RESOURCE HUB *Correspondents*

8th International Conference on *Plasmodium vivax* Research (ICPvR) 2022 - Virtual

Complete series

MESA Correspondents bring you cutting-edge coverage from the 8th International Conference on Plasmodium vivax Research 2022

5 - 8 April 2022

Virtual Conference

The MESA Alliance would like to thank the Organizers and Chairs[1] of the conference who collaborated with the program.

The MESA Alliance would also like to acknowledge the MESA Correspondents Melina Florez-Cuadros (Naval Medical Research Unit N°6 through the Vysnova, Inc. in Peru), Varun Gorki (Panjab University Chandigarh, India), Neha Sylvia Walter (PGIMER, India) and Duru Vincent Chiagozie (Nnamdi Azikiwe University, Nigeria) for their crucial role in the reporting of the sessions.

[1] *Complete list of names at the end of the report.*

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Day 1: 5th April 2022

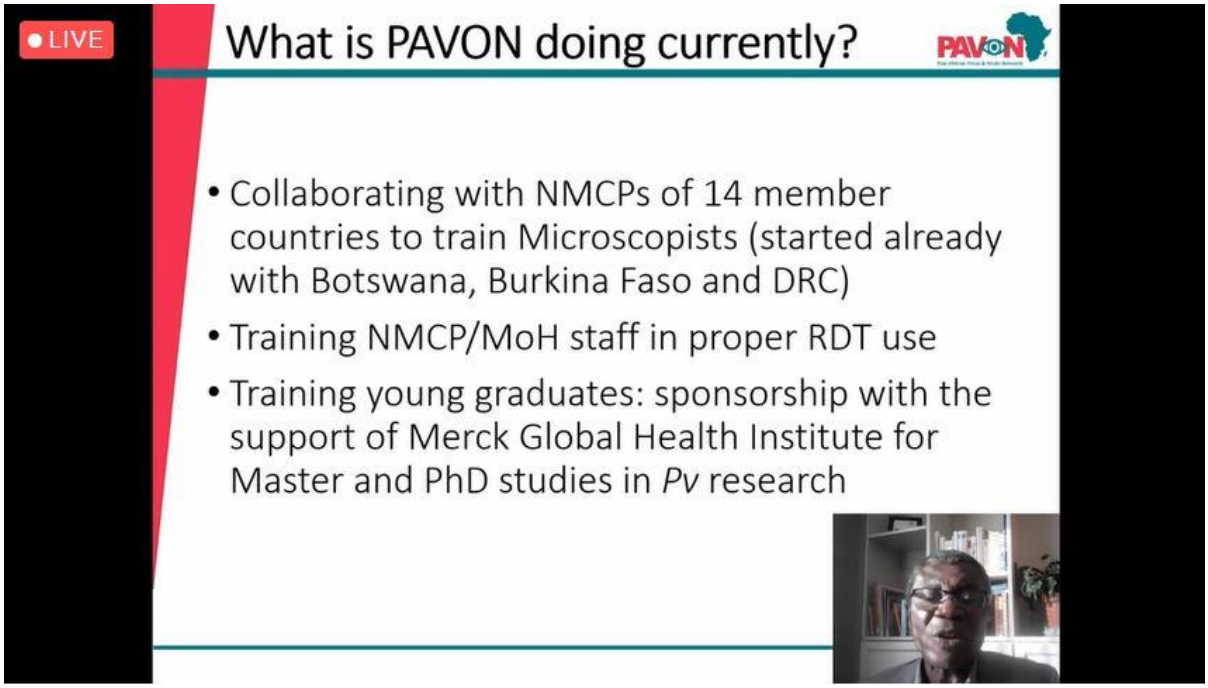
Welcome & Opening Lectures

The organizers of the conference, **Kamala Ley-Thriemer** (Menzies School of Health Research, Australia) and **Leanne Robinson** (Burnet Institute, Australia), opened the 8th International Conference on *Plasmodium vivax* Research (ICPvR) by welcoming the attendees. They greeted over 300 delegates from 37 countries across the globe and energized them to gain the latest insights on *P. vivax* research from 17 distinguished experts on the latest developments in *P. vivax*. The organizers advocated for using the Spatial Chat to make connections, gather information, ask questions, and interact with eminent speakers.

Prof. Jetsumon Sattabongkot Prachumsri and Prof. Isaac Quaye delivered keynote remarks on the first day of the conference, which was chaired by Wai-Hong Tham and Ric Price.

By providing insight into the conference's history, **Jetsumon (Sattabongkot) Prachumsri** (Mahidol Vivax Research Unit, Thailand) illustrated the current models employed for *Plasmodium vivax* research and the challenges undermining different models. She first mentioned a major challenge identified 20 years ago - the development of a method to continuously culture *in vitro P. vivax* blood stages. Therefore, an alternative was proposed to use infected blood as media growth for long term culture. Prachumsri highlighted the advances and challenges that *P. vivax* research faces from mosquito rearing and feeding to the replication of the human liver stage in humanized mice and the controlled human malaria infection (CHMI). The majority of the current models have great challenges since they depend on having a good supply of infected blood (which is not easy to find in non-endemic countries), a stable colony of vectors and sufficient hepatocyte donors. These models are very expensive and require very specific protocols. She concluded her talk by summarizing the key stages of *P. vivax,* the need to improve the current tools and the urgent need for collaboration to develop better models for malaria research. She finished by encouraging young researchers to fill the research gaps already identified.

Issac Quaye (Regent University College of Science and Technology, Ghana) initiated his talk by introducing the Pan African Vivax and Ovale Network (PAVON) established in 2019 in 14 Sub-Saharan African countries with its headquarters in Accra, Ghana. PAVON collaborates with National Malaria Control Programs (NMCPs) on diagnosis, surveillance and education of malaria. Quaye then highlighted the role of asymptomatic infection in the rising burden of *P. vivax* in Africa. He cited the case study of Botswana from 2008 to 2012, where *P. vivax* elimination is impeded by microscopy competence, lack of appropriate equipment, availability of the tools and inadequacy of trained personnel. PAVON has played a crucial role in ameliorating these concerns including: going back to basics, finding new tools to combat *P. vivax* above current strategies tailored to *P. falciparum*, NMCPs routine engagement with local researchers, and training of graduates to drive human capacity building. Finally, he mentioned that the way forward would include devising innovative strategies to tackle asymptomatic *P. vivax* spread in the region.



Session 1: Global burden and epidemiology of *vivax* malaria

Abdisalan Noor (World Health Organization - WHO, Geneva) initiated his talk by presenting the WHO methods for estimating global malaria cases, and mortality with a focus on *P. vivax*. The three methods for estimating cases include: data as reported without adjustment (which only contributes a very small proportion of information), or the adjusted reported data for testing, reporting and treatment rates and the 3rd method which generates the bulk of data comes from the Bayesian model that converts parasite prevalence to incidence. He also highlighted the major challenges to WHO estimation of the global burden of *P. vivax* and the possible solutions targeted at ameliorating these challenges.

J. Kevin Baird (Eijkman Oxford Clinical Research Unit, Indonesia) explained how *P. vivax* infection diagnostic techniques rely on blood for demonstrating parasite presence, but contrastingly, most of *P.vivax* biomass is not necessarily in that tissue. It has been shown that people can have acute and chronic malaria without parasitemia, making us rethink new diagnostic methods and techniques that take into account the bulk of *P. vivax* biomass existing in CD71+ enriched extravascular niches. Those being hidden in extravascular compartments may be more common than we think. Baird left his talk with a valuable question: Is *P. vivax* malaria infection in Africa improbably rare or benign?

Somya Mehra (University of Melbourne, Australia) presented a mathematical model for *P. vivax* transmission that considers the particular hypnozoite stage of the parasite, along with multiple blood stage infection and immunity acquisition. Her model also accounts for three sources of stochasticity: stochasticity in dormancy, variability in the number of relapses from each bite and the randomness in mosquito bites. Their sensitivity analyses show how hypnozoite dormancy dynamics impact transmission, for instance. Their model provides useful insights into the epidemiology of *P. vivax.*

Liony Fransisca (Menzies School of Health Research, Australia) and colleagues reported how health systems strengthening under the Strengthening Health Systems for Effective Primaquine (SHEPPI) project in Papua, Indonesia, shows how simple pragmatic interventions and health system strengthening are feasible and crucial for *P. vivax* elimination in the area. Through the established "malaria corner" the project was able to provide better surveillance data, timely automated report of

cases, improved quality of care and ultimately reduced recurrence and incidence of *P. vivax* in the region, despite certain limitations.

Rapid Fire Presentation - Global burden and epidemiology of *vivax* malaria

#100 **Daniel Villela** (Oswaldo Cruz Foundation, Brazil) commenced his talk by highlighting the increasing proportion of *P. vivax* cases in recent years that accounts for more than 80% of the total in the area. The Poisson model was used and they found differences in expected vs actual malaria cases in several cities. Results showed the increase to be multifactorial, where deforestation, imported cases, agricultural activities, and mining were the main drivers.

#102 **G. Dennis Shanks** (Australian Defence Force Malaria and Infectious Disease Institute, Australia) initiated his talk by stating *P. vivax* malaria was extensively prevalent on the Thai Burma Railway. 56% of smears taken from war prisoners were positive with 26% remaining positive, months later. Relapses were likely a result of sub-optimal treatment, high infection rates and activation of hypnozoites.

#103 **Anaclara Pincelli** (University of Sao Paulo, Brazil) stated that prolonged breastfeeding was found to be a predictor of decreased risk-blood stage *P. vivax* infection for the first two years of life. Researchers used serology to measure the cumulative exposure to *P. vivax* in infants. They concluded that most of *P. vivax* infections in children remain undiagnosed in the Brazilian Amazon and prolonged breastfeeding partially prevented the *P. vivax* infections.

#104 **Gustavo da Silva** (University of Notre Dame, United States) and colleagues tried to understand how travelling for work was related to the malaria peak at the end of each year in the Ethiopian Highland. Travel was a risk factor for infection for both, *P. falciparum* and *P. vivax*, although the majority of infections were for non-travelers.

#105 **Caroline Jasmine Abanto Alvarez** (Cayetano Heredia University, Peru) pointed out that asymptomatic infection contributes up to 80% of the community-wide malaria transmission in Peru. Results showed that experiencing a recurrence with parasite homologous to the ones present in the initial infection and being children between 6 and 15 years were factors associated with experiencing an asymptomatic recurrence.

#106 **Ayleen Alicia Kosasih** (University of Indonesia, Indonesia) presented her study that measured the detectability of gametocytes in untreated P. *vivax* infections during a series of mass blood surveys (MBS). The study results showed marked fluctuations of parasites and gametocytes in the population and suggested new strategies other than MBS for detection and removal of infection reservoirs.

#107 **Angelica Vigil** (Institute of Tropical Diseases - National University Toribio Rodriguez of Mendoza, Peru) stated that in order to describe the situation of malaria in the Amazonas region and determine significant changes in malaria *P. vivax* incidence through time in relation to changes in malaria control policies, the study analyzed the *P. vivax* database reported by the Regional Directorate of Health Amazonas between 2005 and 2020. The results showed changes in malaria cases through time and the need for a sustained malaria control program that could lead to malaria elimination in the region.

Rapid Fire Presentation - Pathology and Pathogenesis of *vivax* malaria

#109 **Katherine Torres** (Cayetano Heredia University, Peru) illustrated the significant role of plasma interleukins and chemokines found in asymptomatic malaria infections: IL-9, IP-10, MIP-b, PDGF-bb and RANTES with a clinical trial performed in Peru. She concluded that the alteration among such cellular immune responses led to immunopathogenesis.

#110 **Aditi Arya** (Indian Council of Medical Research and National Institut of Malaria Research - ICMR/NIMR, India) a research scholar, explained the association of thrombocytopenia in *P. vivax* infected patients with *Pvmsp3α* gene. She urged that the severity of the account of *P. vivax* malaria must be elaborated at a molecular and clinical level.

#111 **Iris Aparici-Herraiz** (Barcelona Institut for Global Health - ISGlobal, Spain) in collaboration with other institutes, emphasized the importance of developing humanized mouse models (Splenic/Bone Marrow) for examining the effect of extracellular vesicles as intercellular communicators in the bone marrow and spleen during *P. vivax* malaria with supported observations.

#112 **Alberto Ayllon-Hermida** a PhD student (Barcelona Institut for Global Health - ISGlobal, Spain) presented the work explaining the use of CRISPR-Cas9 technology to generate transgenic lines of *P. falciparum* which will express the specific genes of *P. vivax*, which favors the parasite to thrive in the environment of bone marrow and spleen.

Session 2: Pathology and Pathogenesis of *vivax* malaria

Steven Kho (Menzies School of Health Research, Australia) commenced his talk by explaining the basics of spleen biology. He presented a cohort study from 2015 to 2017, carried out at Rumah-Sakit-Umum-Daerah (RSUD) hospital, Indonesia with 22 splenectomy cases with no malaria symptoms at the time of surgery. Interestingly, spleen tissue in 95% of the patients showed asymptomatic *Plasmodium* infections (7 *P. vivax*,13 *P. falciparum*, and 1 mixed infection), confirmed by microscopy and PCR. Further, the study also found that *P. vivax* parasites and immature reticulocytes accumulated in the same splenic compartment. These observations suggested that the spleen harbors a cryptic endosplenic lifecycle in chronic *P. vivax infection* and the need to look for biomarkers of hidden parasite biomass in the spleen, where we can find up to 98.7% of it.

A study by **Joao Luiz Silva-Filho** (University of Glasgow, United Kingdom) and colleagues assessed the associations between peripheral parasitemia and total parasite biomass and the host response in patients with uncomplicated *P. vivax* malaria from Manaus, Brazil. Results indicated two types of patient clusters. *P. vivax* high group revealed more severe thrombocytopenia, lymphopenia and neutrophil enrichment in contrast to *P. vivax* low group. Results showed that total parasite biomass,

rather than peripheral parasitaemia, was a better predictor of disturbances in host homeostasis in *P. vivax* patients. This adds support to the theory of a *P. vivax* tissue reservoir, especially in the haematopoietic niches of bone marrow and spleen.

Florian Bach (University of Oxford, United Kingdom) employed controlled human infection models (CHIMs) to elucidate the host immune response to *P. vivax* and *P. falciparum* malaria, employing whole blood RNAseq, plasma protein profiling, and mass cytometry. Results indicate a similarity in the systemic inflammation caused by both species of the parasite in the acute phase. However, they found that *P. falciparum* results in higher levels of T cell activation compared to *P. vivax*, which may be responsible for the differences in the disease severity. They further re-infected the volunteers with both the parasite species to understand the development of disease tolerance. Reinfection was found to be associated with modified T cell response with reduced number and diversity of effector cells upon re-challenge which was linked to lower levels of tissue damage biomarkers, indicating the establishment of disease tolerance.

Lisa Ioannidis (University of Melbourne, Australia) presented her work which used high dimensional single-cell mass cytometry to unravel the complex $CD4^+$ T cell and memory B cells (MBCs) responses that occurred in *P. vivax* infection. Using this approach, they identified and supported the fact that the diverse $CD4^+$ T cells and MBCs populations expressing the class-switched T-bet either mitigated or enhanced the risk of *P. vivax* malaria. She concluded her talk by emphasizing the fact that inflammatory responses to malaria are not always detrimental and can contribute to the control of infection. While Class-switched MBCs and humoral immunity were important for the control of symptomatic *P. vivax* malaria, the cellular immune responses mediated by $CD4^+$ T cells were important for the control of asymptomatic infection of low parasite densities.

This report is brought to you by the MESA Correspondents Melina Florez-Cuadros, Varun Gorki, Neha Sylvia Walter and Duru Vincent C. Senior editorial support has been facilitated by the Organizers and Chairs of the sessions.

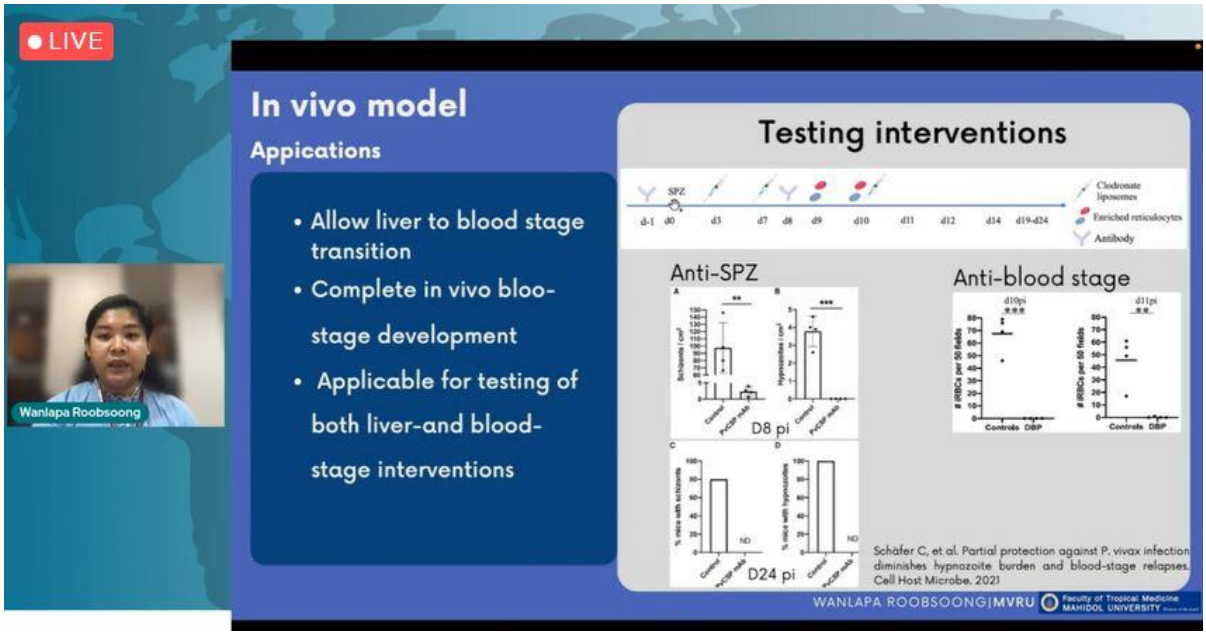
Day 2: 6th April 2022

Keynote presentation

Luzia H. Carvalho (Oswaldo Cruz Foundation, Brazil) initiated her keynote address by giving an overview of what is generally known about the antibody response to the DBPII (Duffy Binding Protein II) and highlighted its genetic variability in endemic areas, which is the major ligand for *P. vivax* invasion of erythrocytes, and also highlighted the potentials of this ligand holds as a vaccine candidate, given its ability to elicit IgG antibodies that are able to block the interaction of DBPII with the Duffy Antigen Receptor for Chemokines (DARC). However, only a low proportion of *P. vivax* exposed individuals actually acquire the strain-transcending blocking inhibitory antibodies (BIAbs) response which she called "the elite responders''. The DBPII 'elite responders' were found to be unrestricted to areas of high transmission alone considering their presence in the Brazilian Amazon areas. Also, using a synthetic DBP immunogen (DEKnull2), associated with strong and persistent naturally-acquired IgG antibody followed by IgM antibodies response to provide a strong and persistent response, suggested promising results. However, there were differences in the gene expression (Allele a/Allele b) in elite responders versus the non-responders. Altogether, these results are encouraging and elicits a potent BIAb response.

Session 3: Invasion and biology of vivax malaria

Wanlapa Roobsoong (Mahidol Vivax Research Unit, Thailand) X-rayed the various experimental models of *P. vivax* culture that have been successfully used in studying the liver stages in their lab. For the *in vivo* models, two humanized mouse models support complete liver stage development and hypnozoite formation. The HC-04 cell line allows *in vitro* schizont and hypnozoite culturing. This cell line also supports *in vitro* reactivation of hypnozoites and genetic manipulation of host hepatocytes.



Considering the large variations of *P. vivax* field isolates, **Gigliola Zanghi** (Center for Global Infectious Disease Research, United States), in collaboration with other groups, developed a Chesson strain liver stage model employing the sporozoites (PvSPZ) of the Chesson strain. Chesson blood stages parasites were propagated in Saimiri monkey and fed to the mosquitoes for PvSPZ production and cryopreservation. The PvSPZ was inoculated in human liver-chimeric FRG huHep mice and the liver-

stage parasites were analysed by single cell RNA-seq. The group observed the exo-erythrocytic schizonts formation over nine days and a typical ratio of 40% hypnozoites: 60% schizonts in the infected livers. The Chesson strain liver stage model will enable the identification of innovative therapies to prevent relapse by providing a better knowledge of hypnozoite biology.

Anthony Ruberto (Center for Tropical and Emerging Global Diseases, USA) presented a study elucidating the molecular signatures of *P. vivax* hypnozoites and human hepatocytes using single-cell RNA sequencing. On the parasite side, the study revealed distinct transcriptional signatures between the hepatic schizont and the hypnozoite stage. On the human side, Ruberto highlighted the transcriptional responses of hepatocytes infected with hypnozoites or schizonts and observed that the pathway associated with immune responses was down-regulated while the energy metabolism genes were enriched. This study offered insights into *P.vivax* liver-stage biology and revealed host and parasite markers that may be targeted for new antimalarials development.

Prasun Kundu (Cambridge Institute for Medical Research, UK) reported a study showing the role of the Plasmodium Tryptophan Rich Antigens (TRAgs) in blood stages invasion. They employed the HEK293E cell system to express 25 full-length TRAgs of *P. vivax.* Polyclonal antibodies were raised and used to localize the proteins. Despite the fact that only half of the *P. vivax* TRAgs were predicted to have an N-terminal signal peptide, immunofluorescence experiments revealed surface staining on merozoites in the *P. knowlesi* model, regardless of signal peptide prediction. Flow cytometry assays, lipid dot blots, liposome binding experiments and transgenic *P. knowlesi* (through deletion of the orthologous gene) were used to confirm TRAg25 lipid binding and its role in the invasion of reticulocytes.

Session 4: Towards vaccine development

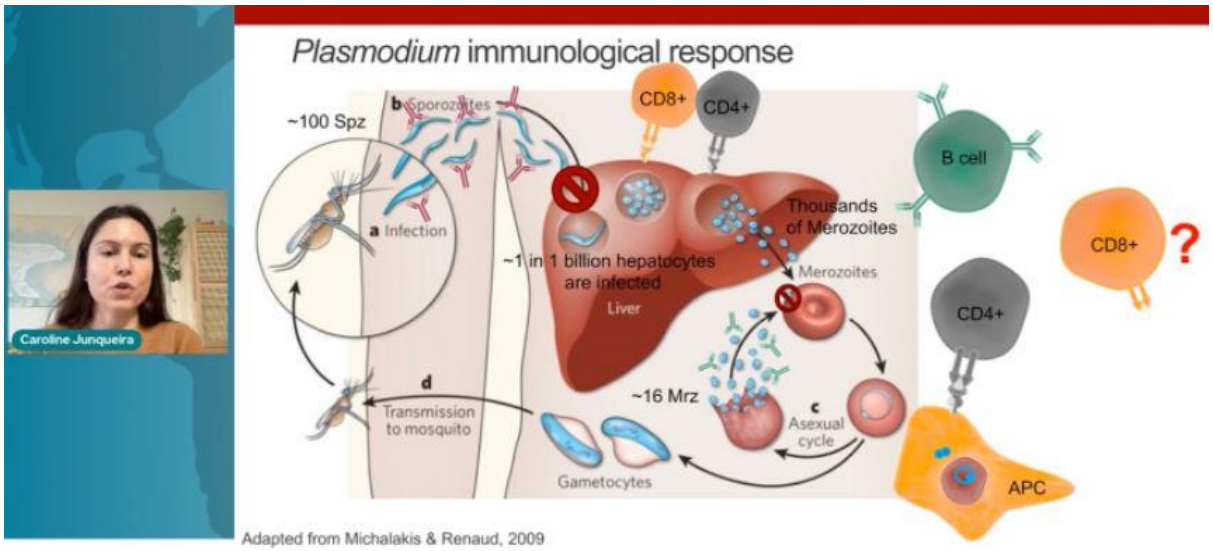
Mimi Hou (University of Oxford, UK) presented research work on testing the clinical efficacy of the PvDBPII malaria vaccine in a controlled human malaria infection (CHMI) study undertaken in the UK. Two formulations of PvDBPII vaccine were developed: a viral vectored and a protein in adjuvant (Matrix-M™). Study findings indicated better immunogenicity and efficacy of a fortuitously delayed dosing regimen of the protein-in adjuvant than the viral vectored vaccines. A 51% reduction in the *in vivo* parasite multiplication rate, and corresponding high antibody titres good effects were observed in this group compared to the control and viral vectored groups. They also established an *in vivo* growth inhibition activity (GIA) assay using *P. knowlesi* transgenic for PvDBP and showed a correlation with vaccine efficacy using this assay, pointing the way to a way of predicting vaccine efficacy in the future.

Ganesh Ram Visweswaran (Seattle Children's Research Institute, United States) called attention to the matter that secondary infections caused by hypnozoites relapsed in *P. vivax* can account for 90% of clinical cases, which also impact transmission. They reported the use of the FRG humanized mouse liver model to assess the ability of CSP antibodies to arrest schizont development and hypnozoite formation. The experiments showed that mice infused with CSP antibodies had reduced relapses when the antibody was administered at a suboptimal dose.

Sumana Chakravarty (SANARIA, United States) and colleagues reported the development of a resource to provide live *Plasmodium vivax* sporozoites, akin to the *P. falciparum* product (*Pf*SPZ). The SANARIA group aimed to generate stocks of aseptic, purified, vialed cryopreserved *Pv*SPZ that meet the regulatory criteria for human use. They reported the successful production of PvSPZ using a *Saimiri boliviensis* primate model to produce patent infection with *P. vivax* gametocytes, then used the infected blood to transmit to *Anopheles stephensi* mosquitos before harvesting viable sporozoites

from the salivary glands of the mosquitos. This resource promises the possibility of a continuous supply of *P. vivax* sporozoites.

Caroline Junqueira (Oswaldo Cruz Foundation, Brazil) and her group used immunopeptidomics analysis on human leukocyte antigen (HLA) class I molecules expressed on the surface of *P. vivax*-infected erythrocytes to identify more than 450 peptides that belong to 167 parasite proteins, from samples of seven patients. These proteins included housekeeping proteins, proteins conserved across species and common among the patients studied. After assessing the immune response to these peptides and also, MHC restriction analyses, they conclude that *P. vivax* infected reticulocytes present peptides on both classical and non-classical MHC-I molecules. As a result, *P. vivax* infected reticulocytes are recognized and killed by the CD8+ T cells. In addition, the study group hypothesized that since most of these proteins are housekeeping proteins, they will likely be presented by infected hepatocytes, too.



Rapid Fire Presentation - Invasion and biology of vivax malaria

#200 **Katlijn De Meulenaere** (Institute of Tropical Medicine Antwerp - ITM Antwerp, Belgium) stated how the identification of new receptor-ligand pairs for the invasion process of *P. vivax* is very crucial to uncovering vaccine candidates targeting the parasite erythrocyte stage. They identified a new band3-mediated pathway in *P. vivax,* with pvTRAg genes being the best ligand candidate. They also showed that linking invasion phenotypes with transcriptomes from the same parasite can inform host-parasite interactions involved in invasion.

#201 **Caitlin Bourke** (Walter and Eliza Hall Institute - WEHI, Australia) elucidated how the researchers performed a full-length RNA sequencing on an mRNA molecule, inclusive of the 3' and 5' untranslated regions (UTRs) that flank the coding sequence, in order to better understand and interpret the *P. vivax* transcriptome from a sporozoite point of view. The study cataloged isoform variations present in the sporozoites and provided crucial insights into the *P. vivax's* sporozoite transcriptional landscape.

#203 By using the cell-line which overexpressed the EphA2 against *P. vivax*, **Sittinont Chainarin** (Mahidol Vivax Research Unit, Thailand) strengthened the concept of EphA2 as a hepatocytic receptor for *P. vivax* infection and provided a platform for more research into the biology of exo-erythrocytic *P. vivax*-host interactions.

#204 **Susanne W. Warrenfeltz** (Center for Tropical and Global Diseases, United States) on behalf of the VEuPathDB Team elaborated on, and encouraged researchers to access PlasmoDB and VectorBase databases. They are totally free, easy to use and have the information of genomic-scale datasets and data mining tools of 112 organisms including *Plasmodium* species and their vectors.

Rapid Fire Presentation - Towards vaccine development

#206 **Herbert Opi** (Burnet Institute for Medical Research and Public Health, Australia) shared that his group has developed novel luminex assays for quantifying functional immunity in *P. vivax* infections that helped in assessing immune responses to multiple antigens simultaneously. They were able to identify known and novel antibody targets and functions, useful for vaccine development.

#207 **Utpalendu Paul** (Vellore Institute of Technology, India) et al. used reverse vaccinology to find highly antigenic *P. vivax* proteins specifically of Sal1 species and analyzed clinically extracted *P. vivax* protein using immunoassays. Four proteins (MSPI, MSP8 putative, MSP10 putative and Asparagine-rich protein) were identified as highly antigenic and are potential vaccine candidates.

#208 **Sataporn Thongpoon** (Mahidol University, Thailand) and the group identified and characterized transmission-blocking vaccine candidates from plasma of patients naturally infected with *P. vivax*. In addition to an already known vaccine candidate Pvs230, new transmission-blocking vaccine candidates were identified.

#209 **Sheena Dass** (Harvard T.H. Chan School of Public Health, United States) et al. selected eight antigen *P. vivax* proteins considering their functional roles in erythrocytes invasion and relatedness with *P. cynomolgi* and *P. knowles*i. Four antigenic antibodies displayed >50% inhibition in both sister species of which high priority antigen Pv_P108 showed the highest level of invasion inhibition in all three species. Thus the proposed model of using *P. cynomolgi* and *P. knowlesi* can facilitate identifying vaccine candidates for *P. vivax*.

#210 **Luna de Lacerda** (Fiocruz, Brazil) presented her study that evaluated immune response of 50 *P. vivax* peptides using *P. yoelii* mice modelsL3 and S2 were the most relevant Pv peptides that induced immune response to Py infection and decreased parasitemia, thus can be potential vaccine candidates

#211 **Thomas Martinson** (Oregon Health & Science University, United States) along with his group used the *P. cynomolgi* in Rhesus macaques to assess CD8 T cell killing capacity against iRBCs. They found that CD8 T cell response is dependent on the MHC-E molecule. This validates the human-relevant model for *P. vivax* studies.

#212 **Guilherme Castro** (Instituto René Rachou, Brasil) presented his research on the importance of γδT cells in the lysis and phagocytosis of merozoites and infected erythrocytes in patients with P. falciparum as well as and *P. vivax* malaria.

#213 **Cristiana Ferreira Alves de Brito** (Institute René Rachou - Oswaldo Cruz Foundation,Brazil) et al. Investigated the IgG antibody titres in neotropical primates naturally exposed to *Plasmodium*. Their findings indicated high antibody titres in wild adult monkeys than in the captive ones shedding light on the potential reservoirs of malaria infection and the immune response generated in the natural environment.

#214 **Herbert Opi** (Burnet Institute of Medical Research and Public Health, Australia) and his group assessed the ability of naturally-acquired and vaccine-induced antibodies (Abs) to block the interaction between PvAMA1 and RON2, essential for parasite invasion. The presence of this Abs did

not result in protection against clinical malaria but they resulted to be very very cross-reactive, making this interaction an attractive vaccine target.

#215 **Iris Aparici Herraiz** (Barcelona Institute for Global Health - ISGlobal, Spain) utilized the direct immuno-affinity capture (DIC) technique to identify the $CD71^{+}$-EVs from the infected reticulocytes of *P. vivax* malaria patients. They reported DIC as a robust strategy to identify *P. vivax* immunogenic proteins as antigens for vaccine development.

#216 **David Narum** (National Institute of Allergy and Infectious Diseases - NIAID, United States) et al. developed and characterized Pvs230D1M and Pvs230D1-EPA which are conjugated to nanoparticles. Both had the ability to block *P. vivax* transmission using membrane feeding assays with blood from naturally infected individuals from Colombia as well as in Samiri monkeys. They are planning to test the vaccine candidate (cGMP Pvs230D1-EPA with Matrix-M™ adjuvant) in clinical trials.

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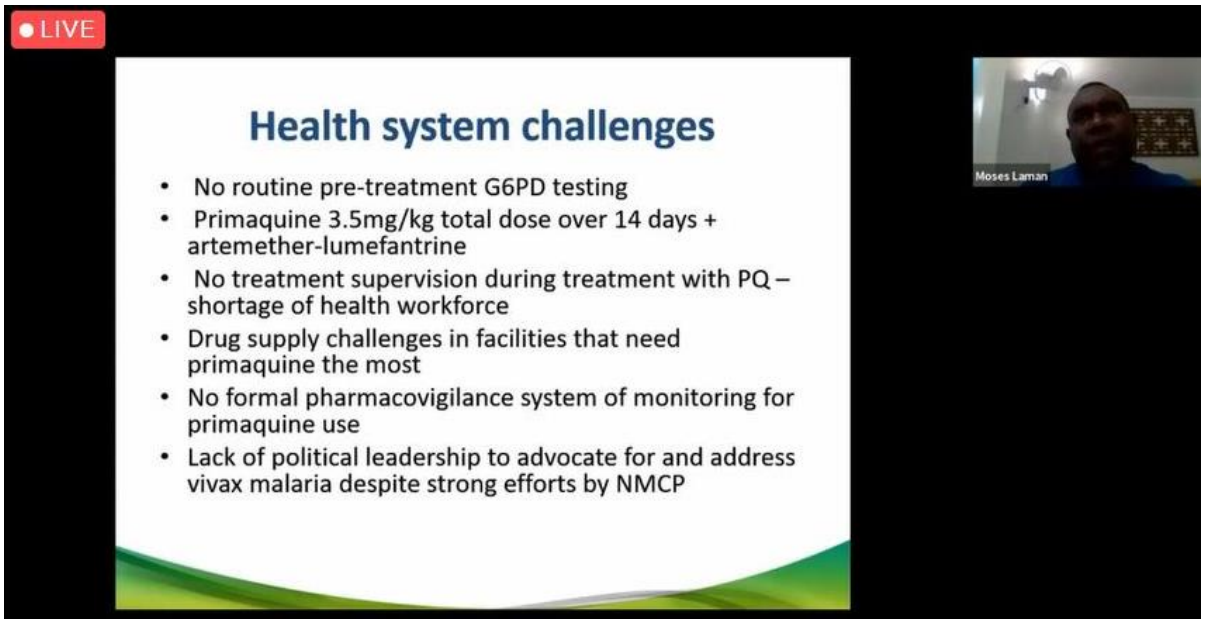
Day 3: 7th April 2022

Keynote Presentation

Moses Laman (Papua New Guinea Institute of Medical Research, Papua New Guinea) initiated his talk by highlighting the high *Plasmodium vivax (P. vivax)* malaria burden in PNG wherein relapses occur in 80% of the cases. He emphasized the challenges in addressing the *P. vivax* malaria burden in PNG which includes lack of G6PD testing, drug supply challenges, absence of a formal pharmacovigilance system for the use of primaquine, shortage of health workforce as well as the lack of political support. He then introduced the National Malaria Control Program's (NMCP's) strategic plan (2021-2025) that aims to achieve routine G6PD testing, review malaria treatment guidelines, ensure the safety of *P. vivax* radical cure and approval for SD biosensor in PNG. He further stressed the point of care of qualitative and quantitative G6PD testing before primaquine administration, improved diagnostics and prescription of WHO recommended drug dose, identification and management of the adverse effects and improved surveillance. He concluded by showcasing the country's readiness to develop a roadmap of tools and strategies to eliminate *P. vivax* malaria and the significance of research in the endemic areas.



Session 5: Treatment of vivax malaria

Rob Commons (Menzies School of Health Research, Australia) presented a systematic review and individual patient data meta-analysis on the optimal primaquine (PQ) dose. He pointed out that primaquine has both pros and cons *viz,* better efficacy, hemolysis and gastrointestinal (GI) symptoms. WHO recommends a dose between 3.5 to 7.0 mg/kg over 14 days. After a screening of potential studies, results from 6879 included patients showed that the lower dose of 3.5 mg/kg of primaquine is suboptima compared to 7.0 mg/kg which reduced the risk of *P. vivax* recurrence by 55%. However, giving a high dose daily may lead to more GI symptoms like vomiting, diarrhoea, and anorexia. Commons finished by highlighting the need to evaluate the hematological benefits of the higher dose to prevent relapses which can lead to recurrent parasite induced haemolysis.

Sze-Ann Woon (University of Western Australia, Australia) and her research group performed an open label, randomized controlled trial in children 6 months to 12 years with uncomplicated malaria and normal G6PD level to evaluate the efficacy and safety of 1 mg/kg twice daily primaquine regimen for

3.5 days. The dose of primaquine exhibited no difference in parasitaemia recurrence in both early (PQ on day 3) and delayed (PQ after 21 days) groups by day 42, though GI symptoms were more common in the early group. She concluded that primaquine treatment must be administered for radical cure in uncomplicated *P. vivax* malaria patients thereby contributing to malaria control.

Marcus Lacerda (Doctor Heitor Vieira Dourado Tropical Medicine Foundation, Brazil) presented the results of the first phase of an operational feasibility study with tafenoquine or primaquine (TRuST). A non-interventional, observational study was carried out to investigate if *P. vivax* patients were treated with tafenoquine or primaquine in accordance with the appropriate level of glucose-6-phosphate-dehydrogenase (G6PD) activity. Data collected from 09/09/21 to 18/10/21 was analyzed from health facilities in Manaus and Porto Velho, Brazil. They observed that appropriate treatment of patients according to G6PD activity was better with tafenoquine than primaquine. No cases of acute hemolytic anemia (AHA) were noted following tafenoquine treatment, unlike primaquine treatment which resulted in 5 cases with some requiring blood transfusions.

Shahin Tajeri (Sorbonne University, France) demonstrated and accentuated the problem that primaquine and tafenoquine are the only chemotherapeutics available for treating *Plasmodium vivax* hypnozoites, which are dormant liver stage and favour the parasite for epidemiological persistence. Due to the risk of fatal haemolysis in G6PD deficient patients, new hypnozoitocidal chemicals are urgently required. He demonstrated infusions prepared from *Artemisia annua* (high artemisinin content) and *Artemisia afra* (low artemisinin content) were effective in *in vitro* studies at non-toxic doses. Confocal microscopic examination after exposure to either infusion showed disruption of the parasites' apicoplast suggesting that a non-artemisinin component in the infusions might be responsible for parasite killing.

Session 6: New advances in diagnostics and surveillance (parasite and G6PD) from discovery to implementation

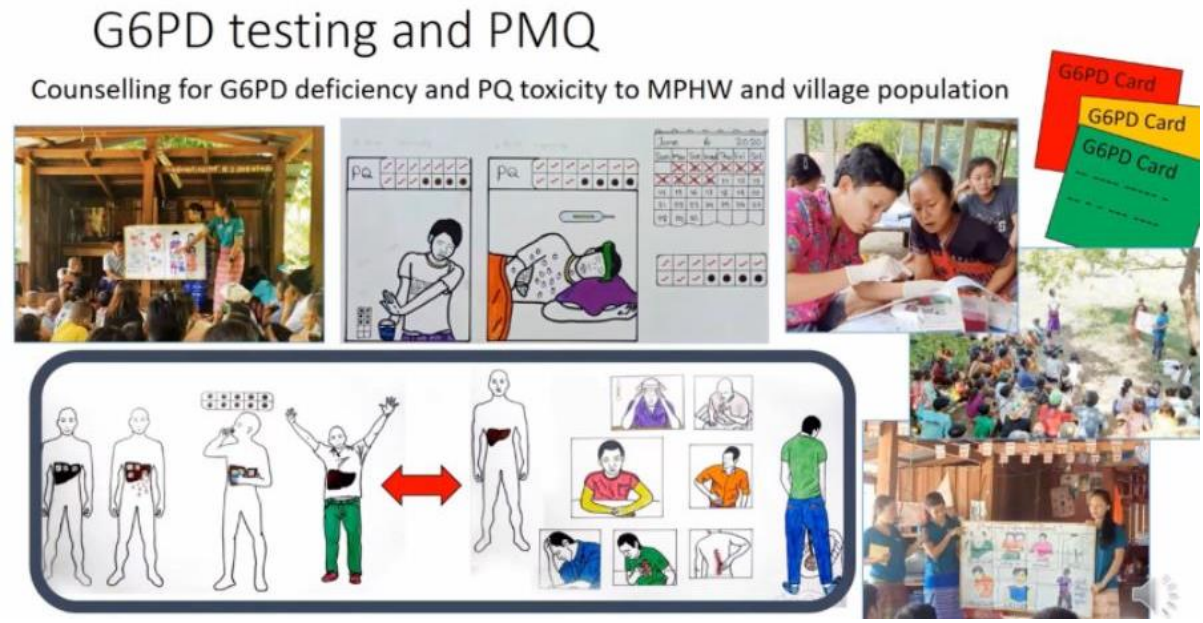
Rhea Longley (Walter and Eliza Hall Institute of Medical Research - WEHI, Australia) initiated her talk by highlighting the complex biology of *P. vivax* which makes surveillance and elimination challenges. In contrast to other malaria species, *P. vivax* forms dormant liver stages, that cannot be diagnosed to date but provide a silent reservoir that complicates *P. vivax* malaria elimination and control. Longley and her team identified a set of 8 serological markers that showed very good performance in predicting a *P. vivax* infection within the past 9 months. Thereby identified individuals have a very high

likelihood of carrying dormant liver stages and can be targeted for treatment. The authors conclude that these antibodies can be used for surveillance to identify hidden *P. vivax* malaria reservoirs.

Benishar Kombut (Papua New Guinea Institute of Medical Research, Papua New Guinea) started the presentation by giving a brief background of *Plasmodium vivax* infection in pregnancy in Papua New Guinea (PNG) and its contributory adverse effects and limitations of current diagnostic tools. Kombut noted that *P. vivax* infections are often sub-microscopic and asymptomatic, identification requires novel ultra-sensitive bedside diagnostics. The aim of this study was to assess the diagnostic performance of the Loop-mediated Isothermal Amplification (LAMP) for detecting *P. vivax* infections during pregnancy in PNG. Using a cross-sectional survey, the study was the first to assess the *Pv* LAMP performance in the field and was able to establish evidence of a high burden of undetected *P. vivax* infections during the first and second trimesters of pregnancy.The evaluated *Pv* LAMP was found to be a promising field deployable tool for detecting sub-clinical *P. vivax* infections in pregnant women with a sensitivity of 83% and specificity of 93%.

Benedikt Ley (Menzies School of Health Research, Australia) highlighted that the main risk factor for 8-aminoquinoline (8AQ) induced hemolysis is G6PD deficiency. Despite 8AQs being in use for over 60 years and G6PD deficiency being one of the most common enzymopathies worldwide, very few cases of 8AQ induced hemolysis have been reported. To address this contradiction, he assessed the G6PD activity of 335 malaria patients when parasitemic and again more than six months later when all patients were aparasitemic. He found that G6PD activity increased in the course of a malaria episode at a clinically relevant level. Dr Ley hypothesizes that this change is due to parasite induced hemolysis. A prospective case control study is required to confirm these findings.

Germana Bancone (Mahidol University, Thailand) assessed the usability of a handheld quantitative G6PD diagnostic (STANDARDTM, SDBiosensor, ROK) that was integrated into a malaria elimination program in the north of Thailand and neighboring Myanmar. In 2020 and 2021, G6PD survey using G6PD biosensor was conducted in around 23 villages and more than 5000 residents participated in this survey. Before this survey, health workers were trained for conducting quantitative tests using G6PD biosensors and counselling sessions were conducted for village population on G6PD deficiency and primaquine toxicity. Results from their study showed G6PD deficiency prevalence of 15.4% thus concluding that the diagnostic test was easy to use, well-accepted, and could reliably identify both intermediate and deficient phenotypes if used with community engagement activities.



Rapid Fire Presentation - Treatment of vivax malaria

#300 **Mohammad Sharif Hossain** (International Centre for Diarrhoeal Disease Research, Bangladesh) conducted a clinical evaluation of primaquine (PQ) adherence in 33 *P. vivax* infected patients. At length, he endorsed minimizing the treatment delay and the use of a short course of primaquine for *P. vivax* malaria found through focus group discussions (FGD) organized during the study.

#301 **Vishnu Teja Nallapati** (Kasturba Medical College, India) presented a systematic review on chloroquine resistance for *P. vivax* which is found to be relatively low in India even though it is increasing. In the 17 studies included in the systematic review, chloroquine resistance was identified in *in vivo* and *in vitro* studies, even though in a very low percentage.

#302 **Bob Taylor** (Mahidol Oxford Tropical Medicine Research Unit - MORU, Thailand) elaborated on how to overcome the challenges of primaquine *viz*, no adapted paediatric dosage, bitterness for paediatric use, low acceptability and poor compliance. New quality assured formulations with a better test are being studied in two field clinical trials in Ethiopia and Burkina Faso.

#303 **John Huber** (Washington University in St. Louis School of Medicine, United States) explored different sources of site-specific biases with an individial-based model to identify recurrent infections, either due to relapse or reinfection. He concluded that transmission intensity causes downward bias which can be mitigated with vector control.

#304 In the qualitative study presented **Annisa Rahmalia** (Yayasan Pengembangan Kesehatan dan Masyarakat, Indonesia) observed substantial obstacles faced by community health workers (CHWs). Eventually, in ethnically diverse locations, recognising social connections (public and private) are the critical aspect of developing an effective community-based malaria strategy.

#305 **Benjamin Bob** (Akwa Ibom State Government of Nigeria, Nigeria) presented the conclusion of a random sample cluster survey (2015-2020) conducted to monitor the healthcare facilities *viz*, proper access to prompt diagnosis, care, treatment of malaria. He suggested that a community delivery mechanism is a cornerstone to achieving the National Malaria Elimination Programme (NMEP) policy goals.

#306 **Leticia Ferreira** (University of Campinas, Brazil) paid attention to the emergence and the spread of drug-resistant parasite strains of *Plasmodium* against artemisinin-based combination therapies (ACTs). Therefore, with the aid of *in silico* i.e., QSAR, *in vitro* and *in vivo* approaches, compounds were designed and tested. Among them, LDT-623 exhibited better EC_{50} and was found active against sexual stages with good synergism with methylene blue and might be considered as a HIT molecule.

#307 **Brice Campo** (Medicines for Malaria Venture - MMV, Geneva) has developed new screening assays to assess hypnozonticidal activity of primaquine and tafenoquine and also reviewed the challenges, success and the progress in *P. vivax* drug development.

#308 Through semi-structured interviews, **Varunika Ruwanpura** (Menzies School of Health Research, Australia) collected the insights of twenty-seven global malaria stakeholders on how to best apply new global guidelines for providing a radical cure for *P. vivax* malaria. Results showed a variety of views on what types of evidence should be assessed to make global recommendations.

#309 **Parinaz Mehdipour** (University of Melbourne, Australia) developed a within-host red blood cell (RBC) model to assess the effect of primaquine administration on the haemoglobin and reticulocyte

profiles of G6PD deficient *P. vivax* malaria patients which can help determine optimum drug dose for these individuals.

#310 **Parinaz Mehdipour** (University of Melbourne, Australia) reviewed the efficacy studies published between January 1999 and March 2020 of uncomplicated *P. vivax* malaria which administered a daily dose of primaquine and concluded that decreased compliance, as well as less monitoring, increase the prospects of relapse of *P. vivax* infection.

#311 **Annisa Rahmalia** (Papuan Health and Community Development Foundation, Indonesia) assessed the feasibility and social acceptability of phone follow-up for monitoring primaquine compliance and established the cost-effectiveness of this strategy in containing *P. vivax* recurrence. Patients prefered calls over visits since there was more flexibility and nurses are seen as trustworthy points of information.

#312 **Megha Rajasekhar** (University of Melbourne, Australia) performed a meta-analysis of clinical efficacy studies published between January 2000 and March 2020 of patients with uncomplicated *P. vivax* treated with primaquine. Findings indicate high daily doses of PQ doses to be associated with poor gastrointestinal (GI) tolerability and severe hematological events.

Rapid Fire Presentation - New advances in diagnostics and surveillance (parasite and G6PD) from discovery to implementation

#313 **Jose Diego Brito-Sousa** (Fundação de Medicina Tropical Heitor Vieira Dourado, Brazil) et al. used the qualitative CareStart G6PD screening test to diagnose Glucose-6-phosphate dehydrogenase (G6PD) deficiency, in order to avoid adverse effects after primaquine treatment. Screening test was found to be highly sensitive (100%) but had low specificity of 68.1% in identifying G6PD deficient patients.

#314 **Shalini Aggarwal** (IIT Bombay, India) et al.'s goal was to find potential biomarkers for diagnosis of *P. vivax*. They did proteomic analysis on patients' samples and found 5 recurring parasite proteins. *P. vivax* tryptophan-rich antigen (PvTRAg) performed the best in further analysis and was purified to be used for diagnostic and prognosis of *P. vivax* infections.

#315 **Benedikt Ley (Menzies School of Health Research, Casuarina, Australia)** assessed the repeatability and inter-laboratory reproducibility of the SD biosensor's quantitative handheld G6PD diagnostic in discriminating between high, intermediate and low G6PD activities and compared to spectrophotometry. Results showed good repeatability and reproducibility of the biosensor, however, clinical trials are needed to assess its performance in practice.

#316 **Angela Devine** (Menzies School of Health Research, Australia) tried to explore the impact of the cost-effectiveness of tafenoquine following quantitative G6PD screening in Brazil. Her study established strong evidence of high likelihood of cost-effectiveness of tafenoquine prescription after quantitative G6PD testing.

#317 **Jason Rosado** (Institut Pasteur, Paris, France) measured antibody responses against 8 serological exposure markers (SEM) to *P. vivax* to identify recently exposed people and determine the transmission dynamics of the parasite in peri-urban and riverine communities in Loreto Peru following implementation of control programs in the region. SEM showed increasing importance for malaria surveillance in the Peruvian Amazon.

#318 **Yanie Tayipto** (Walter & Eliza Hall Institute of Medical Research, Melbourne, Australia) developed an 8-antigen panel that detects total IgG as serological markers of *P. vivax* exposure within the past 9 months. Their results suggest high levels of exposure are required for a sufficient IgG3 response as accuracy of serological markers is affected by the acquisition of antibodies and not just the longevity.

#319 **Kavitha Saravu** (Kasturba Medical College, Manipal, India) sought to evaluate a rapid hemozoin-based malaria detection device (Gazelle™) for rapid detection of *P. vivax* malaria parasites in blood samples. Results from this study showed that the device has good sensitivity, specificity and accuracy.

#320 **Thomas Obadia** (Institut Pasteur, Paris, France) used a serological test and treat method which they termed "*P. vivax* serological test and treat (PvSeroTAT)" considered a safer alternative to mass drug administration in order to minimize the risk of over-treating individuals with drugs which may induce haemolysis. Findings from this study show that PvSeroTAT is predicted to be a safe and efficacious option for targeting the hypnozoite reservoir toward *P. vivax* elimination.

#321 **Abhijit Sharma** (PATH, India) evaluated a novel point-of-care (POC) STANDARD™ G6PD test for the detection of normal, intermediate and deficient populations in East India. With a 100% sensitivity and near-perfect figure for specificity, they concluded that the novel POC met the acceptance criteria for diagnosing G6PD deficiency for individuals with <30% G6PD activity in the study population.

#322 **Emily Gerth-Guyette** (PATH, United States of America) developed and implemented a measurement framework to assess operational feasibility across multiple dimensions including adherence to a revised case management algorithm that includes G6PDclassification, assessment of healthcare provider knowledge, evaluation of training, cost analysis and qualitative exploration of barriers and facilitators that impact G6PD testing.

#323 **Wondimagegn Adissu** (Jimma University, Ethiopia) conducted a systematic review that concluded a low prevalence of G6PD deficiency in Ethiopia with geographic heterogeneity. While the prospective diagnostic study assessed the performance and usability of a new POC, STANDARD™ validated the STANDARD™ G6PD test, albeit with low performance in the classification of G6PD intermediate females.

#324 **Gonzalo Domingo** (PATH, United States) explored how the G6PH being an X-linked disorder influences the performance of diagnosis tests for G6PD deficiency. For a point of care test, the biology of G6PD deficiency supports a high performance to identify G6PD deficiency but lower for identifying intermediate females.

#325 **Allison Golden** (PATH, United States) and her team work demonstrated that the RapidGEN rapid diagnostic test improved the limit of detection for *P vivax* LDH antigen, increasing the chances to find positive cases missed by microscopy. Results should be confirmed with clinical studies.

#326 **Arkasha Sadhewa** (Eijkman Institute for Molecular Biology, Indonesia) et al. found that G6PD deficiency prevalence is quite variable across Indonesia, even in the same region. Ethnic differences might explain this pattern as well, as the variety of methods used to assess the anomaly.

#327 **Manash Shrestha** (Medicines for Malaria Venture, Nepal) used a Theory of Change framework for identifying key research questions in order to achieve timely elimination goals in which 12 Asia Pacific countries participated. Implementation was the most prioritized research area followed by research and development.

#328 **Giulia Pianta** (London School of Hygiene and Tropical Medicine, United Kingdnom) and team created a transgenic line replacing *pkldh* gene with its orthologue from *P. vivax* and *P. ovale* spp. to compare species-specific sensitivity and reactivity of RDTs. The transgenic line can be used as a Secondary International Standard for *P. vivax*, once approved by the WHO.

#329 **Tais Sousa** (Fiocruz, Brazil) described molecular diagnostic methods using the Real-Time PCR (qPCR) and Droplet Digital PCR (ddPCR) that tested blood, saliva and buccal swab. They concluded that Non-invasive DNA sampling can be used for malaria diagnosis by targeting multicopy sequences combined with the highly sensitive ddPCR.

#330 **Shazia Ruybal-Pesántez** (Walter and Eliza Hall Institute of Medical Research, Australia) Used serological exposure markers (SEMs) for the detection of hidden *P. vivax* infections to slow community transmission. Children were classified as recently exposed or not recently exposed. Results show heterogeneous spatial clusters of *P. vivax* infection. It was also predicted that children with recent exposures are at higher risk of recurrent infections.

#331 Using flow cytometry, **Angela Rumaseb** (Menzies School of Health Research, Australia) et al. were able to distinguish between infected and uninfected in parasitized and non parasitized RBCs. G6PD deficient cells were less likely to get infected in the schizont (0.4%) and ring stage (0.2%) compared to normal cells (2% and 0.9%, respectively). Further studies are needed to explore preferential parasitization.

#333 **Agnes Orban** (Budapest University of Technology and Economics, Hungary) et al. quantified hemozoin levels in a small population of patients treated for malaria, using rotating-crystal magneto-optical detection (RMOD)Hemozoin levels were reduced three days post-infection in *P. vivax*. They conclude that hemozoin could be used as a biomarker for hidden *P. vivax* infections or in the onset of relapses.

This report is brought to you by the MESA Correspondents Melina Florez-Cuadros, Varun Gorki, Neha Sylvia Walter and Duru Vincent C. Senior editorial support has been facilitated by the Organizers and Chairs of the sessions.

Day 4: 8th April 2022

Session 7: Transmission - from gametocyte biology to transmission studies and entomological surveillance

Fitsum Girma Tadesse (Armaeur Hansen Research Institute, Ethiopia) presented his talk on the *Plasmodium vivax* and *Anopheles stephensi* in the Horn of Africa. He described this region as an epicenter of emerging challenges for example Pfhrp2/3 deletion, artemisinin resistance and *An. stephensi* emergence in urban settings, which is a dominant vector in India and Persian Gulf. This new scenario for *An. stephensi* threatens 126 million people living in urban areas in Africa, moreover this vector is resistant to insecticides and very efficient in transmission of both *P. vivax* and *P. falciparum.* He estimated an elevation in malaria incidence even in low transmission areas post *An. stephensi* invasion. He also explained the changing parasite dynamics in urban areas Ethiopia, Djibouti and Sudan with a high proportion of *P. vivax* malaria infections.

Rajnikant Dixit (Indian Council of Medical Research and National Institute of Malaria Research - ICMR/NIMR, India) presented his talk on the interactions between mosquito gut- microbiome and the development of *Plasmodium vivax*. He illustrated that the vector competency of a mosquito is modulated by its gut microbiota. Gut-meta-transcriptomic study revealed the vector immune evasion by the parasite during its invasion phase by suppressing microbiota. Further, RNAseq analysis highlighted the genetic changes in the parasite enabling it to manipulate mosquito's metabolism for its own survival and transmission.

Wang Nguitragool (Mahidol University, Thailand) et al. wanted to understand how patients with low parasitemia contribute to *P. vivax* transmission, as well as the transmission capacity of mosquitoes under such circumstances. They performed serial dilutions of 8 patients' samples for feeding membrane assays, to evaluate parasite density and mosquito infectivity counting the oocysts present in the gut. Infection rate and intensity were positively correlated with parasite density. Even though the study demonstrates the ability of *P. vivax* to infect mosquitoes at a very low parasitemia, there is a large variation among cases and no immunity was taken into account. Further analyses are needed.

Clara Champagne (Swiss Tropical and Public Health Institute, Switzerland) illustrated the role of mathematical transmission modelling to estimate the risk and impact of various malaria control interventions against *P. vivax*. The model can further be used to compare strategies for disease elimination. She introduced a model for *P. vivax* dynamics, which also included interventions for case management. She also presented the applications of the model in Panama to identify sustained areas of transmission and an R package available online, which can be used to apply the model to data. To conclude, she pinpointed certain limitations of the model *viz.* simplified model for treatment delays and valid for low transmission areas where immunity does not play a major role.

Session 8: Genomic epidemiology and parasite evolution

Ian Cheeseman (Texas Biomedical Research Institute, San Antonio, United States) aimed to develop a single-cell genomics approach to understand how malaria parasites adapt to new environments. Cheeseman conducted a single-cell sequencing of recurrent *P. vivax* primary and secondary infection from patients from Thailand and identified de novo mutations (DNMs) in the *P. vivax* malaria isolates. More than half of the identified DNMs were tight clusters suggesting a cryptic relatedness. Results showed no correlation between the mutations and the formation of the hypnozoite stage. Also that DNMs were under a strong positive selection and they had functional relevance with recurrent mutations in the ApiAP2 gene family. He compared this result with another *P. falciparum* infection from patients from Malawi and found some parallel targets of mutation in both species. Cheeseman's study reveals information on relatedness and process of adaptation in malaria infection.

Johanna Helena Kattenberg (Institute of Tropical Medicine Antwerp, Belgium) started her talk by noting that molecular tools were important in characterizing changing transmission intensity and were also able to guide malaria control by distinguishing local and imported cases and also sources of reintroduction. She designed novel within-country single nucleotide polymorphism (SNP) barcodes for Vietnam and Peru using whole genome data. SNPs were selected and genotyped using a highly multiplexed *P. vivax* Ampliseq NGS assay including amplicons targeting some drug resistance genes. These were applied to sample isolates from travellers to routine samples collected at local sentinel sites in Vietnam and to retrospective samples from Peru. Kattenberg's study highlighted higher diversity and increased resolution for within-country spatio-temporal surveillance.

Lucas Buyon (Harvard TH Chan School of Public Health, United States) summarized that owing to the lack of *P. vivax* culture, the molecular determinants of chloroquine and other antimalarials resistant to *P. vivax* are uncertain. *pvmdr1*, an orthologous to *pfmdr1*, reflected both polymorphisms in sequence and expression level. Notwithstanding decades of work into *pvmdr1*, no conclusive link has been found between *pvmdr1* polymorphisms and drug resistance. To elucidate that, they analyzed 24 *pvmdr1* haplotypes alleles and an endogenous *pkmdr1* gene was replaced with *pvmdr1* alleles in *P. knowlesi* transgenic lines were then tested for resistance to a variety of antimalarial drugs and found

certain haplotypes resistant to mefloquine, dihydroartemisinin, and lumefantrine. Furthermore, they had no evidence that *pvmdr1* haplotypes alone confer a substantial shift in chloroquine susceptibility.

Mariana Kleinecke (Menzies School of Health Research and Charles Darwin University, Australia) described that the information for a genome-wide panel of microhaplotypes can be obtained from amplicon-based sequencing assays whose additional analyses and improvements are being made, by eliminating superfluous markers to get a final panel of 100 microhaplotypes. In brief, a single Illumina rhAmpSeq multiplex was designed and they took the 192 *P. vivax* samples (10 countries) with 16 control samples to conduct the Multiplex and indexing PCRs. To inform performance of these potential assays, a MiSeq run was conducted and analysed with a standard pipeline consisting of bwa, samtools, picard and GATK. A majority of the assays performed well with samples from 10 countries, and *Plasmodium* species were reliably distinguished using mitochondrial markers. Globally, population genetic investigations revealed a high level of diversity, as well as a geographic grouping.

Rapid Fire Presentation - Transmission - from gametocyte biology to transmission studies and entomological surveillance

#400 **Lincoln Timinao** (PNG Institute of Medical Research, Papua New Guinea) performed direct skin feeding assays on 11 asymptomatic *Plasmodium* carriers in Madang Province and also on clinical samples and found *P. vivax* to be more infectious in both the populations and that asymptomatic individuals can still transmit malaria.

#401 **Camila Fabbri** (Fiocruz, Brazil) assessed asexual and sexual stage maturation exposed to methylene blue, using three different. Methylene blue inhibited schizonts with a lower IC_{50} than chloroquine also inhibited the ookinetes and even though it didn't decrease the infection rate in direct feeding it reduced the oocyst intensity.

#402 **Wakweya chali Gerba** (Armauer Hansen Research Institute, Ethiopia) 241 *P. vivax* patients were recruited for blood collection and follow-up. Results show that 84 patients had a recurrence episode during the study. In addition, 45% of mosquitoes fed with infectious blood were infected.

#403 **Sofia Forcellini** (Instituto Carlos Chagas/ Fiocruz, Brazil) studied the antigenicity of the Plasmodium vivax gametocyte protein Pvs47. 30% of 40 plasma samples showed IgG againts Pvs47. Naturally acquired antibodies recognize the recombinant protein Pvs47.

#404 **Lorena Tapia** (Naval Medical Research Unit Nº6, Perú) and her group tested three *P. vivax* adapted strain lines to recreate the life cycle in vivo. After unsuccessful attempts to reach blood

stage infection, they inoculated all three strains in one splenectomized monkey in hope to obtain hybrids with increased fitness. Infection was a success and three more monkeys were infected through a mosquito bite. Whole genome sequencing is under way.

Rapid Fire Presentation - Genomic epidemiology and parasite evolution

#405 **Alebachew Messele Kebede** (Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Ethiopia) conducted an in-depth genomic analysis of Ethiopian *P. vivax* genomes. This highlighted adaptations of potential public health concerns in a setting with moderately high and stable transmission of *P. vivax* malaria and underscored the need for enhanced *P. vivax* interventions and surveillance to identify and contain new adaptive variants that threaten control and elimination efforts.

#406 **Jillian Grassia** (Duke University, United States) leveraged a simulation model for *P. vivax* that incorporates realistic features of parasite life history and epidemiology as well as studies that expected genetic diversity of the parasite population. This model can be used to test the impact of intervention on parasite genetic diversity through simple adjustments to parameters.

#407 **Alison Paolo Bareng** (Deakin University, Australia) sought to determine the global distribution of antigen diversity and to identify gene regions with signatures of immune selection amongst leading *P. vivax* vaccine candidate antigens and sero-surveillance markers. The result from this study will guide researchers in designing widely effective vaccines and serological tools against *P. vivax.*

#408 **Suman Tamang** (Indian Council of Medical Research - ICMR and National Institute of Malaria Research - NIMR, India) highlighted the importance of molecular markers to confirm drug resistance and determine it trends. concluded in his presentation that isolates obtained from Goa exhibited mutant genotypes [*Pvdhfr* (80%) and *Pvdhps* (52%)] in *P. vivax* with novel *k*12 mutation in contrast to Delhi isolates [*Pvdhfr* (13%) and *Pvdhps* (0%)] which is an indicator of the emergence of sulfadoxine-pyrimethamine drugs resistance.

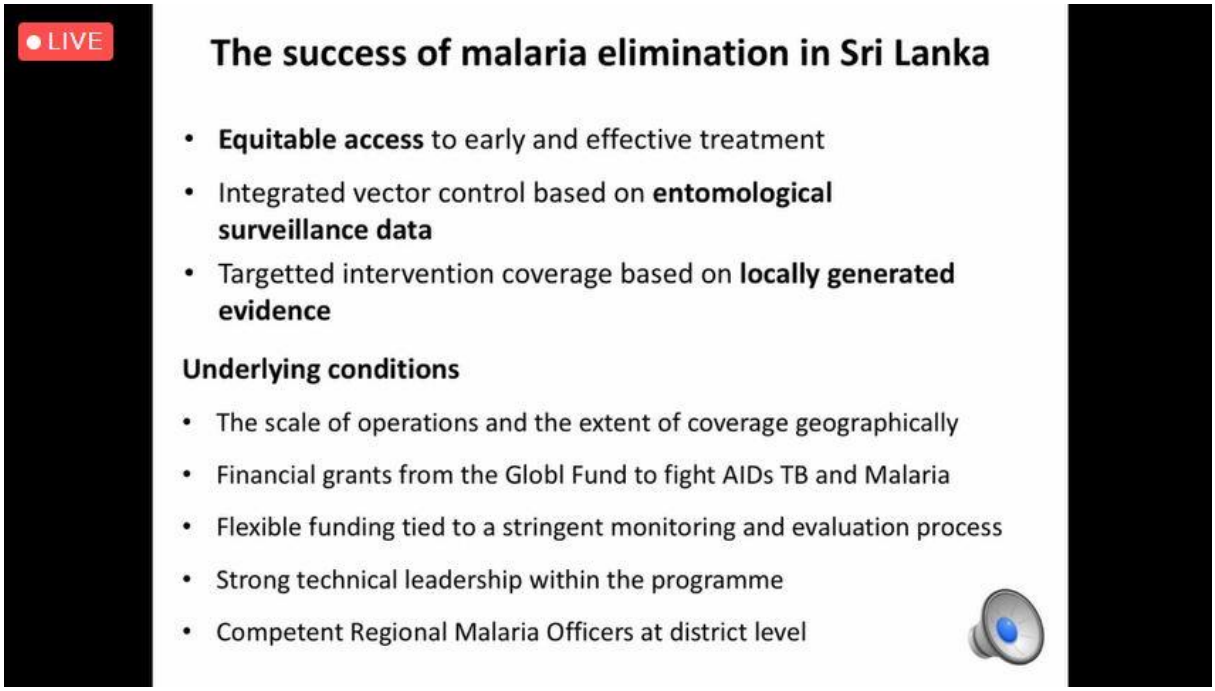
#409 **Catarina Bourgard** (University of Campinas, Brazil) illustrated the methodological outline made for RNA-seq from *P. vivax* isolates with better RNA extraction (yielding ~ 100 $pg.\mu L^{-1}$), suitable for Illumina sequencing, SMART-Seq and Ultra-Low Input RNA library which would solve the problem of getting RNA from low parasitaemia and would have a favourable influence on *P. vivax* research.

#410 Noelia Coronado (Universidad Peruana Cayetano Heredia, Peru) pointed out that the genes encoding MSP8 and MSP10 of *P. vivax* have a lot of genetic diversity with no selection pressure. To reveal the impact of geographic origin of the parasite on diversity and potentially host antibody response, genetic analysis of more sequences should be done.

Richard Carter memorial lecture

Time bears witness to deeds undertaken with perseverance, especially those promoting peace and health for mankind. The Richard Carter Memorial Lecture was announced to commemorate malariologist Professor Richard Carter who contributed so much to the study of malaria and *Plasmodium vivax* and who died in September last year. **Jane Carlton** (New York University, United States) gave a summary of Dr. Carter's life and work at the University of Edinburgh and the National Institutes of Health, U.S.A., describing his intellectual curiosity, passion for research, and keen mentorship of students, closing with thoughtful remarks from several of his colleagues. **Kamini Mendis** (University of Colombo, Sri Lanka), a long-time collaborator, presented the Richard Carter memorial lecture concerning malaria elimination in Sri Lanka between 2000 and 2015. Dr. Mendis

highlighted that access to effective and early treatment, integrated vector management and targeted interventions based on the local evidence were the cornerstone for successfully eliminating malaria in Sri Lanka. The pre-elimination phase was initiated in 2008 and the number of cases gradually declined until the last case was reported in 2012 during a very well controlled outbreak in the south of the country. Sri Lanka received the WHO certification as malaria free in 2015. She quoted Dr. Richard Carter that as more interventions are targeted to control *P. falciparum* the residual *P. vivax* burden typically increases. She reviewed the features of *P. vivax* which give it a survival advantage and emphasized that preventing relapses, efficient diagnosis and vector control comprise an effective strategy to contain and eliminate *P. vivax* malaria. Dr. Mendis concluded that malaria can be eliminated even with the currently available tools, but that the strategies and interventions must accord to the reality of local areas, and that prompt action is required which should be targeted efficiently.



Closing Ceremony

The participants and scientists of the four-day event were thanked by the organizers Kamala Thiemer and Leanne Robinson for their participation and interaction with speakers after their presentations and during the spatial chat. The organizers warmly expressed gratitude to the MESA team and their correspondents for making this event available as a recording on the website. The winners of the best talk and poster awards were also announced with prizes. The organizers were pleased to confirm that the next ICPvR will be hosted in India.

This report is brought to you by the MESA Correspondents Melina Florez-Cuadros, Varun Gorki, Neha Sylvia Walter and Duru Vincent C. Senior editorial support has been facilitated by the Organizers and Chairs of the sessions.

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