# 9th International Conference on *Plasmodium vivax* Research (ICPvR 2025)

# MESA Correspondents Report



**Written by** Nallapati Vishnu Teja, Varijakshi Gutthedhar, and Priya Kumari.

**Senior editorial support** has been facilitated by Dr. Manju Rahi, Dr. Dhanpat Kochar, and Dr. Ashis Das.

MESA Correspondents bring you cutting-edge coverage from the 9th International Conference on Plasmodium vivax Research (ICPvR 2025)

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# Pre-conference Workshop: Tuesday, 11<sup>th</sup> February 2025

**Workshop 1 – The pathophysiology of severe vivax malaria**

Nicholas Anstey (Menzies School of Health Research, Australia) discussed the worldwide impact of *Plasmodium vivax* malaria, which is mostly reported in India and Indonesia. He emphasized its chronic nature, frequent relapses, and potential for serious illness. A key focus was the hidden parasite biomass in the spleen, constituting 98% of the total parasite burden in chronic infections and 88% in acute cases. Anstey discussed that *P. vivax*-infected red blood cells (RBCs) accumulate in small blood vessels, while uninfected RBCs lose deformability, resulting in endothelial activation, thrombotic microangiopathy, and impaired circulation. Rosette formation complicates anemia by causing uninfected red blood cells (RBCs) loss by splenic trapping and relapses. Organ-specific complications can involve splenic rupture, more prevalent in adults and occurring more commonly with *P. vivax* than *P. falciparum*, with thrombocytopenia, which raises the risk of severe illness in both adults and children. Other organ-specific complications, such as acute respiratory distress syndrome (ARDS) and acute kidney injury (AKI), show geographical patterns, with over 75% of AKI cases reported from India. Anstey concluded the talk by highlighting that pregnant women have an elevated risk of severe *P. vivax* malaria, with studies indicating higher risks among women in Brazil, India, and the Thai-Myanmar border area.

**Workshop 2 – Multi-organ dysfunction in vivax malaria and the comparison of the phenotype with severe falciparum malaria and mixed infection malaria**

Dhanpat Kochar (Sardar Patel Medical College, India) highlighted the increasing incidence of severe malaria observed in *Plasmodium vivax* infection. Kochar discussed the differentiation between sequestration and non-sequestration forms of severe malaria. The presentation included case reports, with *P. vivax* mono-infection who presented with high-grade fever, jaundice, renal failure, severe anemia, and thrombocytopenia. Another case involved a pregnant woman with anemia and more reports include pulmonary edema/ARDS, and hypoglycemia, leading to coma and severe dysfunction. Kochar highlighted a case of post-malaria neurological syndrome (PMNS) in a patient who developed bilateral facial palsy on the 14th day of recovery. Kochar also discussed research findings indicating that cerebral involvement, though rare in *P. vivax* malaria, can lead to life-threatening complications. He emphasized that *P. vivax* malaria frequently presents with thrombocytopenia, though no clear correlation exists between parasite density and platelet count. He noted that infections during pregnancy contribute to maternal anemia, preterm birth, fetal loss, and low birth weight. The key takeaway from Kochar's talk was that submicroscopic infections in pregnancy often go undiagnosed, leading to untreated placental malaria, which significantly increases the risk of fetal and maternal mortality.

**Workshop 3 – Management of severe vivax malaria**

Kavitha Saravu (Kasturba Medical College, India) emphasized the World Health Organization (WHO) definitions of severe *Plasmodium vivax* malaria based on various clinical and laboratory features. These criteria include impaired consciousness, prostration, multiple convulsions, pulmonary edema, acute respiratory distress syndrome, acute kidney injury, abnormal bleeding, hypoglycemia, metabolic acidosis, hyperparasitemia, renal impairment, and pulmonary edema. Saravu's interactive approach, combined with real-life case discussions, effectively highlighted the complexities of clinical management, including the challenges presented by recurrent malaria. Saravu presented a case in which a patient experienced multiple recurrences of malaria within eight months. Additionally, Saravu pointed out common pitfalls in the management of *P. vivax* malaria, such as: presumptive

treatment without proper diagnosis, misidentification of infecting species or gametocytes as active infections, misclassification of severe *P. vivax* infections, failure to provide radical cure with primaquine and G6PD testing, assuming all treatment failure cases are due to resistant malaria, and presuming all recurrent cases of malaria are resistant or complicated. Her insights into these challenges are critical for improving the management and treatment outcomes for patients with severe *P. vivax* malaria.

## Workshop 4 – Clinical outcomes and long-term consequences of severe malaria

Sanjay Kochar (Sardar Patel Medical College, India) discussed the complications of severe *Plasmodium vivax* malaria, including anemia, kidney and lung injury, jaundice, cerebral malaria, post-malarial neurological syndrome, bleeding, shock, and splenic rupture. Kochar also highlighted that patients post-splenectomy, are at increased risk of severe malaria, recurrent relapses, infections, and hemolysis, necessitating constant monitoring and preventive strategies such as prophylaxis and vaccinations. Long-term consequences were addressed, such as congenital and neurodevelopmental disabilities, cardiac complications, chronic kidney disease (CKD), post-malarial syndrome, hepatic dysfunction, chronic liver disease, persistent inflammatory response and autoimmune disorders, metabolic dysregulation and increased risk of diabetes, neurological complications, maternal anemia, premature labor and low birth weight, fetal loss, fatal respiratory complications, and relapsing malaria. To illustrate post-malarial syndrome, he presented a *P. vivax* case of renal failure requiring hospitalization and hemodialysis, which later led to chronic fatigue, joint pain, cognitive issues, and sleep disturbances. Kochar concluded by stating that severe malaria has a major socio-economic impact beyond health, particularly affecting vulnerable populations, and cited a case of a patient who struggled with hospitalization expenses.

## Workshop 5 – Diagnostic modalities in vivax malaria

**Vishnu Teja Nallapati** (Kasturba Medical College, India) addressed the challenges of diagnosing *Plasmodium vivax* malaria and discussed current diagnostic approaches, highlighting their advantages and disadvantages. Nallapati presented various methods, including microscopy, quantitative buffy coat (QBC), and rapid diagnostic tests (RDTs). Molecular diagnostic techniques such as nested polymerase chain reaction (PCR), quantitative PCR (qPCR), Loop-Mediated Isothermal Amplification (LAMP), and the mini dot blood nucleic acid lateral flow immunoassay were also discussed. Additionally, he emphasized the potential of using serological surveillance methods such as enzyme-linked immunosorbent assay and immunofluorescent assay (IFA) to identify individuals exposed to *P. vivax*. The presentation was followed by a microscopic demonstration of the ring form, schizont, and gametocyte stages of *P. vivax*, along with hands-on experience in conducting an RDT. This combination of theoretical insights and practical demonstrations made the information more tangible and beneficial for the audience.

# Day 1: Wednesday, 12th February 2025

**Registration and Inauguration**

The Indian Council of Medical Research (ICMR) – Vector Control Research Centre (VCRC), Puducherry hosted the 9th International Conference on *Plasmodium vivax* Research (ICPvR) 2025 at Trafalgar Square, Ocean Spray Resort, Puducherry, India. The conference brought together over 256 participants from around the world, including 120 international attendees. The conference's theme was "*Plasmodium vivax* – Science and Tools for Elimination." Opening remarks were delivered by several distinguished speakers: Manju Rahi (Convenor), Ashwani Kumar (Conference Chairperson), Amit Sharma (Co-Chairperson), Rajani Ved (Guest of Honour), Rakesh Sehgal (Guest of Honour), Tanu Jain (Chief Guest), Rajiv Bahl (Director General, Indian Council of Medical Research) and Saima Wazed (Regional Director, World Health Organisation, South-East Asia). All speakers acknowledged the conference theme, emphasizing the significant challenges in different facets of malaria elimination and the urgent need for innovative tools to support national malaria elimination programs related to *vivax* malaria.

**Session 1 – Keynote lecture and plenary sessions**

**Nick White** (Mahidol University, Thailand) discussed the geographical distribution, origin, and spread of *Plasmodium vivax* malaria. He highlighted the current diagnostic methods, their limits of detection, and the drawbacks associated with these modalities. White pointed out that severe vivax malaria (SVM) may result from overdiagnosis. In comparison to *Plasmodium falciparum* (*P. falciparum*) malaria, uncomplicated vivax malaria is characterized by lower parasite densities, reduced pyrogenic diversity, and greater red cell loss per parasitized erythrocyte. *P. vivax* malaria has a higher incidence in children and is more transmissible than *P. falciparum* malaria. The likelihood of disease progression to severe complications such as hepatic dysfunction, renal dysfunction, metabolic acidosis, severe thrombocytopenia, and cerebral dysfunction is much lower in *P. vivax* malaria than in *P. falciparum* malaria. Vivax malaria during pregnancy is linked to complications such as abortion and low birth weight. Additionally, the burden of asymptomatic malaria may represent the hidden extent of *vivax* malaria, often described as the "unseen iceberg". White concluded by emphasizing that early detection and treatment are crucial for the elimination of malaria.

**Dhanpat Kochar** (Sardar Patel Medical College, India) emphasized key epidemiological changes in *Plasmodium vivax* malaria during global malaria elimination programs, noting a higher incidence of severe cases in India and Indonesia. He addressed the changes in the ratio of short-incubation *P. vivax* in temperate regions to long-incubation in sub-tropical and tropical areas. Kochar emphasized well-documented cases of severe *P. vivax* malaria since 2005, accompanied by consequences including cerebral malaria, convulsions, jaundice, renal failure, multiorgan dysfunction, and post-malarial neurological syndrome. Particularly, mortality from severe *P. vivax* was higher in children compared to adults. Thrombocytopenia was stressed as a significant yet currently unrecognized complication of severe malaria. Additional challenges in malaria elimination, such as sub-microscopic infections in pregnant women and Duffy-negative individuals were also underscored due to their role as hidden reservoirs contributing to persistent infections.

**Kevin Baird** (Oxford University Clinical Research Unit, Indonesia) challenged the belief that *Plasmodium vivax* causes only benign malaria. A clinical audit from Papua showed a case fatality rate of 0.022% for *P. vivax*, with severe cases occurring even in regions without *P. falciparum*. In South Korea, severe *P. vivax* infections occur despite good healthcare and

nutrition, indicating the parasite alone can cause life-threatening illness. Baird emphasized that *P. vivax* has a strong tropism for extravascular erythropoietic tissues, including the bone marrow, spleen, and liver, making peripheral parasitaemia an unreliable indicator of severity. This hidden biomass correlates with severe disease, challenging conventional diagnostics. Baird also discussed that our current methods fail to detect *P. vivax* in deep organs, likely underestimating its burden. He further added that the parasite's preference for immature reticulocytes suggests that stress erythropoiesis may expand its biomass dangerously. Until better diagnostic tools emerge, our understanding of *P. vivax*'s global impact remains incomplete, requiring a reassessment of its burden and consequences.

**Session 2 – Unveiling Plasmodium vivax dynamics: transmission, genomics & surveillance**

**Tanu Jain** (National Centre for Vector Borne Diseases Control – NCVBDC, India) discussed India's progress towards malaria elimination, along with other diseases like Kala Azar and Lymphatic Filariasis. She reviewed malaria elimination efforts from 2015 to 2023, addressing challenges in different categories. Jain presented the National Strategic Plan for Malaria Elimination (2023-2027), which emphasizes four key strategies: transforming surveillance, ensuring universal access to diagnosis and treatment, enhancing malaria control measures, and accelerating the path to malaria-free status, highlighting an 80.5% reduction in malaria cases and a 78.3% reduction in deaths. Jain also pointed out the challenges of controlling *Plasmodium vivax* malaria, with an 80% relapse rate due to the hypnozoite stage. She discussed diagnostic methods, including microscopy, rapid diagnostic tests (RDTs), and new tools like tafenoquine and G6PD testing. Jain also underscored the critical role of grassroots health workers in achieving malaria elimination.

**Leanne Robinson** (Burnet Institute, Australia) highlighted the global burden of *Plasmodium vivax* malaria, noting its dominance in the Asia-Pacific and South America. The hidden burden of *P. vivax* persists despite declining malaria cases, with 80% of infections resulting from relapses and many remaining undetectable due to current diagnostic limitations. As prevalence decreases, a growing proportion of infections become sub-patent, with 60-80% falling below the detection limits of microscopy and rapid diagnostic tests (RDTs), especially in low-transmission areas. To tackle *P. vivax* challenges, strategies include next-generation RDTs with fivefold improved sensitivity, G6PD-guided 7-day high-dose primaquine (PQ7) and tafenoquine (TQ) treatment for safe radical cure, and *P. vivax* serological testing and treatment (Pv SeroTAT) for detecting hypnozoite reservoirs. Studies like SCOPE, TRUST, ARCTIC, and EFFORT support policy-driven implementation, highlighting feasibility, cost-effectiveness, and public health benefits in co-endemic settings.

**Harsha Kota** (Indian Institute of Technology Delhi – IIT Delhi, India) presented a study on the impact of climate change on malaria incidence, focusing on developing an accurate malaria forecast model for Goa, a historically endemic region with significant case reduction over the past decade. Using data from 2010–2019 from the National Centre for Vector Borne Diseases Control (NCVBDC) and ECMWF RE Analysis V5, temperature and precipitation variations in North and South Goa were analyzed. The study applied seasonal Mann-Kendall tests to identify strong climate trends and Pearson's correlation to assess climate prediction accuracy. In forecasting, time series models like ARIMA and SARIMA, as well as machine learning models such as Support Vector Machine, Random Forest, and XGBoost, alongside hybrid models combining ARMA and machine learning methods, were used. The findings revealed that extreme weather events had a greater impact on malaria incidence than standard weather conditions. Kota emphasized that history-informed machine learning models performed best in forecasting malaria and highlighted the importance of integrating

climate data into predictive models to improve malaria forecasting accuracy and strengthen proactive health measures.

**Sanjay Kochar** (Sardar Patel Medical College, India) presented a study from the Bikaner district, Rajasthan, India, investigating the influence of asymptomatic and submicroscopic *Plasmodium vivax* infections on elimination efforts. Out of 2,325 individuals tested for *P. falciparum* and *P. vivax* using rapid diagnostic tests (RDTs), 131 tested negative. Polymerase chain reaction (PCR) of these negative samples identified *P. vivax* in three cases, with no *P. falciparum* detected. Peripheral blood smear identified gametocytes in a single sample, necessitating a thorough examination of over 200 high-power fields. These findings highlight the limitations of the current diagnostic techniques in identifying low-density infections. Kochar concluded by emphasizing the need for improved diagnostic tools, enhanced surveillance, and targeted policy interventions.

**Session 3 – Rethinking Relapse: In vivo Studies & Human Challenge Models (1/2)**

**Jean Popovici** (Institut Pasteur, Cambodia) presented a study investigating *Plasmodium vivax* relapses in Cambodia, focusing on hypnozoite regulation and reactivation. The study highlighted geographic variation in relapse timing, with 50% of relapses occurring within 4 weeks in Thailand and 60% in Cambodia. A clinical trial compared primaquine (PQ) doses of 3.5 mg/kg and 7.0 mg/kg over 14 days, revealing that a higher dose was necessary to prevent recurrence within 90 days. Popovici noted that no evidence of artesunate failure was observed, as recurrence clearance was as rapid as initial infections. Whole-genome sequencing (WGS) of *P. vivax* is ongoing to further investigate relapse mechanisms. The study also identified a consistent increase in reticulocyte count 13-14 days before relapse, suggesting a link between erythropoiesis and hypnozoite activation. These findings, Popovici explained, provide new insights into *P. vivax* relapse patterns and potential biomarkers, which may help refine treatment strategies and improve malaria control efforts.

**Kavitha Saravu** (Kasturba Medical College, India) discussed the clinical presentations of acute malaria and the challenges associated with differentiating *P. vivax* malaria from *P. falciparum* based solely on clinical features. She summarized the World Health Organization (WHO) criteria and various definitions for severe vivax malaria, accompanied by a case study highlighting severe vivax malaria. Key complications of malaria include cerebral malaria, renal failure, lung complications (such as ARDS), and severe anemia. Notably, more than 50% of cerebral vivax malaria cases and over 70% of renal manifestations have been reported from India. In a series of postmortem examinations of malaria cases, lung complications were found to be the most common findings. Saravu emphasized the urgent need for new definitions and criteria for vivax malaria in clinical practice. Additionally, she identified the prevention of vivax malaria and its potential relapses in children and pregnant women as a top priority.

**Chetan Chitnis** (Malaria Parasite Biology and Vaccines Unit, Institut Pasteur, France) discussed *P. vivax* vaccine candidates and the current challenges. Chitnis emphasized the importance of erythrocyte-binding proteins. Specifically, sulfated Y41 plays a crucial role in the binding assay of *P. vivax* Duffy Binding Protein (PvDBP)-II with the Duffy antigen receptor for chemokines (DARC). In a trial examining the efficacy of the PvDBP II vaccine in protecting against *P. vivax* blood-stage challenges, a parasite multiplication rate (PMR) reduction of 53% was observed in volunteers who received the PvDBP II vaccine using a delayed dosing regimen of 0, 1, and 14 months. However, no significant impact was noted in the group that received the regimen of 0, 1, and 2 months. Other immune correlates: FcγR and C1q binding

suggest that antibody-dependent cellular mechanisms and complement activation may also contribute to protection.

**Session 4 – Rethinking Relapse: In vivo Studies & Human Challenge Models (2/2)**

**Kamala Thriemer** (Menzies School of Life Sciences, Australia) presented a study on *Plasmodium vivax* malaria relapse prevention in Ethiopia, Pakistan, Indonesia, and Cambodia. Patients received schizontocidal treatment with either high-dose primaquine (PQ7 – 7 mg/kg for 7 days), a single dose of tafenoquine (TQ), or low-dose primaquine (PQ14 – 3.5 mg/kg for 14 days), all unsupervised. PQ7 and TQ were more effective than PQ14 over six months. TQ was effective with chloroquine in Pakistan and Ethiopia, artesunate-pyronaridine in Cambodia, and DHA-piperaquine in Indonesia. No hemolysis occurred with PQ7 or TQ in patients with >70% G6PD activity, ensuring safety and tolerability.

**Maria Ome-Kaius** (Papua New Guinea Institute of Medical Research – PNG IMR, Papua New Guinea) presented the SCOPE study aimed at evaluating the feasibility of point-of-care G6PD testing combined with a short course of PQ for the radical treatment of *P. vivax* malaria. The study is divided into two stages. In Stage 1, a safety review of PQ treatment was conducted at two clinics in Indonesia and two in PNG. Stage 2 will assess the feasibility and cost-effectiveness of the intervention at 6 clinics in Indonesia and 4 clinics in PNG, scheduled for 2025. The rates of adverse events of special interest (AESI) and serious adverse events (SAE) were generally lower than those reported in previous PQ clinical trials as well as the overall risk of hospitalization. She concluded that findings from both stages will help inform Ministry of Health consultations on updating vivax malaria guidelines and scaling up interventions.

**Rintis Noviyanti** (Eijkman Research Centre for Molecular Biology, Indonesia) presented a study assessing a newly developed microhaplotype assay on samples from a therapeutic efficacy study conducted in Timika, PNG, a region with high malaria transmission. Noviyanti explained that the assay generates identity by descent (IBD) values, which quantify the proportion of shared DNA fragments between parasites. An IBD of 100% indicates a clonal relationship, 50% suggests sibling parasites and 25% corresponds to half-siblings. Among 84 analyzed samples, the study identified one case with approximately 100% IBD, likely representing a relapse, and another with ~6.25% IBD, suggesting a possible reinfection. These findings highlight the utility of microhaplotype assays in distinguishing relapse from reinfection, contributing to more precise malaria surveillance and control strategies.

**Madan Mohan** (Vector Borne Diseases, Health & Family Welfare Department, India) presented recent trends in *P. vivax* malaria and the challenges associated with it in a district predominantly affected by *P. falciparum* malaria. Secondary epidemiological data from routine malaria surveillance and the Durgama Anchalare Malaria Nirakaran (DAMaN) system were collected and analyzed. In certain *P. falciparum*-dominated sub-centers under the DAMaN Mass Screening and Treatment initiative, *P. vivax* cases surged to over 90% in 2024. By September 2024, the overall incidence of malaria had risen fivefold, with 1,841 cases reported compared to just 349 cases in 2023. To address this unprecedented *P. vivax* increase in the *P. falciparum*-endemic regions, new approaches such as TrueNat®️ could be considered for detecting sub-microscopic *P. vivax* malaria at the primary healthcare level. Additionally, administering a single dose of TQ for a radical cure may be beneficial for the national malaria program.

**Pubudu Chulasiri** (Ministry of Health, Sri Lanka) studied imported *P. vivax* cases from 2013 to 2023, documenting, treating, and monitoring them for relapses over a 12-month period.

His analysis showed that *P. vivax* accounted for 40.5% of imported malaria cases, with its proportion decreasing from 54.7% in 2013 to 6.45% in 2023, likely reflecting India's declining malaria burden. Despite effective treatment, three cases experienced relapses, indicating a delayed illness onset compared to *P. falciparum*. Chulasiri also noted the higher prevalence of *P. vivax* gametocytes, which increases transmission potential and poses a greater risk of re-establishment than *P. falciparum*. These findings highlight the challenges in eliminating *P. vivax*, particularly in regions receiving imported cases. They also reinforce the importance of continued surveillance, effective relapse prevention, and international collaboration to prevent resurgence in malaria-free areas.

**Stephanie Zobrist** (Diagnostics – PATH, United States) presented a study that assessed the STANDAR G6PD test for identifying severe anaemia in malaria case management. The STANDARD test was conducted on capillary specimens, alongside a comparator point-of-care hemoglobin test: the HemoCue 201+ (HemoCue AB, Sweden). A total of 951 participants were enrolled, 466 with available test results. Nearly one-quarter (24%) of the participants had moderate or severe anemia. When compared to the reference complete blood count (CBC) assay, the STANDARD G6PD test showed similar performance and patterns of misclassification as the HemoCue, when used in a controlled clinical evaluation. No true cases of severe anaemia were misclassified by the STANDARD Test but it tended to overestimate moderate and severe anaemia. HemoCue misclassified two severe anemia cases as moderate but showed a higher overall positive predictive value (PPV) for severe anemia. The STANDARD G6PD test reliably identifies severe cases. Zobrist emphasized its importance in remote areas with limited assessment options.

# Day 2: Thursday, 13ᵗʰ February 2025

**Special Invited Session**

**Andrea Bosman** (Global Malaria Programme, World Health Organization – WHO, Switzerland) discussed the new WHO recommendations regarding near-patient G6PD tests and radical treatment for *Plasmodium vivax* and *P. ovale*. In G6PD testing studies, severe adverse events, mainly severe hemolysis, occurred in 11.2% of deficient individuals but were nearly absent in those with normal levels. Bosman emphasized the WHO recommendations of using semi-quantitative near-patient tests with fixed standards for deficient, intermediate, and normal G6PD activity to help inform the administration of specific treatment regimens. Bosman highlighted the practical guidance of near-patient G6PD testing in managing the anti-relapse treatment of *P. vivax* and *P. ovale* with primaquine (PQ) and tafenoquine (TQ). The dosing recommendations are as follows: 1 mg/kg/day of PQ for 7 days or a single dose of TQ should only be administered to individuals whose G6PD activity is above the threshold corresponding to greater than 70% of normal levels. For those with G6PD activity above the threshold of 30% of normal levels, 0.5 mg/kg/day of PQ for 14 days, or 0.5 mg/kg/day for 7 days, can be given to prevent relapses of *P. vivax* and *P. ovale*. Since WHO recommendations are a living document, dosing guidelines for radical cure, such as for malaria caused by *P. vivax* and *P. ovale*, can change based on new evidence and ongoing research.

**Session 5 – Targeting vivax: Decoding Host-Parasite Interactions**

**Manoj Duraisingh** (Harvard T.H. Chan School of Public Health, United States) emphasized that a blood-stage vaccine is a potential tool for malaria elimination, as vaccination with *Plasmodium vivax* Duffy-binding protein (DBP) has been shown to inhibit parasite growth during controlled human malaria infection. However, challenges persist, such as tissue sequestration and reduced accessibility to drugs in younger reticulocytes. The study presented explored alternative invasion pathways, essential ligands, and critical receptors using *P. knowlesi* models. The hypothesis posits that targeting ligand-receptor interactions could lead to effective therapeutic strategies like vaccines or monoclonal antibodies. Parasite biomass, associated with disease severity, indicates sequestration in tissues like bone marrow and spleen, where parasites preferentially invade younger reticulocytes. Screening revealed that reticulocyte binding-like (RBL) and erythrocyte binding-like (EBL) superfamilies mediate host cell selection, with *P. vivax* relying on DARC and transferrin receptor (TFRC) for invasion. Antibody inhibition assays in *P. knowlesi* and *P. cynomolgi* identified potential antigen targets. Duraisingh concluded that targeting multiple pathways could improve vaccine efficacy and support the development of resistance-proof therapeutics.

**Vineeta Singh** (Indian Council of Medical Research – National Institute of Malaria Research – ICMR-NIMR, India) talked about the molecular markers and genetic diversity of *Plasmodium vivax* infections in India, highlighting challenges in malaria control. India contributes nearly half of the global *P. vivax* burden, with high genetic variability, multiple malaria vectors, and varying relapse phenotypes that complicate elimination efforts. Singh analyzed drug resistance mutations in *Pvmdr1, Pvdhfr, Pvdhps, Pvk12*, and *Pvcrt-o* using PCR and sequencing. A phylogenetic analysis mapped genetic diversity among Indian isolates, offering insights into regional variations. At Safdarjung Hospital, a large tertiary care hospital in Delhi, Singh's study found no correlation between thrombocytopenia or cytokine levels and parasitemia, while severe *P. vivax* cases rose from 32.8% in 2020 to 65.7% in 2024. The study highlighted the increasing severity of *P. vivax* infections and the urgent need for intervention. Singh emphasized the need for molecular surveillance, improved diagnostics, and targeted interventions to combat *P. vivax* malaria effectively and prevent its resurgence.

**Carmen Fernández-Becerra** (Barcelona Institute for Global Health – ISGlobal and Germans Trias i Pujol Research Institute – IGTP, Spain) discussed insights into the mechanisms of human malaria pathology through the study of extracellular vesicles (EVs). She supported the idea that there are subpopulations of *P. vivax*-adherent parasites present in the microvasculature of the human spleen. The cytoadherence of *P. vivax*-infected reticulocytes to human spleen fibroblasts (hSFs) is facilitated by plasma-derived EVs. Immunofluorescence analysis showed that the *P. vivax* spleen-dependent protein 1 (PvSDP1) is located on the surface of infected red blood cells in a transgenic line, a finding that was later confirmed in natural infections. Plasma-derived EVs from individuals infected with *P. vivax* (PvEVs) significantly enhanced the cytoadherence of the 3D7_PvSDP1 transgenic line to hSFs, and this binding was inhibited by the presence of anti-PvSDP1 antibodies. Single-cell RNA sequencing of hSFs treated with PvEVs revealed an increased expression of genes related to adhesion. This highlights the significance of parasite spleen-dependent genes and EVs from natural infections in establishing intrasplenic niches in *P. vivax*, posing a major challenge for malaria elimination.

**Chris Drakeley** (Armauer Hansen Research Institute – AHRI, Ethiopia and London School of Hygiene & Tropical Medicine – LSHTM, UK) presented a multisite study in Ethiopia examining factors influencing *Plasmodium vivax* transmission to mosquitoes. The study found that transmission is driven by parasite and gametocyte densities, with both symptomatic and asymptomatic infections contributing to the infectious reservoir. Recurrent infections remain highly infectious, further sustaining transmission. Additionally, mixed and heterologous infections complicate disease dynamics, while drug efficacy and immune responses play a role in transmission. Drakeley concluded that targeting both infection types with genetic and immunological insights is essential for malaria control and elimination.

**Ashis Das** (Birla Institute of Technology and Science – BITS-Pilani, India) presented the difference in the transcriptome profile of Plasmodium interspersed repeat (pir) genes in *Plasmodium vivax* cerebral malaria and hepatic dysfunction cases, namely hepatic dysfunction and cerebral malaria focusing on the vir genes, some of which have been postulated to be involved in cytoadherence. The study involved 23 patients, with 17 categorized as complicated cases (12 with hepatic dysfunction and 5 with cerebral malaria) and 6 as uncomplicated. Real-time qPCR confirmed the upregulation of two genes and the downregulation of two others. The upregulation of these vir/pvpir genes may be important in disease pathogenesis, particularly in their role in cytoadherence to host cell receptors.

**Session 6 – Immunology Unmasked: Natural Infections, Cryptic Reservoirs**

**Luzia H. Carvalho** (Molecular Biology and Immunology of Malaria – BMIM, Brazil) presented a study on *Plasmodium vivax* vaccine strategies, focusing on Duffy Binding Protein II (DBPII), a key protein for red blood cell invasion via the Duffy Antigen Receptor for Chemokines (DARC). While people in malaria-endemic areas develop antibodies against DBPII, only some produce broadly protective antibodies, limiting immunity. To address this, DEKnull variants were designed to target conserved DBPII regions, inducing stronger and broader immunity. Long-term studies show that some individuals maintain immune memory, while others lose it or fail to develop a response. Notably, IgM antibodies play a crucial role in malaria immunity, challenging the belief that IgG is the main protective antibody. While DBPII remains a promising vaccine target, its strain-specific nature reduces effectiveness. Carvalho concluded by stating that future vaccine strategies should focus on optimizing DEKnull-2 to enhance cross-strain protection, ensuring long-lasting immunity against diverse *P. vivax* variants in endemic populations.

**Hernando del Portillo** (Barcelona Institute for Global Health – ISGlobal and Germans Trias i Pujol Research Institute – IGTP, Spain) presented on the role of reticulocyte-derived extracellular vesicles (EVs) in establishing cryptic *Plasmodium vivax* infections within human bone marrow and spleen. Del Portillo's study employed organ-on-a-chip technology to replicate the bone marrow and spleen, demonstrating that EVs from malaria patients, but not from healthy individuals or *P. falciparum* patients, induced erythropoiesis and facilitated parasite migration to the bone marrow. High EVs concentrations were found in malaria patient plasma, suggesting a mechanism for parasite survival and persistence. Del Portillo's team successfully generated the erythroid lineage on the chip, with cell viability exceeding 95% after five days. Ongoing single-cell RNA sequencing and spatial transcriptomics aim to uncover the signaling pathways involved in these interactions. Additionally, a humanized mouse model using CD34+ cells and fetal tissues, providing insights into parasite dynamics and tissue-specific growth, was also developed. The findings highlighted the importance of bone marrow and spleen as reservoirs for malaria persistence and pointed to EV-mediated signaling as a potential target for therapeutic interventions.

**Flora Kano** (Molecular Biology and Immunology of Malaria – BMIM, Brazil) stated that the DEKnull-2 vaccine, designed by altering polymorphic residues, targets conserved B-cell epitopes, generating high-titer, stable antibodies. The study hypothesized that T follicular helper (Tfh) cells and B-cell interactions in persistent responders (PR) enhance protective antibody responses. A long-term follow-up in the Brazilian Amazon categorized individuals into persistent, temporary, and non-responders based on DBPII-erythrocyte binding inhibition. PR had more activated and Th2-like Tfh cells, providing stronger B-cell help. PR also had more Tbet+ atypical memory B cells, secreting IgG with superior binding and inhibitory activity than classical memory B cells. Kano underscored the role of these immune subsets in a stable, strain-transcending antibody response and their relevance for next-generation DBP vaccine development as part of a multi-stage, multivalent malaria vaccine strategy.

**Steven Kho** (Menzies School of Health Research and Charles Darwin University, Australia) presented the first prospective study quantifying splenic biomass in acute *Plasmodium vivax* malaria. Due to limited spleen histopathology in untreated *P. vivax* cases, Kho compared blood markers in splenectomized and spleen-intact patients in Timika, Indonesia, using ELISA for plasma lactate dehydrogenase (LDH) and microscopy for circulating *P. vivax* biomass. Kho found that circulating parasites were higher in patients without a spleen, while total parasite biomass was greater in those with an intact spleen, indicating a large hidden reservoir. A case study further confirmed these findings, highlighting the spleen's role in *P. vivax* infections.

**Upninder Kaur** (Post Graduate Institute of Medical Education & Research – PGIMER, India) investigated the role of polymorphisms in *TLR1*, *TLR6*, and *TLR9*, with *TLR9* T-(1486)-C in the promoter region being the most frequent, particularly in complicated cases. Cytokine levels (IFN-γ, TNF-α, IL-6, IL-10) measured via ELISA showed a significant link between *TLR9* polymorphisms and cytokine production, with lower IL-10 and IFN-γ levels in *TLR9* (1486T) carriers, indicating an altered immune response. These findings highlighted *TLR9*'s role in immune modulation, influencing disease severity and offering potential targets for vaccine development and treatment strategies.

**Session 7 – Bridging the Gap: Advanced Model Systems for *Plasmodium vivax***

**Anthony Ruberto** (University of Georgia, United States) discussed the challenges and opportunities in studying *Plasmodium vivax* liver stages, particularly hypnozoites, to aid drug discovery. The challenges include the lack of continuous blood-stage parasite cultures,

limited genetic tools, and difficulties in identifying the mechanism of action (MoA) of compounds targeting hypnozoites. Additionally, the host hepatocyte signal may mask the hypnozoite protein signal, making it harder to detect. Chemoproteomic profiling revealed disruptions in proteins related to biological processes in infected hepatocytes. The development of an *in vitro P. vivax* liver stage model is crucial for testing compounds that target hypnozoites and understanding both parasite and host responses. Ruberto highlighted the success of the first non-8-AQ hit project for *P. vivax* hypnozoites, which established the foundation for further research and potential drug development strategies for anti-hypnozoite therapies.

**Melanie J. Shears** (University of Washington, United States) discussed the development of humanized mouse and nonhuman primate models for studying *Plasmodium vivax*, focusing on vaccine and monoclonal antibody (mAb) development. The FRG humanized liver mice model, engrafted with human hepatocytes, supports liver stages and hypnozoite formation. Using PvCSP mAbs, researchers observed reduced schizont formation and relapse rate. In FRG-NOD mice, depleting macrophages and neutrophils enabled complete blood-stage cycles and gametocyte observation, with PvDBP mAbs reducing parasitemia by over 95%. HIS-HEry mice, with human hematopoietic stem cells, offer insights into bone marrow and transmission-blocking strategies. Shears also highlighted the macaque model with *P. cynomolgi*, a close relative of *P. vivax*, which mimics relapse and reticulocyte preferences. The novel Pc-PvCSP challenge line facilitates testing of PvCSP-based vaccines, while the PbViVac transgenic parasite, capable of infecting human liver cells but not RBCs, shows promise for vaccine development. Shears concluded that these models are essential for advancing therapeutic interventions against *P. vivax* malaria.

**Robert W. Moon** (London School of Hygiene and Tropical Medicine – LSHTM, United Kingdom) presented a study using transgenic *Plasmodium knowlesi* to explore the role of *P. vivax* duffy-binding protein (DBP) and reticulocyte binding protein 2b (RBP2b) in erythrocyte invasion. Both unmodified *P. knowlesi* and modified lines with the *P. vivax* DBP orthologue showed preferential binding to one allele, with a stronger effect in *P. knowlesi*. This research offers insights into DBP-based vaccine design for both species. The study also enabled the characterization of parasite-derived full-length RBP2b, aiding in the validation of human monoclonal antibodies. Using orthologous replacement techniques for non-Laveranian parasites like *P. vivax* continues to be a valuable tool for studying invasion mechanisms, identifying vaccine antigens, and validating inhibitory antibodies. The inclusion of PvRBP2b is crucial for understanding *P. vivax* invasion, especially its restriction of reticulocytes.

**Jenna Oberstaller** (University of South Florida, United States*)* highlighted the importance of understanding *Plasmodium vivax* biology for malaria control, emphasizing the parasites' genetic diversity. The study utilized *P. knowlesi* as a model for genome-wide functional characterization through piggyBac transposon mutagenesis, saturating 98% of genes to assess their essentiality for blood-stage growth. Results revealed significant metabolic differences despite conserved genes, indicating species-specific adaptations for survival in different hosts. For instance, genes essential in *P. falciparum* were dispensable in *P. knowlesi*, demonstrating metabolic rewiring. The study also identified parasite-specific pathways involved in intracellular organization, receptor signaling, and nutrient acquisition. Validation experiments showed that genes like APkicm1 and APkrp6 differ in their importance across species. Oberstaller concluded that such genetic rewiring affects drug target efficacy, underscoring the necessity of testing antimalarial drugs across multiple malaria species to ensure broad-spectrum effectiveness. Ongoing work aims to further dissect these metabolic pathways for potential therapeutic interventions.

**Jeremy Salvador** (Institut Pasteur du Cambodge, Cambodia) highlighted that *P. vivax* primarily resides in the bone marrow (BM), with low parasitemia in peripheral blood but high density in organs such as the spleen and BM. The BM provides a unique environment with hypoxia, mesenchymal stem cells (MSC), and soluble factors like hematopoietic growth factors and metabolites. The study hypothesized that specific BM conditions support *P. vivax* survival and growth. Short-term high-oxygen exposure did not significantly impact parasite survival, development, or oxidative stress markers *in vitro*. Further, experiments with MSCs and a conditioned medium showed no notable effect on parasite survival. However, a high concentration of BM serum tended to reduce *P. vivax* viability. Preliminary results suggest that while hypoxic conditions might reduce oxidative stress, the BM microenvironment is not essential for *P. vivax* development *in vitro*. The study continues with additional biological samples to confirm these findings.

**Clara Champagne** (Swiss Tropical and Public Health Institute – Swiss TPH, Switzerland) evaluated *Plasmodium vivax* elimination in Honduras using mathematical modeling to assess intervention strategies in *Gracias a Dios*, the most endemic region. The study considered improving case management through increased healthcare access, reducing treatment delays, and enhancing primaquine adherence, along with minimizing imported cases from Nicaragua. The presentation underscored that increasing access to care and better adherence to primaquine significantly contribute to reducing malaria cases. Champagne stressed the importance of reducing case importation for successful malaria elimination and emphasized the need to strengthen health systems and enhance cross-border collaboration to achieve this goal in Honduras.

## Session 8 – Duffy Enigma: Epidemiology & Invasion Mechanism

**Isaac Quaye** (Regent University College of Science and Technology, Ghana) presented research demonstrating *Plasmodium vivax* infection in Duffy-negative individuals, challenging the notion of their inherent resistance. The study demonstrated that *P. vivax* bypasses Duffy receptor dependency by selectively invading CD71+ reticulocytes, which are highly enriched in the spleen and bone marrow. Furthermore, the absence of atypical chemokine receptor 1 (ACKR1) in Duffy-negative individuals disrupts neutrophil homeostasis, leading to reduced immune cell counts and potentially heightened susceptibility to infections. *P. vivax* exploits immunosuppressed niches in the bone marrow and spleen, establishing reservoirs that support persistence and transmission. These findings redefine malaria epidemiology, demonstrating that Duffy negativity does not confer immunity against *P. vivax*. Quaye concluded that understanding ACKR1's role in immune regulation and parasite invasion is crucial for refining malaria control strategies, particularly in high-prevalence regions such as Africa.

**Amy Ibrahim** (London School of Hygiene and Tropical Medicine – LSHTM, UK) presented a study on Duffy-negative erythrocyte invasion in *Plasmodium knowlesi*. The *P. knowlesi* A1-H.1 parasite line was exposed to a mix of Duffy-negative and Duffy-positive erythrocytes and after six months, a stable *in vitro* line (Z1H1) was established with Duffy-negative erythrocytes. Genome sequencing revealed that the invasion pathway is independent of the Duffy antigen receptor for chemokines (DARC) binding and is driven by genomic recombination between the DBPα and DBPγ genes. This study demonstrates *P. knowlesi's* ability to invade Duffy-negative erythrocytes, with implications for parasite control. It also offers a new tool for studying Duffy-negative invasion in *P. vivax*, which lacks a stable *in vitro* culture system.

**Johnsy Mary Louis** (Kangwon National University, South Korea) presented a study on identifying and characterizing how Duffy binding ligands and receptors facilitate invasion in non-Laverania *Plasmodium* species. The study confirmed that Duffy Binding Protein (DBP)-DARC interactions are critical for invasion in *P. vivax*, *P. knowlesi*, and *P. cynomolgi*. Co-immunoprecipitation (Co-IP) results showed species-specific binding: PvDBP-RII interacts with human DARC, PcyDBP1-RII with human DARC, and PkDBPα-RII with both human and monkey DARC. Structural analysis revealed mutations in PkDBPα-RII that affect affinity and key residues like D24 in DARC influence binding. These findings suggest that specific DBP residues affect binding affinity, host selection, and interaction preferences, which could inform vaccine design for *P. vivax* and related species.

**Ababio Grace K** (Regent University College of Science and Technology, Ghana) presented a study on Duffy gene polymorphisms in Ghana, Namibia, and Zambia. The research identified -67T>C and 125 G>A mutations, with uninfected individuals showing a fixed Duffy-negative genotype, while infected individuals displayed variations. This suggests *P. vivax* may use co-infections and alternative invasion pathways. Grace K also highlighted that *Plasmodium* infections may trigger genetic changes near codon 125, while ACKR1 may also impact hematopoiesis and immune responses, underscoring the need for further research on parasite-host interactions.

# Day 3: Friday, 14th February 2025

**Session 9 – Diagnostics Unlocked: Omics, Single-Cell & Serological Advancements**

**Liliana Mancio-Silva** (Institut Pasteur, France) discussed the unveiling biology of *Plasmodium vivax* hypnozoites with single-cell transcriptomics. Mancio-Silva highlighted gaps in understanding the molecular basis of dormancy, the need for better *in vitro* systems, and the importance of identifying specific biomarkers for hypnozoites. Using micropatterned co-cultures (MPCCs) of human hepatocytes, her team employed single-cell transcriptomics to uncover a 10-gene hypnozoite transcriptional signature. This research revealed that hypnozoites rely on transcriptional and translational repression to maintain dormancy and that proteolytic activity and RNA-binding proteins are essential for this process. Mancio-Silva also noted the role of the hepatocyte circadian rhythm in regulating the permissiveness to infection, suggesting that the timing of infection may influence parasite dormancy. These findings offer new insights into the host-parasite interactions that govern relapse and could lead to novel biomarkers and drug targets to prevent malaria relapse.

**Ivo Mueller's** (Walter and Eliza Hall Institute of Medical Research – WEHI, Australia) talk focused on a novel serological approach for *Plasmodium vivax* testing and treatment, called PvSeroTAT. Muller discussed the challenge of hidden *P. vivax* infections, which hinder malaria elimination, and the importance of targeting this hidden reservoir. Serological markers can identify both current and past infections, with antibody responses helping to classify individuals with recent exposure. Mueller highlighted antigen genetic diversity's limited impact on classification and antigen expression optimization for better efficacy. PvSeroTAT, a novel intervention, reduces overtreatment and resource use compared to mass drug administration (MDA). The strategy significantly impacts low transmission settings, reducing the risk of recurrent *P. vivax* infections by 53%. Seropositive individuals were found to be 7.5 times more likely to experience recurrence, while untreated seropositive individuals had a 19-fold higher likelihood of relapse. The approach includes risk stratification to target interventions effectively, alongside monitoring and evaluating ongoing malaria elimination programs.

**Erica Pasini** (Biomedical Primate Research Center, Netherlands) presented a study aimed at identifying new diagnostic targets for *Plasmodium vivax* hypnozoites. The study compared *P. cynomolgi* (a hypnozoite-forming parasite) and *P. knowlesi* (a non-hypnozoite-forming parasite) using optimized liver-stage cultures for metabolomics analysis. The study detected 567-653 metabolites in *P. cynomolgi* cultures and 513 metabolites in *P. knowlesi*. Nineteen hypnozoite-related metabolites were detected, and pathway analysis confirmed their relevance. The next step involves down-titrating *P. cynomolgi* infection in rhesus monkeys, collecting serum samples at different time points, and applying a sensitivity-based metabolomics approach to validate these metabolites for developing a rapid diagnostic test for hypnozoite infection in *P. vivax*.

**Zach Popkin-Hall** (University of North Carolina, United States) presented a study on high-throughput genotyping of *Plasmodium vivax* in the Peruvian Amazon using molecular inversion probes (MIPs). The study designed four MIP panels targeting geographically differentiating single nucleotide polymorphisms (SNPs), rare and common neutral SNPs, and genes of biological interest, including erythrocyte invasion ligands and antimalarial resistance orthologs. The panels were applied to 866 *P. vivax* infections, successfully genotyping 685 samples. Key findings included the identification of clonal transmission networks, copy number variation in key proteins, mutations in antimalarial resistance genes, and balancing selection in potential vaccine targets. Popkin-Hall emphasized the study's

importance, showing that MIPs can comprehensively assess genomic epidemiology in *P. vivax*, offering valuable insights for malaria control and vaccine development.

**Session 10 – Climate Change & Malaria: An Emerging Threat**

Michael White (Institut Pasteur, France) delivered a comprehensive talk on mathematical modelling for measuring radical cure efficacy in *Plasmodium vivax* malaria. White explained that different perspectives influence efficacy measurements: biologists focus on hypnozoite clearance, clinical trial lists assess recurrence reduction, modellers estimate relapse rate reduction, and patients seek symptom resolution. White emphasized that transmission intensity affects efficacy estimates and highlighted challenges in distinguishing relapses from reinfections. He outlined three approaches to address this: relocating patients to areas without reinfection, genotyping recurrent infections, and applying mathematical models to clinical trial data. Using Bayesian statistical methods, White's team analyzed data from Ethiopia, demonstrating that adherence to primaquine (PQ) and higher doses significantly improve radical cure effectiveness. White stressed the need for operational studies, including cluster randomized trials like PvSTATEM, to evaluate population-level impacts. White concluded by advocating for safe, widespread PQ use with G6PD testing to reduce malaria transmission and support elimination efforts, offering valuable insights into future strategy.

**Isabel Byrne** (London School of Hygiene and Tropical Medicine – LSHTM, UK) presented research on the spatial heterogeneity of *Plasmodium vivax* and its implications for randomized controlled trials (RCTs), using the PvSTATEM study as a case example. Byrne emphasized the influence of climate change on malaria transmission, noting *P. vivax*'s broader environmental range due to temperature and elevation sensitivity. Byrne explained that cluster randomized trials (cRCTs), while the gold standard for population-level intervention evaluation, often fail to account for environmental and spatial factors during randomization. Her team applied geostatistical simulations using household PCR prevalence data from Ethiopia and Madagascar to assess spatial heterogeneity's impact. Byrne demonstrated that vegetation and elevation significantly influenced malaria prevalence, with spatial models outperforming non-spatial approaches, and emphasized the need to consider environmental factors when interpreting trial results. Byrne explained that the study simulates environmental conditions to test randomization strategies and improve interventions, highlighting the need to consider climate-driven shifts in malaria transmission for effective control and elimination.

**Sagar Pandhare** (Indian Institute of Technology Bombay – IIT Bombay, India) presented a study on modeling malaria trends in Dhalai, Tripura, India, using climate data. Three approaches were explored for analyzing temporal patterns: additive decomposition, holt-winters exponential smoothing, and seasonal local regression; finding that seasonal regression provided the most accurate forecasts. The findings revealed that malaria cases peak during the monsoon, with major outbreaks in 2014 and high cases in 2018-2019. Ridge regression identified temperature, normalized difference vegetation index (NDVI), and humidity as key factors influencing malaria trends. Pandhare emphasized the importance of continuous monitoring and predictive modeling to enhance malaria control and facilitate early prevention strategies.

**Closing remarks**

The 9th International Conference on *Plasmodium vivax* Research 2025 (ICPvR 2025) successfully concluded in Ocean Spray, Puducherry on February 14, 2025, after three days of

impactful discussions and research presentations. The feedback received from the delegates indicated that the Conference was rich in scientific content with ample opportunities for cross-talks and networking between groups working in different domains. The closing ceremony recognized leading malaria researchers, oral sessions and poster participants, and key contributors, with awards presented for best turbo talks and poster presentations, along with special acknowledgments for young and distinguished researchers. **Dr. Manju Rahi**, Director, Indian Council of Medical Research–Vector Control Research Centre (ICMR-VCRC) and Convenor of ICPvR 2025 expressed gratitude to the international and national scientific committees, sponsors (BMGF and ANRF) and organizing teams from ICMR-VCRC for their support and thanked all participants for their contributions. The conference officially concluded with the announcement of ICPvR 2027, which will be held in Peru, continuing efforts toward malaria elimination. With renewed enthusiasm, participants bid farewell, looking forward to future collaborations and advancements in malaria research.

# Annex 1: Turbo Talks

*\* These summaries were provided by the organizing committee.*

**Session 2:**

**Amala R** (ICMR - Vector Control Research Centre, Puducherry, India) presented a study identifying 11 Indian districts as persistent Plasmodium vivax malaria hotspots, crucial for national elimination efforts. Utilizing 24 years of malariometric data, they pinpointed these areas, including Kolkata Municipal Corporation, based on consistently high case numbers and slide positivity rates. The study evaluated the impact of the 14-day primaquine (PQ-14) regimen and long-lasting insecticide-impregnated nets (LLINs) on P. vivax cases. Nationally, both interventions significantly reduced P. vivax cases. However, Kolkata remained a challenge, maintaining a disproportionately high burden. The research highlighted P. vivax's critical role in hindering malaria elimination, despite P. falciparum's larger overall contribution and emphasized the need for targeted interventions in these hotspots, including enhanced vector control, directly observed treatment and strengthened surveillance, to support India's national malaria elimination strategy.

**Cindy Chu** (Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit - LOMWRU, Lao PDR) presented data from a longitudinal cohort study of healthy volunteers previously treated with chloroquine for vivax malaria. Enrolled participants were given primaquine and followed until P. vivax recurrence. The study revealed a significantly lower overall incidence of vivax malaria after radical cure compared to historical data. Primaquine failure, defined as vivax recurrence within four months, was 75% less than predicted by a model using contemporary data and assuming constant hypnozoite burden. These results indicate that decreased transmission correlates with reduced hypnozoite burden, suggesting lower doses of 8-aminoquinolines may be sufficient in pre-elimination settings.

**Session 4:**

**Dalia Diaz-Delgado** (Institute of Tropical Medicine Antwerp - ITM, Antwerp, Belgium) presented a high-resolution transcriptome time course of the asexual blood stage of Plasmodium knowlesi A1H1, focusing on the schizont stage, to enhance its utility as a P. vivax model. Tightly synchronized P. knowlesi cultures were sampled at five time points (5, 14, 20, 24, 27 hpi), with schizont stages purified using Nycodenz gradients and sequenced after human globin mRNA depletion. Transcriptional profiles of invasion-associated gene families in P. knowlesi were explored, finding different expression profiles among them. The transcriptional patterns obtained were compared to available transcriptomic data from two P. vivax isolates, revealing similar intraerythrocytic expression profiles between ortholog genes, but with P. knowlesi genes often expressed later in the schizont stage. The study provided valuable data for understanding P. knowlesi gene expression during the asexual blood stage, which will serve for assessing similarities of expression between ortholog genes with P. vivax, therefore helping refining the use of P. knowlesi for studying P. vivax genes in vitro.

**Ashwarya Kumari Sihag** (ICMR-NER RMRC, Dibrugarh, Assam, India) presented the findings from Dr Ipsita Pal Bhowmick's group work in Tripura, India, highlighting the rise of P. vivax cases, contrasting with the previous P. falciparum dominance. Active and passive surveillance, combined with molecular testing, revealed a significant hidden burden of asymptomatic and submicroscopic P. vivax infections at the beginning, followed by the increase of P. vivax symptomatic cases from late 2021 onwards. This phenomenon was

observed at all levels ranging from Hamlets, Subcentres, and PHCs to different Districts. Mutations in Pvmdr-1 genes raise the possibility of drug resistance which can have contributing factors to P. vivax rise along with other possibilities. The study highlights the importance of molecular surveillance to understand P. vivax dynamics and address potential drug resistance.

**Session 5:**

**Sophia M. DonVito** (London School of Hygiene and Tropical Medicine - LSHTM, United Kingdom) employed transgenic Plasmodium knowlesi to dissect the roles of P. vivax Duffy binding protein (DBP) and reticulocyte binding-like protein 2b (RBP2b) in erythrocyte invasion. Both unmodified P. knowlesi and modified lines expressing P. vivax DBP exhibited preferential binding to the FyB allele of DARC, with a stronger effect observed for P. knowlesi DBP. This research provides valuable insights for DBP-based vaccine design. Additionally, orthologous replacement techniques facilitated the characterization of full-length PvRBP2b, aiding in the validation of monoclonal antibodies. These studies are crucial for understanding P. vivax invasion mechanisms, particularly its reticulocyte restriction, and demonstrate the continued utility of orthologous replacement in live parasite studies.

**Maisa Araujo** (Fiocruz Rondônia, North of Brazil) demonstrated a novel vector control strategy targeting Plasmodium vivax transmission in *Anopheles darlingi*, the primary Amazonian vector. Using sugar bait treated with atovaquone (ATQ) in membrane feeding assays, the study revealed a significant reduction in infection prevalence and the elimination of oocysts in mosquito midguts. ATQ also decreased sporozoite prevalence and intensity. Importantly, mosquito survival remained unaffected over 14 days, though engorgement rates were lower in treated mosquitoes. These findings highlight ATQ's potential as a *P. vivax* transmission-blocking agent in *An. darlingi*, encouraging further exploration of similar compounds for vector control.

**Léa Baldor** (Institut Pasteur du Cambodge, Phnom Penh, Cambodia) presented evidence that Plasmodium vivax utilizes PvDBP gene amplification as a defense against host immunity. Their research revealed a significant correlation between high malaria prevalence and the prevalence of parasites with multiple PvDBP gene copies. Notably, individuals exhibiting strong binding inhibitory antibodies (BIabs) were more frequently infected with these multi-copy parasites. Their study showed that these amplified parasites can successfully infect hosts despite high BIab levels, demonstrating a clear in vivo protection mechanism. This suggests that PvDBP gene duplication allows parasites to evade naturally acquired immunity. Baldor emphasized the implications for vaccine development, highlighting the need to address this immune evasion strategy in future PvDBP-based vaccines.

**Session 6:**

**Gabriela Fernandes** (René Rachou Institute, Fiocruz Minas, Brazil) examined the immune response to EBP2, a novel P. vivax erythrocyte binding protein, in individuals with long-term malaria exposure in the Brazilian Amazon. A 15-year follow-up study involving 363 individuals demonstrated significantly higher anti-EBP2 IgG antibody levels compared to anti-DBPII, indicating a preferential immune response to EBP2. Antibodies against DBPII exhibited more rapid decay than those against EBP2. Preliminary cellular assays revealed a high frequency of EBP2-specific atypical B cells (Tbet+), supporting EBP2 as a promising candidate for a multi-antigen P. vivax vaccine.

**Katherine Torres** (Universidad Peruana Cayetano Heredia, Lima, Perú) presented findings from a comparative analysis of antibody responses (IgM, IgG, IgG1, and IgG3) against 34 P. vivax antigens in asymptomatic (n=27) and symptomatic (n=35) individuals from the Peruvian Amazon, compared to healthy controls (n=9). The study revealed a trend of higher IgG and IgG3 antibody responses in the asymptomatic group, enabling improved discrimination. Multiple comparison analysis identified P. vivax Sporozoite invasion-associated protein 2 (SIAP2, PVX_088860) as a key antigen for differentiating asymptomatic P. vivax infections in this region.

**Session 9:**

**Taruna Kaura** (Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India) presented the challenges of Plasmodium vivax elimination in Punjab, North India, despite low annual parasite incidence due to risk of reemergence because of regular influx of imported cases. The study highlighted difficulties in diagnosing as in low malaria endemic areas microscopists are less acquainted with malaria microscopy. Therefore, with the help of machine learning tools malaria microscopy can be strengthened. In this study, YOLOv8 algorithm was trained to identify the various blood stages of Plasmodium vivax and P. falciparum based on their morphology using Giemsa-stained thin peripheral blood films (PBF) of malaria-positive patients. It was found that the ML-based tool showed a sensitivity of 97.11%, specificity of 99.8%, and accuracy of 95.39% for the detection of malarial parasites and was able to differentiate between parasite species and stages. Thus, this innovative and cost-effective ML-based approach can improve malaria diagnosis and will ultimately strengthen the global progress towards malaria elimination.

**Paul Daly** (Vector-Borne Diseases and Tropical Public Health, Burnet Institute, Australia) presented their team's work on developing evidence-based guidelines for P. vivax serological testing and treatment (PvSeroTAT) in the Asia Pacific. Recognizing P. vivax's challenge to 2030 elimination goals, the study focuses on validated serological markers to identify high-risk populations for relapse. The Vivax Serology Partnership (VISPA) conducts policy consultations in vivax-endemic countries like Lao PDR, Cambodia, the Philippines, and LATAM region to assess the acceptability and feasibility of PvSeroTAT. Lao PDR has implemented PvSeroTAT for outbreak control and hotspot targeting. In the Philippines, serological markers are used to understand residual transmission. In-depth qualitative interviews and policy consultations explore health systems barriers and enablers to PvSeroTAT. The findings will inform country-specific roadmaps for PvSeroTAT implementation.

**Rama Adiga** (Nitte deemed to be University, NUCSER, Mangalore, India) presented a novel, field-deployable diagnostic method for Plasmodium vivax detection using Recombinase Polymerase Amplification (RPA). This isothermal technique, utilizing RPA-specific primers for the MSP1 gene, requires only a heating block, making it suitable for point-of-care diagnostics in resource-limited settings like India. The PCR amplified product can be visualized on a simple lateral flow strip, currently under development. The assay demonstrated high sensitivity and specificity for P. vivax detection.

**Session 10:**

**Gowthami Arumugam** (Kasturba Medical College, Manipal, India) spoke about assessing the impact of climate variables on malaria incidence in coastal Karnataka, India. The research focused on Dakshina Kannada (D.K.) and Udupi districts, analyzing data from 2019 to 2023. The study revealed Plasmodium vivax as the predominant species, comprising 86% of

cases. Malaria prevalence peaked between June and September, correlating with increased rainfall and relative humidity, and decreased temperatures. D.K. showed a strong positive correlation between rainfall and malaria cases, while Udupi showed a weaker correlation. Relative humidity positively correlated with malaria cases in both districts. Mean temperature negatively correlated with cases. The findings underscore the link between climate factors and malaria transmission, emphasizing the need for incorporating climatic data into public health planning. Continuous monitoring and environmental management are crucial for reducing P. vivax burden.

**Sharlot Fosah** (University of Buea, Cameroon) addressed the impact of global warming on malaria incidence in Bonaberi and Tombel. Utilizing observed parasite ratio (PR) data, precipitation data from FEWS NET, and temperature data from ECMWF ERA-Interim, the study employed VECTRI model simulations and CORDEX projections under RCP2.6 and RCP8.5 scenarios. Results indicated PR peaks of 0.8 in Tombel and 0.9 in Bonaberi, with future projections showing PR increasing to 0.95 in Bonaberi and 0.9 in Tombel, highlighting the climate change-driven increase of malaria.

**Britny Johnson** (Cornell University – Department of Entomology, United States) investigated the nuanced impact of relative humidity on key thermal performance traits of *Anopheles stephensi*, a critical factor in malaria transmission dynamics. By subjecting 700 mated female mosquitoes to 35 distinct environmental conditions, varying temperatures (16°C to 38°C), and relative humidity (30% to 90%), the study meticulously tracked egg production, survival, and bloodmeal consumption. The resulting thermal performance curves revealed that lower humidity enhanced egg production at higher temperatures, while higher humidity bolstered survival at elevated temperatures. This highlights the necessity of incorporating humidity alongside temperature in malaria transmission models to improve predictive accuracy, particularly in the context of climate change.

**Syed Shah Areeb Hussain** (ICMR – National Institute of Malaria Research, New Delhi, India) spoke about specialized hybrid models for accurate malaria incidence forecasting in India. Their research combined trend and seasonality components with machine learning algorithms, addressing the challenges posed by climate change on malaria seasonality. District-wise monthly malaria incidence data from six Indian states was utilized, identifying key predictors using recursive feature elimination and SHAP. Various models, including regression, time series, machine learning, deep learning, and hybrid models, were trained and tested. Hybrid models, particularly SVM-ARMA and XGB-ARMA, provided the most accurate and precise forecasts (RMSE 0-0.44). Time-series models performed well with seasonality, but machine-learning models offered greater consistency. Deep learning models tended to overfit. Their study concluded that integrating time-series components into machine learning models enhances forecast accuracy, offering a valuable tool for malaria elimination planning and epidemiological analysis.in understan

# Annex 2: List of posters presented

*\* This list was provided by the organizing committee.*

**Day 1:**

| Title | Presenter | Poster_ID |
|---|---|---|
| Comparison of diagnostic performance between hemozoin-based magneto-optical detection (Hz-MOD) assays and rapid diagnostic tests (RDTs) for malaria detection: a systematic review and meta-analysis | Vishnu Teja Nallapati | T1_01 |
| Exploring novel proteins for diagnosis of *Plasmodium vivax* infection. | Ms. Shradha Bhatnagar | T1_03 |
| Extracellular Vesicle Cargo in Severe or Uncomplicated *P.vivax* clinical isolates. | Ms.Shreya Bhatnagar | T1_04 |
| Single-cell insights into the erythrocytic stages of *P. vivax* natural infections | Mrs. Erin Sauve | T1_05 |
| Identifying novel antigenic markers for improved detection of *Plasmodium vivax* malaria | Dr. Bhuvan Dixit | T1_06 |
| Pv3Rs: An R package to estimate the probability that a *Plasmodium vivax* recurrence is a recrudescence, relapse, or reinfection | Dr Aimee R Taylor | T1_07 |
| Evidence-Based Clinical Trial Design: A Modelling Study of the *Plasmodium vivax* Serological Testing and Treatment in Ethiopia and Madagascar (PvSTATEM) Cluster-Randomised Trial | Dr. Constanze Ciavarella | T1_10 |

**Day 2:**

| Title | Presenter | Poster_ID |
|---|---|---|
| P. vivax chloroquine resistance in Papua New Guinea in 2023 | Mrs. Erin Sauve | T1_11 |
| Characteristics of *Plasmodium vivax* apicomplexan amino acid transporter 8 (PvApiAT8) in the cationic amino acid transport | Mr. Ernest Mazigo | T1_12 |
| Severe Hemolysis on the First Dose of an 8-Week Primaquine Regimen for Radical Cure of *Plasmodium vivax* Infection in a Child with Severe G6PD Deficiency | Prof. Erni J. Nelwan, MD.,Ph.D. | T1_13 |
| Methaemoglobin as a surrogate marker of primaquine antihypnozoite activity in *Plasmodium vivax* malaria: a systematic review and individual patient data meta-analysis | Dr Ihsan Fadilah | T1_15 |
| Mesenchymal stem cells (MSCs) attenuate cerebral malaria induced neurodegeneration in Plasmodium berghei ANKA infected C57BL/6 murine models | INDU SHARMA | T1_16 |
| The acceptability and feasibility of routine clinical follow-up for short course radical cure treatment for P. vivax malaria: a comparative analysis of Cambodia and Ethiopia | Mrs. Muthoni Mwaura | T1_17 |

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|---|---|---|
| Household economic burden of vivax malaria in Indonesia | Patrick Abraham | T1_18 |
| Investigating the Epigenetic landscape of severe malaria: A Genome-Wide methylation analysis of *Plasmodium vivax* clinical isolates. | Ms. Priyanka Roy | T1_19 |
| Preliminary clinical performance and usability data from Brazil on a new point-of-care test for glucose-6-phosphate dehydrogenase (G6PD) deficiency | Rebecca K. Green | T1_20 |
| DNA-Dependent protease promotes parasite survival during replication stress induced through DNA damaging agents. | Risha Shameem | T1_21 |
| Countrywide Implementation of G6PD testing for *Plasmodium vivax* malaria treatment in Cambodia: Challenges and Potential Solutions | Sarah A. Cassidy-Seyoum | T1_22 |
| Exploring mechanistic insight of newly synthesized nano-formulated chalcones for antimalarial activity | Dr. Shweta Sinha | T1_23 |
| Implementing G6PD testing for *Plasmodium vivax* malaria in Punjab, North India: a pilot study | Urvashi Rahi | T1_24 |
| Survey of Glucose-6-Phosphate Dehydrogenase deficiency, anaemia and malaria parasitaemia among febrile patients in Indonesia | dr. Vincent Jimanto | T1_26 |
| Bioassay guided isolated and structural identification of Phyllanthus emblica compounds and their effective on CQ-sensitive strain of Plasmodium falciparum (3D7) | C. Kamaraj | T1_29 |
| The impact of recurrent vivax malaria and primaquine on haematological recovery | Dr. Megha Rajasekhar | T1_33 |
| Correlation of the platelet indices with severity and outcome in cases of vivax malaria | Dr. Sanjay Kumar Kochar | T1_34 |
| Transmission-Blocking Antibodies and Plant Extracts: Novel Interventions Against Plasmodium Development in Mosquito Vectors | Miss Bharti | T1_35 |
| Distribution of Anopheles Mosquitoes and Plasmodium Infection Rates by Anopheles Species in High-Risk Areas of Malaria in Korea | Monoldorova Sezim | T2_01 |
| Convergent-parallel approach to investigate malaria in Indigenous communities during the COVID-19 pandemic | Susan Cilene Paredes Fernandez | T2_02 |
| Effect of *Plasmodium vivax* (P. vivax) infection on cytokines and chemokines and their role in the degrees of thrombocytopenia. | Marian Marcela Muskus Montiel | T2_03 |
| Mesenchymal stem cells restore erythropoiesis and lymphoiesis during malaria infection. | Reva Sharan Thakur | T2_04 |
| Plasmodium dormancy is signified by epigenetically-regulated RNA-binding proteins of the parasite and associates with a specific cellular state of the host hepatocytes | Dr. Abhishek Kanyal | T2_05 |
| Identifying novel drug targets in Type II Fatty Acid Synthesis and Lipid Biosynthesis pathway to combat malaria | Ms. Akanksha Singh | T2_06 |

| Title | Presenter | Poster_ID |
|---|---|---|
| The PvRBP2b-TfR1 interaction is not essential for reticulocytes invasion by *Plasmodium vivax* isolates from Cambodia | Dr. Brice Feufack | T2_07 |
| Single-cell optical diffraction tomography to monitor cell host-plasmodium interactions | Mr. Christeen Davis | T2_08 |
| Identification of proteins induced at low Temperature from Plasmodium | Miss Diksha Sisodia | T2_09 |
| Inhibition of *Plasmodium vivax* Invasion through Disruption of DBP-DARC Interaction: Identification and Characterization of Novel Compounds | Dr. Hojong Jun | T2_10 |
| Characterization of BolA-like protein involved in [Fe-S] Cluster biogenesis pathway from Plasmodium falciparum and P. vivax | Ms. Kanika Hada | T2_11 |
| Title: PvTRAg26.3 (PVX_112660) - Stomatin along with lipid interaction study - new insight of *Plasmodium vivax* invasion into human reticulocyte/erythrocyte. | Mr. Manish Tripathi | T2_12 |
| Systematic reverse vaccinology screening for novel *Plasmodium vivax* blood-stage vaccine antigens | Dr. Prasun Kundu | T2_13 |
| An in-silico analysis of selected vir/pvpir genes exhibiting transcript upregulation in *Plasmodium vivax* cerebral malaria cases | Mr. Saurabh Singh | T2_14 |

**Day 3:**

| Title | Presenter | Poster_ID |
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| Identification of novel interactions between the conserved antigen Plasmodium TRAg and human erythrocytes | Wang Jong-Lee | T2_16 |
| Characterization of a narnavirus infecting *Plasmodium vivax* parasites and its clinical impact | Ms. Dynang Seng | T2_17 |
| Polymorphisms in *Plasmodium vivax* Reticulocyte Binding Protein 2b (PvRBP2b)-Transferrin receptor-1 (TfR-1) interacting site: a challenge for PvRBP2b-based erythrocyte invasion blocking research | Miss Renuka Harit | T2_20 |
| Harmony in the Heat: Unraveling the Symphony of Heat Shock Protein 70 in Indian Malarial vector- A Molecular Ballet Shaping Mosquito Development and Influencing Plasmodium Transmission | Miss Bharti | T2_22 |
| *Plasmodium vivax* malaria in mobile and migratory populations (MMP) of Punjab, North India | Dr. Abhishek Mewara | T3_04 |
| Quantifying the burden of low-density *Plasmodium vivax* infections across different transmission settings – a systematic review and meta-analysis | Wangdahl A | T3_05 |
| MinION sequencing for rapid surveillance of imported *Plasmodium vivax* cases | Angela Rumaseb | T3_06 |
| Molecular Surveillance of P. vivax in Nepal's Pre-elimination Setting | Dr. Anjana Rai | T3_07 |

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| Assessment of Primaquine Compliance in *Plasmodium vivax* Malaria Patients in Two Districts of Odisha State, India | Dr. Anju Viswan K | T3_08 |
| Molecular analysis on drug-resistant genes of malaria caused by *Plasmodium vivax* isolated from Mangaluru, India. | Dr. Anoopkrishna K | T3_09 |
| Deciphering hidden reservoirs of *Plasmodium vivax* in Ethiopia using identity-by-descent and time-to-event models | Dr. Ashley Osborne | T3_10 |
| Longitudinal study in Dhalai district of Tripura finds Anopheles maculatus emerging as new potential vector with both Plasmodium falciparum and vivax infections | Miss Ashwarya Kumari Sihag | T3_11 |
| Characterizing the CDGSH type NEET proteins as Fe-S binding proteins in Plasmodium | Divya Malik | T3_13 |
| *Plasmodium vivax* population structure and fixation of DHFR/DHPS mutations in areas of high and low connectivity in Latin America | Fredy E. Villena | T3_14 |
| High prevalence of asymptomatic malaria in RDT false negative individuals from selected malaria endemic villages of Kalahandi, Odisha | Dr. Gunanidhi Dhangadamajhi | T3_15 |
| High-altitude malaria transmission: persistence and local spread of Plasmodium vivax in the Ethiopian highland | Gustavo da Silva | T3_16 |
| Application of highly multiplexed ampliseq targeted NGS assays for p. Vivax genomic surveillance use cases in asia and latin america | Dr. Johanna Helena (Eline) Kattenberg | T3_18 |
| A new *Plasmodium vivax* reference genome for South American isolates | Dr. Katlijn De Meulenaere | T3_19 |
| Unraveling the P. vivax connectivity in Latin American using genomic surveillance with the highly multiplexed NGS AmpliSeq assay | Mr. Luis Cabrera Sosa | T3_20 |
| Assessing the performance of SNP barcode panels compared with microsatellites in investigating the population genetics of malaria parasites in the Peruvian Amazon | Mahdi Safarpour | T3_21 |
| Understanding *P. vivax* and *P. falciparum* prevalence trends in co-endemic regions on a global scale using a multi-species modelling framework approach. | Dr. Richard Sheppard | T3_22 |
| The prevalence of *Plasmodium vivax* and G6PD deficiency in the Mandoto district in Madagascar and the Shashemene district in Ethiopia, results of a large observational study in preparation of the PvSTATEM cluster-randomized study. | Rob van der Pluijm | T3_23 |
| Genomic Dynamics of Patient Derived Parasites: Comparative Genomic Hybridization of *Plasmodium vivax* causing severe disease | Miss Sampreeti Tahbildar | T3_24 |

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| Profiling Gametocyte Gene Expression in *Plasmodium vivax* from South Karnataka: Towards Regional Surveillance and Elimination Efforts | Ms. Varijakshi Gutthedhar | T3_26 |
| Genomic and Proteomic Analysis of Subspecies of Anopheles culicifacies: Decoding the Mechanism of Refractoriness | Soumya Ranjan Pradhan | T3_28 |
| Serological responses against *Plasmodium vivax* circumsporozoite protein (PvCSP) from different geographically diverse malaria endemic regions of India | Vikas Kumar | T3_29 |
| Cytosolic iron-sulfur cluster assembly pathway from *Plasmodium vivax* | Miss Sayantani Chatterjee | T3_30 |

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