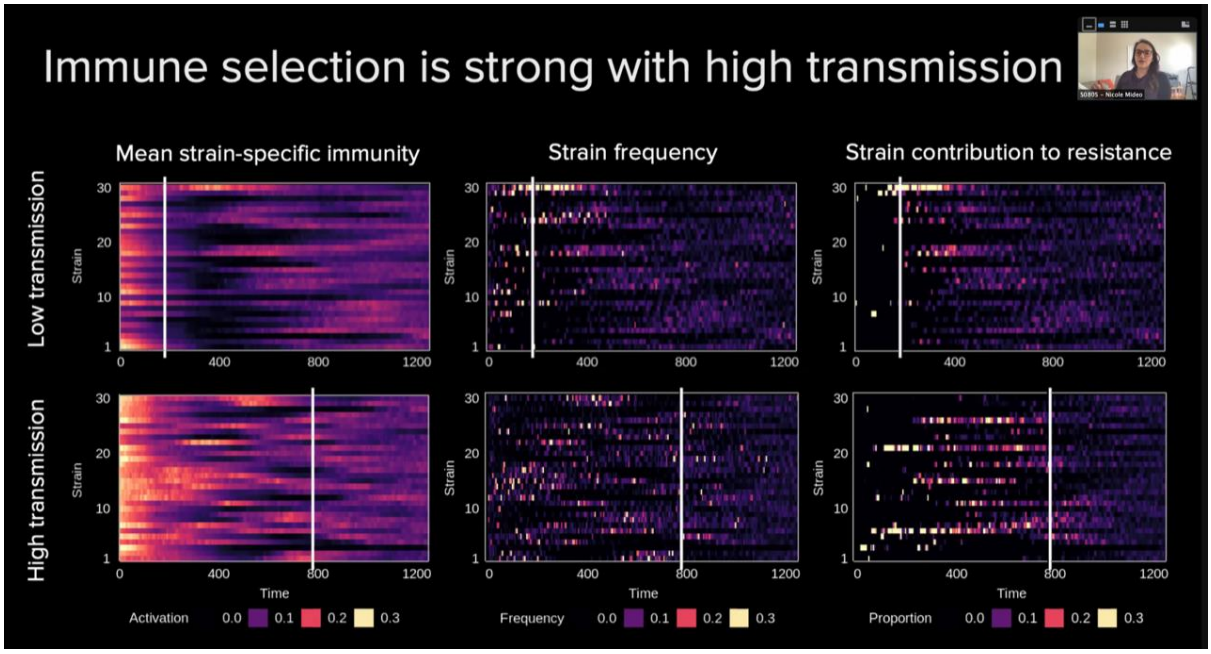
Punam Amratia (University of Florida, USA) started the talk by giving details about a study performed on Hispaniola island, North America. This is the last Island with malaria transmission in the Caribbean region. There is a need to introduce effective malaria control tools, however, prior to this, there is a more urgent need to understand transmission dynamics such as where are the highest and most persistent risks. The study presented used monthly routine case information from 809 health facilities (2014-2017) and 771 (2018-2019). Emphasis was on 2 factors: (1) equitable access to health care and (2) appropriate denominator (catchment vs population) for incidence measurements from each facility. Model infrastructure was designed to provide an annual average of incidence and provide monthly outputs to identify what was the incidence. Yearly incidence models identified several trends, progressive declines over recent years, flow patterns, the origin of cases, and important covariates for transmission: e.g. accessibility, elevation, urban/barren etc. Amratia concluded that maps were proven to be operationally successful and could be used to target areas for spray rounds.

Gillian Stresman (London School of Hygiene and Tropical Medicine, UK) discussed the spatio-temporal transmission dynamics of a recent and contained *Plasmodium falciparum* (*Pf*) outbreak in Praia, Cabo Verde. This setting is unique, as reported incidence has been less than 1% since 2010, so it is likely that nearly all infections in this malaria-naive population would be symptomatic, enabling direct measurement of the reproduction number R_0. To do this, 332 households of the 431 cases were retrospectively geolocated, and R_0 was estimated using the joint likelihood of transmission between two cases based on date and proximity. Stresman found that the outbreak was likely seeded by an infection with an estimated R_0 of 12. Through the epidemic, R_0 ranged from 0 to 12, and the mean serial interval between care-seeking symptomatic cases was 31 days. The reproductive number declined as the outbreak progressed and was suppressed by indoor residual spraying. Stresman's work is an interesting case study that provides insight into the transmission dynamics of *P. falciparum* and effective containment strategies in elimination settings.

Isabel Fletcher (London School of Hygiene and Tropical Medicine, UK) presented the link between high temperatures, rainfall and malaria transmission in Venezuela. She also explains that satellite imagery can clearly elaborate on land cover changes over time, and that deforestation is one of the most implicated causes for disturbing the environment. The aim of the study was to explore how the re-emergence of malaria in this country is amplified by environmental degradation, to what extent mining activities have contributed, and to identify the interaction between climatic factors and changing landscapes in determining malaria risk. A spatiotemporal Bayesian hierarchical modelling framework incorporating monthly case counts from 1996-2016 in Bolivar state, population estimates, spatial random effects, temporal random effects (allow accounting for seasonal peace and neighbourhood effects in different regions, random heterogeneity e.g. bednets), climate variables, deforestation and urbanization identified from satellite land cover map, mining sites, and healthcare accessibility was used. The results demonstrated that forest loss is one of the drivers of malaria and increased mining is also associated with increases in *Pf* and *Pv* malaria. Another contributor to transmission is the current the El Niño conditions, as well as poor healthcare access. However, urbanization showed trends toward reducing transmission. Fletcher concluded that mining and temperature, both higher in deforested areas where microclimatic changes have occurred, favoured the increase of malaria transmission and have driven re-emergence of the disease.

Nicole Mideo (University of Toronto, Canada) closed the session by presenting work on modeling drug resistance evolution in malaria parasites. She noted that parasite drug resistance generally arises rarely and in low-transmission settings. New resistance is unlikely to arise in high-transmission settings due to within-host competition and highly immune hosts, but interactions between immune-driven selection and drug resistance have rarely been investigated. To address this, Mideo presented a multiscale model tracking individual mosquitoes, hosts, and parasites. The model showed that immune selection in high-transmission settings enables sensitive parasites to remain in circulation,

even when resistant parasites have an advantage. However, in low-transmission settings with weaker immune selection, resistant parasite strains can dominate without interference. These results demonstrate the evolutionary interplay between host immunity and drug resistance and may explain the counterintuitive origins of drug resistance.



This report is brought to you by the MESA Correspondents Sushma Ambekar, Christine Markwalter, Rosheen Mthawanji and Nkahe Diane Leslie with mentoring and editorial support from Joanne Power, Elena Gómez Días and Sarah Reece.

Day 3: 24th March 2021

Session 9 - Drug and vaccine development and resistance

Session chair **Lorena Coronado** (Instituto de Investigaciones Científicas y Servicios de Alta Tecnología, Panama) welcomed participants to one of the final conference sessions covering malaria "drug and vaccine development and resistance".

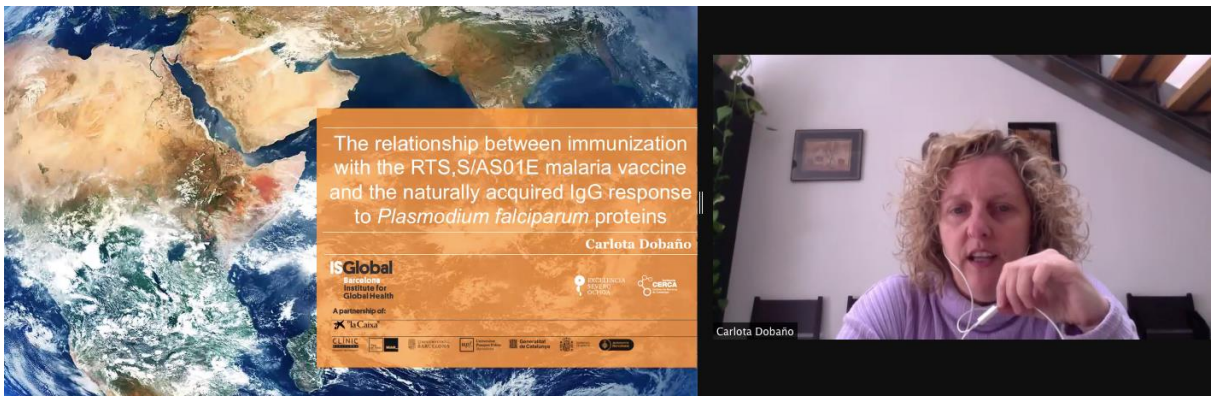
Caroline L. Ng (University of Nebraska Medical Center, USA) opened the session with a reminder that women have made unparalleled contributions to the malaria field such as Tu Youyou's 1972 discovery of artemisinin which changed the malaria treatment landscape. The emergence of artemisinin resistance threatens worldwide progress in reducing the malaria burden. Ng's work focuses on leveraging the roles that the proteasome plays in *Plasmodium* parasite development to identify novel treatments and targets. She found that *P. falciparum*-specific proteasome inhibitors synergize with 4 malaria treatments that activate the unfolded protein response, including dihydroartemisinin, OZ439, methylene blue, and b-AP15. This synergism results in sensitization of parasites to these compounds in the presence of proteasome inhibitors. Additionally, Ng found that mutations in the proteasome β2 subunit sensitize parasites with the resistance-conferring C580Y K13 propeller gene mutation to DHA and OZ439, suggesting a direct link between the proteasome and artemisinin resistance. These results are exciting because they offer a potential path toward overcoming drug-resistant malaria.

Dominique Fatima Voumbo-Matoumona (International Centre for Medical Research in Franceville - CIRMF, Gabon) - This speaker was unable to attend.

Eline Kattenberg (Institute of Tropical Medicine in Antwerp, Belgium) presented an efficient, cost-effective, targeted, and multiplexed next-generation sequencing assay called PfAmpliseq which can be used to characterize *Plasmodium falciparum* population structure, drug resistance, polymorphic antigens, and hrp2/3 deletions. The assay was specifically tailored to *P. falciparum* parasites in the Peruvian Amazon, where *P. falciparum* prevalence has recently increased, and where there has been no molecular surveillance for drug resistance since ACTs were introduced in 1999. Kattenberg applied PfAmpliseq to retrospective samples (2003-2018). She observed high genetic diversity prior to 2005, which later declined with prevalence. No validated resistance-associated K13 mutations were observed. Other markers suggested that parasites have developed resistance to sulfadoxine/ pyrimethamine and chloroquine, but remain sensitive to mefloquine. She also found that the prevalence of hrp2/3 deletions has been increasing over time. Her future plans for the project is to apply the same approach to additional samples from a wider geographic area. However, the true strength of PfAmpliseq is in its modularity; other targets of interest can be easily added, and the assay can be specifically tailored to geographic regions where other resistances exist, making it a powerful tool for robust surveillance of *P. falciparum* drug resistance evolution and population structure.

Merel Smit (Radboud University, Netherlands) presented exciting preliminary results on the first in-human evaluation of the *P. falciparum* transmission-reducing monoclonal antibody (mAb) TB31F. This antibody targets the C-terminal 6-cysteine (6C) domain of pfs48/45, a gametocyte surface protein that plays an essential role in male fertility. Previous studies of TB31F have demonstrated more than 80% transmission reducing activity (TRA) based on standard membrane feeding assays. Smit aimed to extend this work to humans by assessing the safety, serum pharmacokinetics, and direct feeding assay TRA of TB31F in malaria-naive Dutch volunteers. She described the preliminary results from 5 treatment groups; 4 of which received one-time intravenous TB31F at different doses, and 1 of which received subcutaneous TB31F. Volunteers were followed at intervals over 3 months. Safety, half-life, and TRA results were extremely promising, and future work involved extending TB31F half-life through Fc modification.

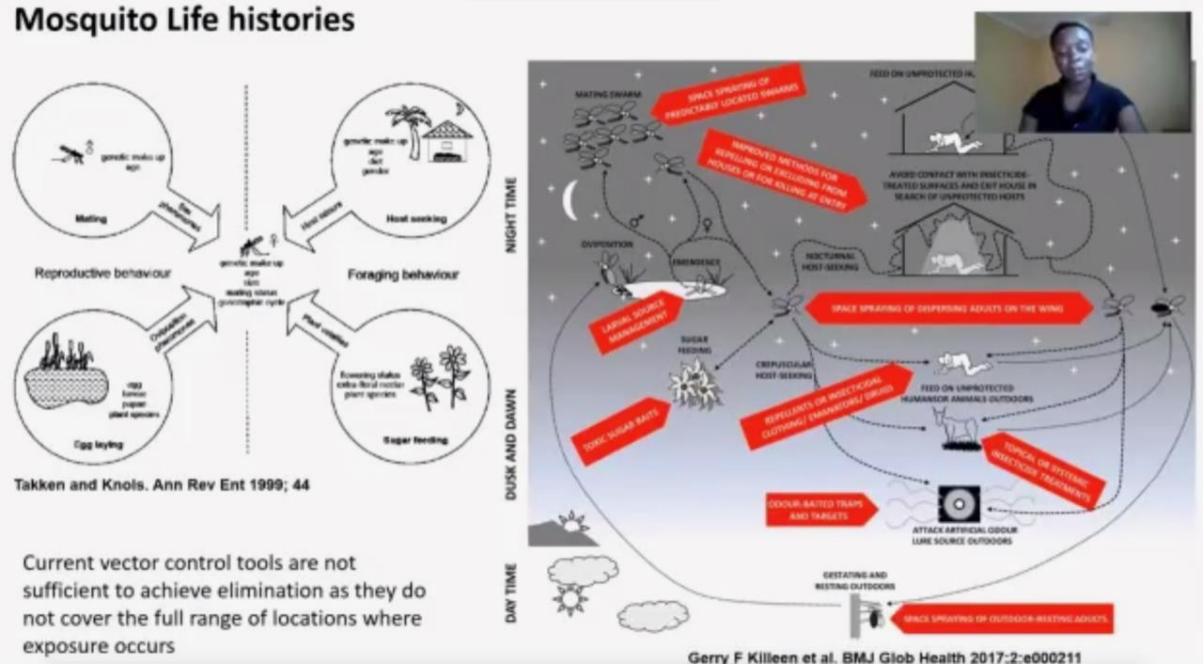
Carlota Dobaño (Barcelona Institute for Global Health - ISGlobal, Spain) closed the session by describing the interplay between RTS,S/AS01E vaccine-induced immunity and naturally acquired IgG. She examined these relationships using protein microarrays to measure IgG reactivity to 1000 immunoreactive *P. falciparum* antigens (representing 763 unique genes) in serum and plasma samples from infants and children enrolled in Phase 3 clinical trial of RTS,S/AS01E. These samples were collected longitudinally from disparate sites around the African continent. In total, seroreactivity was measured in 6,695 samples, producing a rich, high-dimensional data set. Using t-stochastic neighbour embedding (t-SNE) to visualize the data, Dobaño examined IgG responses in malaria cases and controls in the unvaccinated and vaccinated groups to identify trends based on malaria transmission intensity, age, and time since vaccination. Next, she plans to apply machine learning and modeling to identify multi-antigen antibody signatures of protection common across transmission intensities and age groups.



Session 10 - Vector biology and control

Silvie Huijben (Arizona State University, USA) and **Jewelna Efua Birago Akorli** (University of Ghana, Ghana) chaired the session and introduced speakers. Talks in this session focused on efforts to control malaria vectors. Speakers also highlighted the need for innovation and improvement to combat increasing insecticide resistance.

Evelyn Olanga (University of Malawi, Malawi) started her talk by remarking that long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are the front line tools used in vector control. However, since 2015 progress has stalled and one of the contributing factors is insecticide resistance. This threatens the goal set for malaria-endemic countries, by the WHO, of reaching a 90% mortality reduction by 2030. Olanga further stressed the urgent need for novel tools to overcome insecticide resistance and residual transmission. To address these challenges there are a number of novel tools being explored: eave tubes with insecticide-treated nets, odor- baited traps that mimic human odor, attractive toxic sugar bait (ATSB), endectocides and *Wolbachia* bacteria releases that infect ovaries of *Anopheles* mosquitoes. Other tools being tested are dual active ingredient LLINs, spatial repellents and transfluthrin-treated materials. Overall, even after these novel tools are fully established, each country will have to develop its own vector control strategy because the heterogeneity in environments and settings across countries mean that a single solution is unlikely to be appropriate.



Manuela Carnaghi (University of Greenwich, UK) is working towards understanding the stimuli that drive the host-seeking behavior of mosquitoes. Due to the ever-increasing resistance to current vector control tools, novel alternative methods must be explored. The group aims to address this issue by understanding what characteristics make a target most attractive to mosquitoes. This will help develop outdoor traps as well as surveillance tools that are easy to use in the field. An important factor for such traps to work is the need to attract far-away mosquitoes. So this raises the question "What are the short-range and landing behaviors of *Anopheles* mosquitoes?". Experiments to answer this question were conducted in a wind tunnel and attraction to several cues were tested. This included thermal, visual and olfactory aspects of host cues, and roles of the spatial orientation and size of the target. Their results indicated that host odor is crucial for eliciting landing behavior. Thermal cues increased landing, even if the surfaces were transparent. Carnaghi concluded that there is a complex integration of information and a synergistic integration of information taking place that influences the behavior of the mosquitoes.

Beth Crawford Poulton (Liverpool School of Tropical Medicine - LSTM, UK) discussed the problem of growing insecticide resistance leading to the need to improve methodologies for vector-based interventions. Currently, the front-line tools against vectors are long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) that use pyrethroids. Understanding resistance and testing new insecticides require high-throughput assays to study resistance. However, the current WHO larvicide susceptibility assay poses many disadvantages, including laborious rearing requirements, long hours, investigator bias, and low-throughput results. Poulton and her group have therefore developed a quick and unbiased assay, called the INVertebrate Automated Phenotyping Platform (INVAPP), based on an assay being used to study nematodes. It involves capturing the movement of the larvae before and after incubation with the insecticide and carrying out Paragon analysis using Python to calculate a normalized movement index. This system is thus convenient and effective for screening large compound libraries (e.g. the Medicines for Malaria Pathogen Box) and resistance profiling. Looking to the future, Poulton aims to take this approach forward through integration into a smartphone app to improve accessibility.

Barikissou Georgia Damien (University of Abomey-Calavi, Benin) explained that the objective of her group's project was to develop and validate an approach to evaluate the post-deployment

effectiveness of malaria control in all settings. Specifically, the evaluation of post-deployment LLINs in Benin. Currently, the national policy recommends an integrated control approach involving vector control, case management, and chemoprevention. The analysis which considered all age groups, classified infections as positive or negative based on an RDT or microscopy test. In rural areas, LLINs alone gave protection against malaria and combining indoor residual spraying (IRS) further increased protection (to 50%). The study concluded that interventions alone are not enough to combat malaria, thus, there is a need for an operational evaluation of the intervention strategies.

Hilary Ranson (Liverpool School of Tropical Medicine, UK) presented research on the emergence of new insecticide resistance mechanisms in Burkina Faso. Ranson started by giving a brief background of insecticide resistance. She expressed that pyrethroids were developed in the 1970s and usage started in the 1990s. Resistance has been observed for a long time and different mechanisms have been studied since the 2000s. The different mechanisms for insecticide resistance include target-site mutations, and metabolic and behavioral resistance. Consequently, the survival of mosquitoes following pyrethroid exposure has dramatically increased over the last 10 years. Ranson presented experiments testing the impact of mosquito exposure to insecticide-treated or untreated nets in experimental huts. They found that there was no difference between treated or untreated nets and that community protection is lost due to insecticide resistance. In 2019 the ministry of health in Burkina Faso introduced next-generation nets with piperonyl butoxide and interceptor G2 in areas with very high pyrethroid resistance. Lab colonies were established from mosquitoes collected in this area and used for RNA-sequence analysis. An increase in the frequency of the new knockdown resistance (kdr) mutation, L995F haplotype, was observed in *Anopheles coluzz*i. In conclusion, Ranson showed that the southwest of Burkina Faso is a hotspot for pyrethroid resistance in malaria vectors, that resistance is reducing the efficacy of core intervention tools, and that resistance mechanisms vary between mosquito species.

Poster Session and General Assembly

After the closing sessions, there was a virtual Poster session and to end the first Women in Malaria Conference a General assembly was arranged with the aim to shape the future of the community. The organizers closed the conference thanking everyone involved in making a reality the conference and all the attendees from all over the world.

This report is brought to you by the MESA Correspondents Sushma Ambekar, Christine Markwalter, Rosheen Mthawanji and Nkahe Diane Leslie with mentoring and editorial support from Joanne Power, Elena Gómez Días and Sarah Reece.

Discover more content in the Resource Hub

www.mesamalaria.org