21st International Congress for Tropical Medicine and Malaria (ICTMM 2024)

MESA Correspondents Report

Written by Syaiful Rizal, Nurul Izza Zakaria, Muhammad Hafizu Sulaiman, and Sam Jian Hung.

Senior editorial support has been facilitated by Prof. Indra Vythilingam, and Prof. Balbir Singh.

MESA Correspondents bring you cutting-edge coverage from the 21st International Congress for Tropical Medicine and Malaria (ICTMM 2024)

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Sarawak, Malaysia

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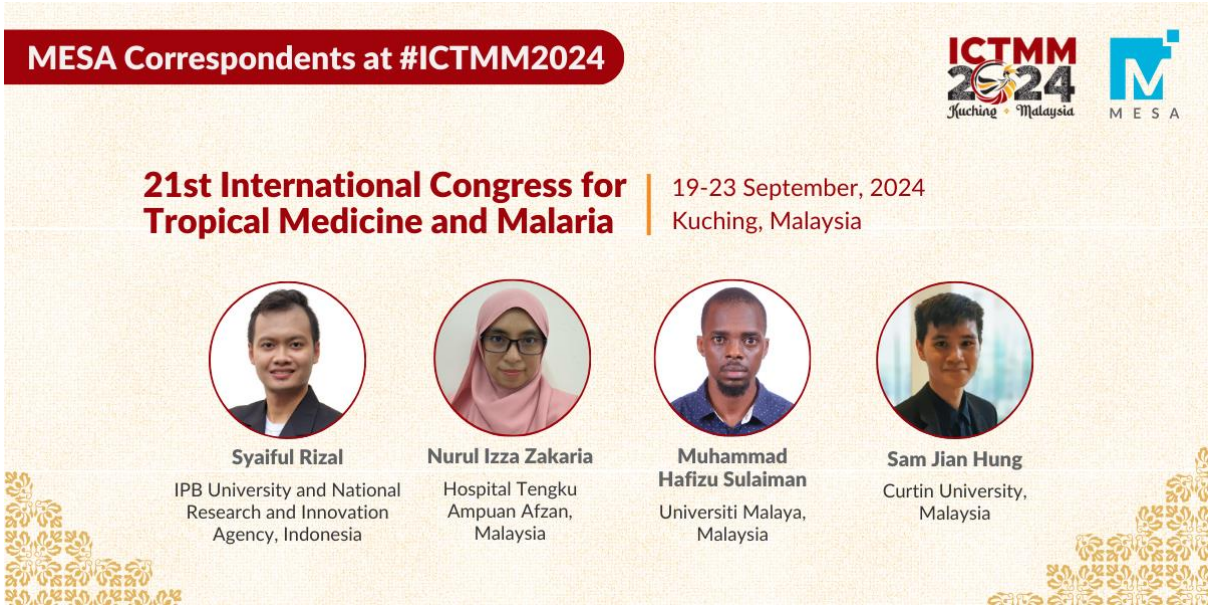

MESA - the malaria knowledge hub, would also like to acknowledge the MESA Correspondents Syaiful Rizal (IPB University and National Research and Innovation Agency, Indonesia), Nurul Izza Zakaria (Hospital Tengku Ampuan Afzan, Malaysia), Muhammad Hafizu Sulaiman (Universiti Malaya, Malaysia) and Sam Jian Hung (Curtin Univerity, Malaysia) for their coverage of the conference.

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Day 1: Thursday, 19th September 2024

Opening Remarks

The Malaysian Society of Parasitology and Tropical Medicine (MSPTM) hosted the 21st International Congress for Tropical Medicine and Malaria (ICTMM) at Borneo Convention Centre Kuching, Malaysia. The conference welcomed over 1200 participants from more than 58 countries. The opening ceremony started with the organizing chair **Siti Nursheena Mohd Zain** (University of Malaya, Malaysia) highlighting the theme of the conference which was "Global Responses and Interdisciplinary Research Towards Eliminating Tropical Diseases". Nursheena underscored the importance of interdisciplinary research, where combining expertise, resources, and innovative ideas are crucial in tackling formidable health challenges. The congress was then officiated by The Honourable Datuk Amar Professor Dr Sim Kui Hian, Deputy Premier of Sarawak and Minister for Public Health, Housing and Local Government Sarawak. Prof Sim emphasized on the main objective of the congress being the eradication of tropical diseases which is a vital part in achieving the United Nations Sustainable Development Goals (SDGs) and achieving healthy lives for people of all ages. Lastly, an important outcome of the opening ceremony was the Memorandum of Understanding (MOU) signing for academic and research cooperation between the Asian Alliance of Societies for Tropical Medicine and Parasitology.

Keynote 1 - The M&Ms: Malaria, Man and Monkeys

Balbir Singh (Universiti Malaysia Sarawak, Malaysia) talked about zoonotic malaria in Southeast Asia, starting with the discovery of a large number of human infections in Sarawak, Malaysian Borneo with *P. knowlesi*, a parasite of macaques. The morphology of *P. knowlesi* is very similar to *P. malariae*, and only molecular techniques like PCR could identify the Plasmodium species precisely. This led to the realization that many cases thought to be *P. malariae* were actually *P. knowlesi*. Singh went on further to talk about the timeline of human infections with simian malaria parasites and highlighted the growing problem of zoonotic malaria. He added that *P. knowlesi* is just the tip of the iceberg, and it's a case of the harder you look, the more cases you find. Besides, recent studies were showing human infections with other simian Plasmodium species such as *P. cynomolgi*, *P. inui*, *P. coatneyi*, *P. fieldi* and *P. simiovale*. Reasons for the increase in zoonotic malaria included increased awareness, the use of molecular detection methods, changes in land use, deforestation leading to changes in vector species, increased vector population, changes in vector bionomics, adaptation of parasites, and human-to-human transmission. He highlighted the challenge of controlling zoonotic malaria, noting that outdoor-feeding vectors pose the greatest risk to those engaged in forest activities. Singh emphasized that bed nets and indoor residual spraying are largely ineffective against zoonotic malaria, and our focus should be directed at finding novel vector control methods and at methods targeting the huge reservoir of monkeys infected with malaria parasites.

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Day 2: Friday, 20th September 2024

Malaria – Diagnostics 1

Himanshu Gupta (GLA University, India) presented a study that investigated the role of miR-3158-3p which is released upon organ damage as a biomarker for the diagnosis of severe malaria. He pointed out that the increase in fatality due to severe malaria is associated with sequestration of *P. falciparum*-infected erythrocytes that leads to organ dysfunction. Using quantitative reverse transcriptase PCRs, the study validated the potential of miR-3158-3p as a biomarker in severe malaria cases as well as its correlation with the severity in patients. Gupta revealed the observation of high levels of miRNA in patients with cerebral malaria compared to patients with non-severe malaria. Change in the micro-RNA levels suggests its potential as a new tool and a cheaper alternative to neuroimaging techniques in the diagnosis of severe malaria.

Mohammad Shoyaib Khazi (London School of Hygiene and Tropical Medicine – LSHTM, United Kingdom) presented a study that assessed factors affecting non-usage of bed nets by women of the reproductive age group in India. Khazi highlighted the role of bed nets in the fight against malaria which led to their study on factors affecting usage among women within the reproductive age in the country. The study utilized data from the National Family Health Survey that assessed annual parasite index at a community level and demographics at individual levels of over 700,000 participants. He stated that the study found a correlation between bed-net usage with age, education status, and annual parasite index. According to him the high non-usage in urban areas, in regions with high API, and in women with low levels of formal education is of concern. Mohammad emphasized the need for continued enlightenment on the importance of using bed nets through informal education, mass media, and social media among populations in India to maximally harness the benefits of the bed nets in the fight against malaria in the country.

Chiging Tupe (ICMR National Institute of Malaria Research, India) presented a study on the mosquito protein ferritin's effect on Plasmodium survival in malaria vectors. The protein which plays a crucial role in iron metabolism is reported in the study to exhibit a diet-dependent expression in Anopheles mosquitoes used in the study. Tupe added that the study targeted various stages of the mosquito life cycle using ferritin selective and broad iron chelators. Iron chelators in their study were interestingly found not only to have an inhibitory effect on Plasmodium survival but also to cause larvicidal effect and decrease in oocytes in female mosquitoes owing to its role in the transport of iron to ovaries. Tupe highlighted the importance of iron chelators and suggested their use in new drug development for malaria control.

Huai Chang (Nagasaki University, Japan) presented on the development of a system that will evaluate expression of an exogenously introduced Schizont Infected Cell Agglutination (SICA) protein on red blood cell surface caused by *Plasmodium knowlesi* infection. He highlighted the importance of understanding the molecular basis of *Plasmodium knowlesi* pathogenicity and virulence owing to its increasing cases and associated mortality in Southeast Asia for an effective intervention strategy. Chang reported an observed sequestration of *P. knowlesi*-infected red blood cells that is associated with disease severity in patients. They interestingly discovered a protein, which mediated the adhesion of *P. knowlesi* to infected red blood cells in human umbilical vein endothelial cells, and named it SICA-HUVEC. He added that the discovery could be useful for screening of chemicals that can be used in inhibiting the expression of parasite-derived molecules on infected red blood cell surfaces with the aim of reducing the parasite's virulence in patients.

Davinder Kaur (Postgraduate Institute of Medical Education and Research, India) presented a study that investigated Toll-Like Receptors (TLR) polymorphism from 65 clinical blood samples positive for *Plasmodium vivax* using PCR-RFLP. The study further identified levels of cytokines from serum of the samples using ELISA to investigate the association between TLR polymorphism and cytokine levels. The study not only detected 4 types of polymorphisms from the samples but also revealed an association between the polymorphism and cytokine levels. Kaur described this relationship as an indicator that could be used in vaccine development and in designing new tools for the control of malaria.

Rahul Pasupureddy (ICMR National Institute of Malaria Research, India) expressed the importance of malaria protease in the degradation of host's red blood cells (RBC) during the erythrocytic phase of Plasmodium infection. Pasupureddy presented a study that investigated the interactions of malarial proteases, falcipains, with their natural substrate haemoglobin and their inhibitor using a combination of bioinformatics and mutagenesis analysis. The study revealed the role of a single amino acid in mediating interaction of the malarial protein falcipain with haemoglobin in RBCs. He revealed how their study characterised novel interactions of FP2 with haemoglobin and also described this phenomenon as potential chemotherapeutic applications in the future.

Peeyush (ICMR National Institute of Malaria Research, India) addressed the challenges of mosquito identification that often required expert knowledge and an ample amount of time. Peyush presented a study that focused on creating an AI-powered application that can easily be used to identify mosquitoes. Peeyush's study used 950 laboratory-reared mosquitoes of different species. Peeyush added that quality data images and optimized model configurations are key to the development of the AI application. One of the limitations of the current development of the application is the requirement of in-depth morphology for species-specific identification. Peeyush indicated that source, resolution, and model complexity are critical to achieving higher accuracy. Future work will focus on expanding the dataset and refining model configurations to enhance accuracy, providing practical applications in mosquito surveillance and vector control.

Parsakorn Tapaopong (Mahidol University, Thailand) presented a study that investigated the trend in *Plasmodium knowlesi* surface protein polymorphism in Thailand over a span of 20 years. Samples at different time periods from the study were compared for the polymorphism which revealed that samples from recent years expressed fewer haplotypes than samples from previous years after sequencing the 42-kDa region of pkmsp1. He linked the observed reduction to mutation which was mediated by adaptive response of the parasite to evade host immunity caused by its increased widespread transmission across the country and beyond. He concluded that findings in their study will contribute to understanding the evolutionary changes of the parasite which could pose a significant threat for malaria control strategies.

Jing Wen Hang (National University of Singapore - NUS, Singapore) presented a study discovering malaria's complexity via volume electron microscopy (vEM). Hang noted the role of parasite-host interaction for diagnostic methods and treatment of malaria. Their study investigated the application of vEM in studying different developmental stages of *Plasmodium falciparum* and *Plasmodium knowlesi*. The presented method is expected to provide a 3D visualisation of the parasite's structures with high resolution and identification of ultrastructural features. Weng added that the technique helped in a better understanding of parasites morphology, host-parasite interaction, drug development as well as malaria treatment and prevention. Despite its potentials, she also pointed out the pitfalls of the method which include it being sluggish and the difficulty in interpreting data of complex 3Ds and the requirement of specialised skills and advanced computational tools.

Symposium P03 – At the Cusp of Malaria Elimination: What are the Lessons Asia can Share with Africa?

Kimberly Fornace (National University of Singapore – NUS, Singapore) reviewed the factors that drive and distribute simian malaria. In doing so she identified priorities for surveillance and control, further discussing the implications for global malaria control and elimination policies. Non-human primates play a significant role in zoonotic malaria in Malaysia, particularly in Sabah and Sarawak. It is known that *Plasmodium knowlesi* is the most prevalent among non-human primates. She conducted a literature review of 148 unique surveys, which included 6,322 non-human primates (mostly long-tailed macaques and some pig-tailed macaques) tested for *P. knowlesi* using molecular methods. Human-mosquito-human transmission was demonstrated experimentally once, but there was no evidence of widespread non-zoonotic transmission of *P. knowlesi* based on systematic reviews and mathematical modelling. However, there remains a possibility for this to occur in the future. Environmental changes, shifting levels of immunity, and the absence of current control measures present challenges to the elimination of zoonotic malaria. Fornace reiterated the need for innovative surveillance and robust control measures to be implemented globally.

Lieven Vernaeve (Malaria Consortium, United Kingdom) presented a success story from his project on reducing residual malaria cases in Cambodia. Cambodia has a strategic plan for malaria elimination for 2015-2028. As part of this, Village Malaria Workers (VMWs) and Mobile Malaria Workers (MMWs) were established in 2001 and 2009, respectively, to provide preventive tools, diagnose malaria early, and provide treatment to hard-to-reach populations. The MMWs are locally recruited members of the community who are well-known by their peers and have knowledge of mobility patterns, local culture, and language. As of June 2024, there are 95 active MMWs in six northern provinces. Diversified activities have been conducted, offering quality mobile malaria services, such as screening co-travelers, active fever screening, passive case detection at the MMWs' homes, empowering communities through community dialogues, and distributing long-lasting insecticidal nets (LLINs). Vernaeve highlighted MMWs in the six northern provinces of Cambodia who significantly contributed to malaria case screening, as part of provincial malaria data. He concluded by drawing attention to its possible integration with other public health issues which could contribute to a more sustainable impact from the MMWs and stressed on the importance of robust surveillance, and community contributions to the objectives of the National Malaria Program.

Muhammad Mukhtar (Ministry of National Health Services, Regulations, and Coordination, Pakistan) explained the steps Pakistan has taken in an effort to eliminate malaria by 2035. *Anopheles stephensi* has posed a significant burden in Pakistan as a vector of malaria, with its impact starting in the early 2000s. It is known that *An. Stephensi* has undergone behavioural changes (resting, biting, feeding, breeding, and seasonality). Rising temperatures have also contributed to the distribution of *An. stephensi* between 2000 and 2020. In response, the government of Pakistan has developed new strategies to manage this species and has offered to assist Africa in malaria elimination by sharing knowledge and expertise. The efforts include robust vector surveillance, capacity strengthening, policy consultancy, institutional support, innovative technologies like drones and AI, and joint research initiatives.

Poe Poe Aung (Malaria Consortium, United Kingdom) shared her activities in addressing the spread of arboviruses in African and Southeast Asian countries pointing out Resilience Against Future Threats (RAFT) which was launched to help address capacity shortfalls in arbovirus preparedness. They have partners in countries including Thailand and Cameroon, which serve as host countries. They work on new research, decision-making frameworks, provide accessible state-of-knowledge evidence reviews, promote South-to-South exchanges,

and conduct country case studies. During exchange meetings, they share new ideas and surveillance strategies, along with laboratory and field visits. Lessons learned highlight deficiencies in arbovirus outbreak preparedness, surveillance, and control across Africa. Prioritizing community engagement, robust surveillance systems, and translating research into practice will strengthen national programs. She concluded by acknowledging future challenges that included the need for capacity strengthening and the use of context-appropriate tools in Sub-Saharan Africa.

Plenary 1 – Malaria in India: How Shifting Epidemiology and Changes in Parasite Transmission are Challenging the Road to Elimination

Jane Carlton (Johns Hopkins Malaria Research Institute - JHMRI, United States) highlighted her research and discoveries during the 14 years of achievement with continuous, long-term funding, and underscored the importance of continuing basic and translational research on malaria elimination in India. Malaria in India is complex and historically had a high burden from 2005 to 2022, with *P. falciparum* and *P. vivax* being the dominant species. Malaria elimination efforts began in 2015, utilizing methods such as Rapid Diagnostic Tests (RDT), PCR, and microscopy. Due to the high number of asymptomatic and submicroscopic malaria cases, a new elimination initiative called Durgama Anchalare Malaria Nirakaran (DAMaN) Camps, a malaria control initiative in inaccessible areas was launched in 2017, delivering malaria control services to remote villages across the state. To evaluate the effectiveness of DAMaN in reducing malaria prevalence, three arms were deployed to compare malaria prevalence in different villages. The evaluations revealed a high failure rate (70%) in RDTs, primarily due to Pfhrp2 gene deletions in *P. falciparum*, posing a significant threat to malaria elimination efforts. To address these issues, Carlton's team is developing the Vector-Cam app for mosquito recognition using AI algorithms, creating portable labs for sample processing and sequencing with MinION, continuing to assess DAMaN's effectiveness, and identifying better RDT targets in *P. falciparum*. With these efforts, new research and surveillance tools are on the horizon, aiming to enhance vector control and improve malaria diagnosis.

Sponsored session – Advancing malaria research: Innovative solutions from QIAGEN®

Khor Yee Min (QIAGEN, Malaysia) presented the benefits of digital PCR (dPCR) for the detection of microorganisms using QIAcuity. This is a third generation and most advanced PCR technology designed for the detection and quantification of nucleic acids using nanoplates. The technology works based on the real-time PCR chemistry and workflow with the same reagents with added benefit of end-point detection, higher precision, and reproducibility, absolute quantification, binary signal from low abundant molecules as well as end-point detection. Khor outlined four key benefits of the new method which include; specificity in detecting target sequence, sensitivity in detecting genes in small number of targets, 2 hour rapid detection, and ability to carry out multiplex of 5 targets per reaction with an interesting ability to combine both viral and bacterial DNA.

Iara Silman (QIAGEN, Germany) presented a novel approach for Plasmodium species detection using a direct multiplex qPCR technology with QIAprep&amp. Silman outlined the limitations of the various tools used in malaria diagnosis such as the need for expertise, low sensitivity, storage issues, and time consumption. The proposed new technology can be used for the detection of *P. falciparum* for as low as 1 parasite/4uL. The technology is equipped with high sensitivity, compatibility, quickness, and simple workflow to detect DNA from large samples without the need for separate DNA extraction formerly used in other protocols. external and internal laboratory trials revealed high sensitivity in detecting Plasmodium parasites. Silman highlighted the special thing about the new technology to differentiate three

different Plasmodium species with an internal control to monitor internal workflow and one hour result processing time.

Bienvenu Nsengimaana (Infectious Diseases Research Collaboration – IDRC, Uganda) presented the performance of the nanoplate digital PCR technology in genotyping and surveillance of pfhrp2/pfrp3 gene deletion in Uganda. Bienvenu recalled Uganda as the 3rd country with the worst malaria cases in the world. Limitations on the use of previous methods of PCR, qPCR and ELISA in cases of mixed infection and low parasite counts and limitation of the most widely used and cost-effective Rapid Diagnostic Test (RDT) of possible false positive due to pfhrp2/pfrhp mutations lead to the investigation into the presence of the mutation that could have affected reported RDT positive reported cases in the country. Interestingly their study found effectiveness of the technology in detecting the mutation and at the same time reported negligible mutation in the country. He shed light on the importance of rapid diagnostic kits in the detection of malaria, especially in developing countries such as Uganda. Although he described the mutation as uncommon in Uganda, he recommended continuous surveillance of the mutation in the country.

Malaria – Epidemiology 1

Daniel Reegan (ICMR-Vector Control Research Centre, India) discussed the malaria epidemiological data of two endemic coastal localities in India: Besant Nagar (urban) and Pamban (rural), covering the period from 2004 to 2023, and correlated it with the highest recorded maximum temperatures. Malaria prevalence in these two areas was analyzed over the same period. Reegan's analysis showed that malaria cases (*Plasmodium vivax* and *P. falciparum*) increased proportionally as temperatures rose in both areas, particularly during the summer seasons from 2004 to 2011, and subsequently decreased by 2023. Mixed infections of *P. vivax* and *P. falciparum* were detected only in Besant Nagar. Additionally, there was no recorded resistance in *P. falciparum* to artemisinin-based antimalarials in these coastal areas. Effective control measures and surveillance contributed to the successful elimination of malaria. The implementation of the new National Drug Policy successfully reduced the malaria parasite load in the community. Larval control activities and Indoor Residual Spray (IRS) also helped in reducing vector populations.

Alfredo Mayor (Manhiça Health Research Centre – CISM, Mozambique) spoke about the benefits of using malaria genomic surveillance for decision-making in Mozambique. The GenMoz project includes the *Plasmodium falciparum* genomic intelligence initiative in Mozambique. The database builds next-generation sequencing (NGS) capacities, generates data (sampling and sequencing), and promotes the use of malaria molecular surveillance (MMS) data at CISM. In May 2011, GenMoz was initiated, involving ethical approval, field activities, training, MiSeq implementation, the first NGS run, and the creation of the dashboard. GenMoz detected 0.1% *hrp2/3* deletions, helped in detecting resistance to artemisinin combination therapy (ACT) related to the *kelch 13* gene and piperaquine, detected mutations in *pfdhfr/pfdhps* for sulfadoxine/pyrimethamine chemoprevention, analyzes the sources of malaria, and is used in antenatal care (ANC) surveillance, aiding in malaria transmission interventions. The data collected were used to create a genetic dashboard and trimestral brochure. He finished the presentation by highlighting that genomic data has the potential to be also used in future vaccine development.

Md. Hasanuzzaman's (Bangladesh Atomic Energy Commission, Bangladesh) topic aimed at exploring harmala alkaloids as novel antimalarial agents against *P. falciparum* through bioinformatics approaches. Unfortunately, the speaker could not attend the oral session.

Kinley Wangdi (The University of Canberra, Australia) explained how imported cases drive local transmission in Bhutan. Data from Bhutan's Vector-borne Disease Control Program (VDCP) from 2016 to 2020 were analyzed using Hawkes processes to study the role of imported malaria in local transmission. According to the data, 87.4% of the malaria cases were male, 71.1% were infected by *Plasmodium vivax*, and 73.6% of the cases came from India. The models showed that *P. vivax* remains infectious for over 19 days, while *P. falciparum* is infectious for 8 days. The study also suggested that *P. falciparum* transmission was mainly caused by importation, whereas *P. vivax* transmission was driven by relapses. Wangdi recommended that the Hawkes process can serve as an alternative surveillance tool for low-case settings and can be strengthened by incorporating factors such as climate, environment, and control measures, as well as investigating the drivers of *P. vivax* transmission.

Angela Ogechukwu Ugwu (University of Nigeria Nsukka, Nigeria) explained the role of immune-inflammatory markers in children with complicated and uncomplicated malaria in Enugu, Nigeria. The study adopted a case-control design, and eligible children were categorized into three groups: those with complicated malaria, uncomplicated malaria, and healthy children, during the period from June 2023 to November 2023. The immune-inflammatory markers studied were IFN-γ, TNF-α, IL-10, NLR, and MLR. The mean age of the participants was 7.3 ± 3.4 years, and the male-to-female ratio was 1:1. They found that the mean level of IL-10 was higher in cases of uncomplicated malaria, and there was a positive correlation between NLR and IFN-γ. She concluded by stating that complicated malaria was associated with higher levels of pro-inflammatory cytokines, while uncomplicated malaria was linked to higher levels of anti-inflammatory cytokines. Furthermore, NLR correlated positively with pro-inflammatory cytokines and could be useful in evaluating the severity of malaria infection.

Win Htike (Burnet Institute, Australia) discussed the health system needs for malaria control in the Greater Mekong Subregion through a qualitative study. This study was conducted through 39 semi-structured interviews in Laos, Myanmar, Thailand, and Vietnam. It focused on high-level policymakers, decision-makers, and managers from the National Malaria Control Program (NMCP) and technical agencies, as well as field managers and supervisors from NMCPs or the Ministry of Health's Infectious Disease Institute. The study participants were purposively recruited, and the data were analyzed using reflexive thematic analysis. Stakeholders highlighted the importance of continuous funding support for malaria elimination to avoid interruptions in malaria elimination activities and the need for strengthening malaria microscopy. Additionally, he emphasized the necessity of tailored interventions for high-risk groups, dedicated staff, highly sensitive surveillance systems, and political commitment and support for a successful malaria elimination program. It is important to assess whether these requirements are already in place before initiating malaria elimination activities.

Geetika Narang (ICMR-National Institute of Malaria Research, India) explained the longitudinal population analysis and natural selection of the Apical Membrane Antigen-1 (AMA-1) gene in Indian *P. falciparum* isolates. A total of 173 samples were collected from 14 Indian states between 1993 and 2021. The polymorphic profile, structure, and natural selection of the gene were assessed to explore longitudinal variation in *Pfama-1* in Indian *P. falciparum* isolates. The results showed 70 haplotypes arising from 52 polymorphic sites, along with two previously unreported mutations: S498C/G and F505Y. However, no significant positive Tajima's D value was observed, and no distinct genetic pattern in the *Pfama-1* gene structure was detected. Thus, Narang underlined that the genetic structure of *Pfama-1* in Indian isolates is complex, exhibiting a high degree of genetic polymorphism. Also, the extensive antigenic repertoire observed in the gene, both in India and globally, could pose challenges for vaccine

design. Finally, since allele-specific immunity has been observed in the gene, Domain II, which showed relative conservation both globally and across all Indian states, could have implications for vaccine design.

Annisa Rahmalia (Menzies School of Health Research, Australia) discusses the barriers to primaquine safety monitoring by community health workers (CHWs) in Timika, Papua, Indonesia. To set the scene, Rahmalia presented several important points. *P. vivax* forms hypnozoites in the liver, which can cause relapse. Artemisinin treatment only targets the blood stage of the malaria parasite. Primaquine is used to target the hypnozoites and the treatment with primaquine takes longer than the duration of the malaria symptoms. This treatment can hemolyze red blood cells, causing the urine to become dark. The results of trial settings showed that the probability of a six-month recurrence of *P. vivax* is lower with supervised compared to unsupervised treatment with primaquine. However, several barriers exist to implementing the supervision, such as a lower number of CHWs compared to the number of patients, difficulties in locating patients, misconceptions about urine color and malaria, and the fact that direct inquiries about urine color can be considered offensive. She concluded that treatment adherence and primaquine safety monitoring require multiple differentiated strategies to reach patients, along with cultural sensitivity and social embeddedness.

Rita Reyburn (World Health Organization – WHO, Lao P.D.R.) initiated a strategy of *Plasmodium vivax* serological-testing-and-treatment (PvSeroTAT) and genomic analysis for an unusual *P. vivax* malaria outbreak in a malaria-free district of Lao PDR. The Nakai district in Khammuane Province has been considered malaria-free; however, there were 222 *P. vivax* cases reported between July 1 and August 16, 2023. Reyburn conducted a serological and genomic survey in early November 2023, targeting all individuals aged over 18 months. The results revealed that 4% were positive by PCR, 17% by serology, 4% by both PCR and serology, and 17% by either PCR or serology. It was noted that 56% had no previous malaria diagnosis. Through genotyping, the diversity and relatedness between the outbreak infections and those from other sites could be determined. The study concluded that the outbreak in Nakai was unusual in that a large number of *P. vivax* cases were detected suddenly in a previously malaria-free area.

Symposium P21 – Emerging Data on the Latest Available Treatments for the Radical Cure of Plasmodium Vivax

Prayuth Sudathip (Ministry of Public Health, Thailand) presented an assessment of the appropriate use and non-use of tafenoquine (TQ) / daily primaquine based on Glucose-6-phosphate dehydrogenase (G6PD) activity for patients greater than 16 year old with uncomplicated *P. vivax* malaria in Yala and Mae Hong Son provinces. They found high follow-up rates on Day 5 and 14 with four patients hospitalized due to non-drug related causes. No cases of acute hemolytic anemia were detected and no patients with possible signs of hemolysis had autoimmune hemolytic anemia (AHA). Four patients were hospitalized but it was due to non-drug related causes. From this, it was concluded that TQ was correctly used in 100% of the cases based on G6PD activity. Sudathip ended the talk by stating the feasibility of using TQ with G6PD testing at different levels of the health system based on this study.

Ayodhia Pitaloka Pasaribu (Universitas Sumatera Utara, Indonesia) Pasaribu presented a study that investigated the use of a higher dose of primaquine (PQ) with shorter course of 1 mg/kg/day administered in 7 days (PQ7) to improve adherence to the treatment regimen among patients. While this regimen had good efficacy under trial settings, it was with increased risk of gastrointestinal intolerability and hemolysis. To mitigate this effect, a point-of-care G6PD test was used to screen patients prior to PQ administration. A case

management package was developed with improved patient counselling tools and processes, community-based clinical review on day 3, and improved malariometric surveillance and pharmacovigilance. She concluded by highlighting that the combination of point-of-care G6PD testing and high dose short course PQ has potential to be safe and effective if implemented with patient education and early clinical review. As such, more evidence is needed on safety feasibility and cost-effectiveness.

Moses Laman (Papua New Guinea Institute of Medical Research – PNG-IMR, Papua New Guinea) presented on the feasibility of implementing a revised case management package for patients with *P. vivax* malaria at 10 health facilities in Indonesia and Papua New Guinea. The package included a point-of-care G6PD activity test prior to treatment with Primaquine (PQ), and subsequent prescription of high dose (7mg/kg total) PQ over 7 or 14 days to eligible patients. Education before treatment and a community-based clinical review on day 3 to encourage treatment adherence and facilitate early detection and management of adverse events were also implemented. Health workers felt that the effort put in has reduced the risks associated with PQ administration in patients. Moses mentioned that PQ7 treatment was received well by the participants and reported a reduced risk of subsequent illness. The day 3 follow-up was seen as an opportunity for PQ education and patients appreciated the visit. The revised case management package was seen as a positive perception of the study's impact on personal health care experience.

Brice Campo (Medicines for Malaria Venture – MMV, Switzerland) presented a study that assessed the use of Tafenoquine (TQ) as a single-dose treatment administered with chloroquine (CQ) for the treatment of *P. vivax*. The combination strategy of TQ-CQ already showed TQ to be active and to synergize well at 300 mg. In a previous study, TQ was tested with dihydroartemisinin-piperaquine (DHA-PQP) instead of CQ but failed to confirm radical cure. Subsequent pre-clinical studies replicated the trial's results and found that although CQ and PQP synergized with PQ, DHA interfered with this synergy. The basis of reduction in efficacy appears to be pharmacodynamic. Campo concluded that this study showed the importance of synergies and understanding their mechanisms which might prompt the use of *in vitro* and *in vivo* assays for investigation and validation in the interactions of TQ with other potential drugs.

Gonzalo Domingo (PATH, United States) stood in for Emily Gerth-Guyette and doubled down on the safe use of TQ and PQ through G6PD deficiency testing. Domingo then introduced point-of-care G6PD tests that had been integrated into malaria case management to expand and optimize provision of radical cure for *P. vivax*. One such example was the STANDARD G6PD test which underwent operations and implementation research in 21 countries. The test was found to be generally acceptable to use and feasible to implement among both providers and patients at multiple levels of the health care system provided with proper training and supervision. Domingo underlined some implementation challenges such as the need for a referral to a health facility which might impact access, the need for quality control for decentralized testing, the overestimation of G6PD activity as testing is expanded to more users, and gaps between policies. Moreover, he mentioned context-specific implementation challenges that will require contextually specific solutions and mitigations for every country.

This report is brought to you by the MESA Correspondents Syaiful Rizal, Nurul Izza Zakaria, Muhammad Hafizu Sulaiman and Sam Jian Hung. Senior editorial support has been facilitated by Prof. Indra Vythilingam and Prof. Balbir Singh.

Day 3: Saturday, 21st September 2024

Malaria – Molecular Biology

Sarah A. Cassidy-Seyoum (Menzies School of Health Research, Australia) presented the challenges and potential solutions for the countrywide implementation of glucose-6-phosphate dehydrogenase (G6PD) testing for *Plasmodium vivax* malaria treatment in Cambodia. Cambodia aims to eliminate malaria by 2025 despite a high prevalence of *P. vivax* infection. The drug used to kill hypnozoites of *P. vivax* can cause severe hemolysis in G6PD deficient individuals. To address the issue, biosensor G6PD test was implemented and its role in *P. vivax* case management was assessed in three high-burden provinces. The surveillance data was analysed, and 67 interviews and 29 focus group discussions were conducted in 2022 and 2023. From the data, most patients were diagnosed by village malaria workers (VMWs). Only 49% reached the health center because of issues related to cost, patient perception, previous treatment experience, and rumors. 94% accepted the G6PD test. She concluded by pointing out that bringing treatment closer to patients through VMWs would improve access to malaria treatment.

Muhammad Fikri Heikal (Khon Kaen University, Thailand) explored the role of *Opisthorchis viverrini* helminth defense molecules (HDM) in shaping host immunity. HDM is a secretory product of helminth with structural characteristics that mimic the mammalian antimicrobial peptide LL-37. These molecules are conserved across liver fluke species. *Fasciola hepatica* (FhHDM) neutralized lipopolysaccharide (LPS)-induced inflammation while *Clonorchis sinensis* HDM (CsHDM) does not affect LPS-induced inflammation. Distinct migration routes influence immunomodulation strategies among liver flukes. *F. hepatica* suppresses M1 macrophage activation, while *O. viverrini* and *C. sinensis* activate M1 macrophages. Heikal investigated the role of the *O. viverrini* HDM (OvHDM) C-terminal synthetic peptide in LPS-induced inflammation using the in vitro ELISA technique. The results showed that OvHDM increased the level of LPS-induced inflammation. HDM from different parasites showed a distinct effect on LPS-induced inflammation. HDM is utilized by the liver flukes to modulate the immune response to facilitate their survival.

Brendan Crabb (Burnet Institute, Australia) explained how inhibiting lipid transfer and ring development can stop malaria parasites. When the merozoite makes contact with a red blood cell (RBC), it begins to invade the RBC and eventually develops into the ring stage. An aryl-acetamide compound called MMV006833 (M-833) prevents *Plasmodium falciparum* from growing inside the RBC after invasion. It targets the *P. falciparum* steroidogenic acute regulatory lipid transfer protein (PfSTART-1). In the presence of START inhibitors, merozoites are unable to transform into amoeboid rings. Crabb suggested that START is involved in the transfer of lipids necessary to establish the parasite's parasitophorous vacuole membrane and for the internal reorganization of membranes needed for the transformation of merozoites into rings.

Badriah Alkathiri (Chungbuk National University, Democratic People's Republic of Korea) discussed the identification of tick-borne pathogens and endosymbionts in ticks using metagenomic next-generation sequencing (NGS). Ticks are considered the second most significant biological vector, with a high capacity to harbor and transmit various pathogens. Alkathiri assessed tick-borne bacteria in ticks distributed in Korea using 16S rRNA metabarcoding. A total of 6,907 ticks were pooled into 534 pools. DNA was extracted from each pool and subjected to metagenomic sequencing. Verification of *Bartonella*, *Rickettsia*, *Wolbachia*, and *Ehrlichia* was performed using PCR. The results showed that the prevalence of *Rickettsiella* was high at 67.28%, while *Coxiella* was at 22.12%. *Rickettsia*, *Wolbachia*, and

Ehrlichia were also detected in the samples. Further molecular characterization and phylogenetic analysis revealed that 70.45% of *Rickettsia* and 2.60% of *Wolbachia* were found in *Haemaphysalis longicornis*, while 16.81% of *Wolbachia* were found in *Haemaphysalis flava*. The presence of *Wolbachia* in *Haemaphysalis longicornis* and *Haemaphysalis flava* represents novel findings.

Jahnvi Jakhan (ICMR National Institute of Malaria Research, India) addressed that glucose-6-phosphate dehydrogenase deficiency (G6PD-d) is an erythroenzymopathy leading to hemolytic anemia under oxidative stress. Although in other parts of the world, the correlation between G6PD and malaria has been extensively researched and implemented, it is still poorly understood in India. The gap being the failure to elucidate the spectrum of mutation causing G6PdD-d with context to *P. vivax* malaria in India. To address this gap, Jakhan aimed to genotypically correlate G6PD variants found in Delhi and Goa regions of India where such data are missing in *P. vivax* infected individuals. From a total of 46 retrospective samples, molecular analysis revealed the presence of two most common polymorphic markers, 1311 C>T and IVSXI 93T>C mutations. Some studies suggested that the presence of these two mutations in an individual could lower G6PD enzyme activity. Besides, two novel mutations, 1388 G>T and 1398 C>T were also found. The bioinformatic studies showed a higher SIFT and higher MutPred2 score suggesting deleterious nature to the encoded protein leading to a greater propensity to be pathogenic. The limitations of the study include the inability to perform G6PD phenotypic profiling for determination of G6PD-d status as the majority of samples were dried blood spots and also due to small sample size in this study.

Youseuf Suleiman (University of Liverpool, UK) addressed the need for accurate and reliable markers for tick identification through his talk. Challenges included scarcity of genomic data, suitable markers, and the presence of cryptic species with similar morphological features that might lead to misidentification. While a complete nuclear genome is a gold standard, their large genomic sizes (1-7 Gb) have posed difficulties with assembly and annotation. Suleiman then introduced tick mitochondrial genomes (mitogenomes) with their considerably smaller sizes (14-17 Kb), high copy number, and maternal inheritance which was ideal for accurate and reliable taxonomic differentiation. With this, complete mitochondrial sequences of nine tick cell lines derived from *Amblyomma, Rhipicephalus, Hyalomma,* and *Ixodes* spp., sourced from the Tick Cell Biobank were produced. Additionally, the first mitogenome of *Amblyomma variegatum* was produced. From the data, Suleiman could reliably place *Amblyomma variegatum* as sister to *Amblyomma hebraeumand* corroborate the taxonomic placement of the tick cell lines. The study highlighted the significance of mitochondrial genomics in advancing the understanding of tick taxonomy and evolutionary relationships.

Pedro Ferreira (Life and Health Sciences Research Institute, Portugal) discussed the severity of cerebral malaria (CM) and the lack of a comprehensive research approach for studies. Thereafter, Ferreira proposed an in vitro system by using cellular approaches to identify molecular alterations in human brain vasculature cells that resemble the blood-brain barrier in CM. Induced pluripotent stem cells (iPSCs) culture was used to generate the brain organoids, thereafter, embryoid bodies formed after 6 days of spontaneous differentiation. After 11 days, neurospheres were formed using a neural induction medium. Finally, the brain organoids were ready after 30 days of post-differentiation. Through transcriptomic analysis, the specific gene expression profiles in human brain microvascular endothelial cells (HBMEC) activated by *P. falciparum* were characterized. From there, the impact of HBMEC-*P. falciparum*-activated secretomes were evaluated in human brain organoids which showed induced specific and significant alterations in transcriptome, and the slowing down of normal development in brain organoids. This model is novel in investigating factors influencing CM contributing to understanding pathogenesis, brain injury, and dysfunction.

Ase Berg (Stavanger University Hospital, Norway) started off by raising awareness about the onset of malaria before proceeding to introduce circulating markers of extracellular matrix (ECM). Berg explained that the ECM has roles in wound healing, and it contributes to constructive regrowth, without which, scarring and fibrosis occur. A clinical study was done with 205 participants with *P. falciparum* infection from 2017 to 2020. The circulating levels of six ECM remodeling mediators (KL 40, CysB, ENRAGE, CXCL 16, GDF 15, and GAL-3) were quantified by enzyme immunoassay. Results showed that all six components had significantly elevated readings with levels increasing with malaria severity in severe and complicated malaria compared to uncomplicated malaria. Berg added that the increasing levels could even predict death. From further exploration of different ECM parameters versus severity criteria, hyperparasitemia triggered all of the ECM remodeling mediators. Cerebral malaria and renal failure showed elevated levels for all except YKL 40, liver failure with heightened ENRAGE and GAL-3, severe anemia with GDF 15, and hemolysis with GDF 15 and GAL-3.

I Made Susila Utama (Prof Ngoerah Hospital, Indonesia) discussed the recent malaria characteristics at Prof Ngoerah Hospital, Bali. Utama raised the awareness that although Bali was malaria-free, it was still at risk of contracting malaria from travelers. As such, a descriptive study was done in the hospital from 2019 to 2023 regarding patient characteristics, type of *Plasmodium*, severity of infection and treatment outcome. From this, 37 cases were treated, 78.4% (29) being male patients from which up to 24.32% (9) had severe malaria but none died. *P. vivax* infections were the highest, 51.35%, followed closely by *P. falciparum* infections, 45.94%, and the lowest being *P. malariae* (2.71%). From severe malaria manifestations, jaundice was the highest occurrence at 77.77%, followed by renal dysfunction at 44.4%. The current treatment for uncomplicated malaria included treatment with 3 days dehydroartemycine (DHP) and primaquine (PQ) for 1 or 14 days. For severe malaria and *P. vivax* infections, artesunate IV was added into the treatment regime. Finally, for the treatment of *P. malariae*, artesunate IV and 3 days DHP were administered without the inclusion of PQ.

Symposium P08 - Introducing and Scaling up Emerging Diagnostic Solutions for Malaria Case Detection and Management

Win Han Oo (Burnet Institute, Australia) gave an account of the challenges in malaria case detection, pre-existing limitations, and emerging threats in the Asia-Pacific region. Current malaria diagnostic tools include clinical diagnosis, microscopy, rapid diagnostic tests (RDTs), and molecular methods. However, there are specific challenges in the Asia-Pacific region, such as subclinical malaria, which can represent a significant burden due to parasite reservoirs that often go undetected and untreated. Also, drug resistance and the need to detect and treat all malaria cases is another hurdle. Additionally, *hrp2/3* gene deletions cause false negatives in malaria RDTs. Detecting hypnozoites in *P. vivax* is also challenging due to its dormant asymptomatic liver-stage form, to address this, Han Oo introduced the G6PD test, which has proven to be an effective screening tool. All these challenges required the need for a robust quality assurance framework and the need for additional data for comparative performance. He drew attention to how critical case detection is with effective diagnostics, as timely and accurate treatment is necessary to detect and eliminate residual malaria transmission. He concluded by alluding to the limitations of current diagnostic tools at the programmatic level which warrants investment in research for continuous development.

Allison Golden (PATH, USA) discussed new diagnostic tools for case detection and management of malaria touching on the ongoing challenges in diagnosing malaria infections using microscopy and rapid diagnostic tests (RDTs). In *P. falciparum* detection, the emergence of *hrp2/3* gene deletions compromises RDT performance, requiring improvements in sensitivity. Analytical testing of malaria using next-generation RDTs can provide

comparative performance data to evaluate RDT results by benchmarking and running tests with panels, collecting data, and analyzing results for evidence and input. Golden simulated the clinical sensitivity of RDTs using laboratory analytical data compared to WHO-prequalified RDTs and demonstrated that improved RDTs will address *hrp2/3* gene deletions and provide enhanced sensitivity for PfLDH and PvLDH. Quantitative and semi-quantitative point-of-care G6PD tests are also currently available. Golden concluded that high-sensitivity *P. vivax* diagnostics alongside G6PD diagnostic tools can provide access to radical cures.

Yu Nandar Aung (Independent Researcher, Myanmar) analyzed the health economic considerations and value proposition for new diagnostic products to support malaria control and elimination. Malaria is one of hundreds of diseases contributing to the global burden of disease. Deciding its importance when there are competing priorities is based on value for money and affordability. Aung conducted a cost-effectiveness analysis to assess the budget impact of malaria burdens. She also used a case study from Lao PDR to understand the cost-effectiveness of G6PD testing for *Plasmodium vivax* radical cure and its budgetary implications. A decision tree model was used to evaluate the effectiveness of radical cure using primaquine (PMQ) under unsupervised and supervised conditions. The results showed that the supervised intervention was the costliest but also the most effective method. The G6PD quantitative test was found to be cost-effective in the setting of Laos. However, rolling out the G6PD test to all health facilities would be expensive and have a limited impact, while a selective rollout based on *P. falciparum* caseload would have the highest impact on the budget.

Van Ahn Ngo Thi (Health Poverty Action, Vietnam) spoke about the challenges and opportunities for the introduction of new products for malaria elimination in Vietnam, a country with low malaria burden, targeting *Plasmodium vivax* that contributes to most of the malaria cases in the country. It was stated that the number of malaria cases in Vietnam in the last decade had reduced significantly with only 17 out of 46 provinces yet to achieve elimination. To help Vietnam achieve its elimination target, Thi and his team launched a project that covered eight provinces, targeting endemic and border populations near these areas. She stated that the project conducted training for health staff to detect active cases and conduct G6PD testing which enables safe and effective *P. vivax* treatment with primaquines. Within one year they successfully achieved over 70% coverage of their targeted population with the G6PD testing. She mentioned the limited opportunity for application due to low endemicity as one of the challenges for implementing new products such as G6PD in non-endemic regions. She recommended routine training on the operating procedure of new products to mitigate the challenge.

Soy Ty Kheang (Health and Social Development, Cambodia) introduced new diagnostic tools for the radical cure of *P. vivax* in G6PD-d patients and shared lessons learned from Cambodia. The case study in Cambodia focused on equal access to safe radical cures. *P. vivax*, the predominant malaria species in Cambodia accounting for about 90% of cases, and a relatively high prevalence of G6PD deficiency. Key implementers are health centers (HCs) and village malaria workers (VMWs), who perform malaria diagnostic using diagnostic tests (RDTs) and provide first-line malaria treatment with artesunate-mefloquine (ASMQ). Patients detected by VMWs were referred to HCs. The survey enrolled over 1,000 *P. vivax* cases in target HCs from 2019 to 2020, with over 600 participants taking the G6PD test. The study reported over 100 cases of G6PD deficiency among the participants. Treatment with ASMQ from the survey led to a significant drop in hemoglobin levels by more than 25% after one week of the regimen, compared to patients on a two-weeks regimen. Therefore, the study proposed a follow-up one week into an eight-week ASMQ treatment. The health facility-community care model for *P.*

vivax radical cure, including patients with G6PD deficiency, proved to be feasible since the uptake improved quickly after implementation.

Plenary 6 – Genomic Epidemiology and Phenotypic Diversity of Malaria Parasites

David Conway (London School of Hygiene and Tropical Medicine – LSHTM, UK) presented the genomic epidemiology and phenotypic diversity of malaria parasites, noting the significant rise in malaria genomics publications over the past 47 years. He explained that advances in genome sequencing have greatly improved the understanding of molecular infections and cellular processes, contributing to a deeper understanding of the biology and genomics of malaria, in addition to the expansion of population genomic studies. Conway highlighted the variation in guanine-cytosine (GC) content and codon usage among malaria species, emphasizing their influence on genomic traits. He pointed out that chemogenomics is now being used to identify potential targets for antimalarial compounds. He also described how single-cell transcriptomics has provided detailed insights into parasite development, particularly through the expression of Gametocyte Development 1 (GDV1), which promotes sexual commitment and increases the expression of the antigen MSPDBL2. Conway mentioned that the discovery of genomic sequences with multiple copies has enhanced DNA detection which improves diagnostic accuracy. However, he raised concerns over the emergence of *hrp2/3* gene deletions, which compromises the effectiveness of rapid diagnostic tests (RDTs), alongside the rise of partial artemisinin resistance. He further explained that genomic studies have uncovered multiple sources of *Plasmodium falciparum* resistance to artemisinin, stressing the need for population genomic analysis to distinguish subpopulations. Additionally, Conway reported that *P. vivax* has a distinct genomic structure, with SNPs increasing the resolution of sub-populations. Finally, he pointed out that ancient parasite genomes provide insights into historical malaria in Europe, and that *Plasmodium knowlesi* has been divided into three genetic populations. He stressed on the necessity of studying both phenotype and genotype together, which could reveal critical information about parasite evolution and resistance development.

Symposium P23 - Innovative biocontrol approaches for mosquito-borne diseases elimination: Role and factors for success

Dickson Wilson Lwetoijera (Ifakara Health Institute - IHI, Tanzania) raised awareness about worldwide malaria cases. He stated that despite the death rates dropping by approximately 20%, the number of cases in the world still remained more or less unchanged for more than two decades. The progress for malaria eradication had started to reverse because of the impact of climate change, insecticide resistance, urban malaria vectors, humanitarian crisis, insufficient compliance, and others, so there is a need for novel tools for malaria eradication. This is where engineered gene drives come into play for population modification to propagate antimalarial effects. However, its development and implementation require sustained capacity building with regard to African stakeholders. Lwetoijera then elaborated on transmission zero for population modification via mosquito gene drives, the first transgenic mosquito made in Africa by Transmission Zero, the global malaria research programme developing genetic vector control strategies. The modification gene drive technology comprises separate strains for effector and drive functions where the effector trait was responsible for blocking *Plasmodium* transmission and the driver for propagating itself and effector in mosquito populations.

Leo Barack (Asia Pacific Malaria Elimination Network – APMEN, Singapore) emphasized the role of regional leadership as being essential to provide long-lasting, affordable, and sustainable solutions like gene drives. These novel approaches in regional malaria elimination

should include adaptability, scalability, application, cultural sensitivity, and compatibility with context in countries. He highlighted the role of regional leadership being pivotal in making this happen via the promotion of research and development through defined research priorities, resource, and funding allocation. Besides, regional leadership is also crucial in advocacy for policies in terms of its development and implementation as well as establishing regulatory frameworks. Lastly, he stressed the role of coordination and collaboration in determining multisectoral efforts with integration along with joint initiatives and shared knowledge to create platforms for further advancement of research.

Mouhamed Drabo (Imperial College London, United Kingdom) discussed strategies for gaining legitimacy with local communities in research projects. Target Malaria is a non-profit research association consisting of seven institutions from Africa, Europe, and North America. Their goal is to develop and share new, cost-effective, and sustainable genetic technologies to modify mosquitoes and reduce malaria transmission. The principles for engaging with Target Malaria prioritize reaching out to the most relevant groups, involving project representatives in the engagement process, and starting the engagement early while maintaining it consistently. In order to gain community trust, the project is committed to being open and accountable, undergoing external review, sharing relevant study results with stakeholders, and building community knowledge about malaria. Additionally, the project aims to conduct power analyses, establish active and sustainable community participation, and ensure transparency and effective communication. At the same time, it intends to maintain permanent dialogue, create partnerships with local leaders and community organizations, and establish feedback processes.

Cheong Huat Tan (National Environment Agency Singapore, Singapore) shared his experience with the *Wolbachia* project implemented in Singapore. The project aimed to reduce the mosquito population to a level that inhibits disease transmission. It was carried out in several phases in areas with a high risk of dengue and a high population of *Aedes aegypti* mosquitoes. A guidance framework for the sterile insect technique was established to assist in decision-making, testing, and implementation. He mentioned that the assessment of using *Wolbachia* to suppress *Aedes aegypti* should consider effectiveness, safety, and engagement with leaders, experts, and the public collectively. Tan further explained that strong collaborative networks between government agencies, academia, research partners, and industry are essential. To support the implementation of the project, it is important to leverage established infrastructure and networks. The release of male *Aedes* mosquitoes should be tailored to the local environment. Implementing data-driven strategies enables informed decision-making, optimizes strategies, measures effectiveness, and enhances both cost-effectiveness and scalability.

Symposium P20 - Accelerating towards zero vivax malaria – The Vivax Serology Partnership (VISPA)

Rhea Longley (Walter and Eliza Hall Institute of Medical Research – WEHI, Australia) explained the use of *Plasmodium vivax* serological exposure markers to uncover hidden malaria infections. Longley highlighted that resistance to traditional malaria control measures in *P. vivax* facilitated by hidden liver-stage hypnozoites remain a challenge for malaria elimination. Longley and her team explored the ability of serological markers to detect past and present infections in addition to detecting the possibility of recurrent infection. She stated that the prospects in serological markers led to the establishment of vivax serology partnership (VISPA), which aims to provide evidence to introduce serology-based identification that will help in integrating drug treatment interventions into global national policy and develop novel diagnostic tools based on their serological tests.

Rahmat Sagara (Oxford University Clinical Research Unit – OUCRU, Indonesia) discussed his efforts to quantify the impact of novel intervention strategies aimed at accelerating *Plasmodium vivax* elimination in the high-malaria-endemic region of Papua, Indonesia. He reported that Papua has the highest number of malaria cases in Indonesia. Sagara outlined Indonesia's malaria program strategy, which includes universal access to healthcare, surveillance, policy development, and empowering communities. He explained that multiple interventions are being used to eliminate the malaria burden, as part of an acceleration plan. Mathematical modeling was employed to estimate the impact of expanding malaria control efforts which included simulations of a range of intervention scenarios using serological diagnostic tools that quantified their impact, identified the optimal deployment strategies, and determined the required levels of G6PD testing. Based on his analysis, Sagara estimated the death rate, maximum age, and the entomological inoculation rate (EIR) at equilibrium. He concluded that, according to Indonesia's elimination plan, the country is expected to be malaria-free by 2030.

Rintis Noviyanti (National Research and Innovation Agency – BRIN, Indonesia) reported on her study using serological markers to predict *Plasmodium vivax* relapses in a cohort of returning Indonesian soldiers. She explained that malaria elimination efforts are hindered by *P. vivax* due to its ability to form asymptomatic, dormant hypnozoites in the liver, which can reactivate and cause relapses. Noviyanti's primary objective was to validate a sero-diagnostic test to identify soldiers who were carrying these dormant forms of the parasite. She mentioned two battalions of soldiers from Surabaya and Malang, deployed to the malaria-endemic region of Papua, who were involved in the study. According to her findings, some soldiers, particularly from the Malang battalion, experienced up to three malaria relapses within a six-month period. Plasma samples from these soldiers were analyzed using Luminex assays to detect antibody responses to *P. vivax* proteins. Noviyanti reported that a total of 592 samples were initially collected, with 553 remaining viable for analysis. She highlighted that median antibody titers for eight *P. vivax* biomarkers were measured over time, comparing those who relapsed to those who did not. Noviyanti concluded that these serological markers have the potential to serve as a novel public health intervention, aiding malaria elimination efforts by identifying individuals at risk of relapse.

Ayleen Alicia Kosasih (Oxford University Clinical Research Unit – OUCRU, Indonesia) presented the efficacy of serological screening and treatment to prevent *Plasmodium vivax* incidence among school children in North Sumatra, Indonesia in a randomized-controlled trial. Detection and treatment remain pivotal for the control and elimination of malaria in both endemic and non-endemic regions. This led to their study that evaluated the effectiveness of serological test and treatment (PvSeroTAT) intervention over a period of a year, with the aim of reducing malaria incidence among school children in the country. The study enrolled over 1,000 participants and the method was found to effectively treat *P. vivax* seropositive subjects and interestingly reduced the rate of recurrent parasitemia by over 50%. In addition, Kosasih pointed out that the method predicted children who were more likely to have recurrent *P. vivax* infection. She concluded that the intervention can help reduce recurrent *P. vivax* infection and reiterated that scaling up the intervention will help in halting malaria transmission in the region.

Rachael Farquhar (Burnet Institute, Australia) described the efforts to develop evidence-based policy and guidelines for the introduction of *Plasmodium vivax* serological testing and treatment strategies in the Asia Pacific region. She highlighted *P. vivax* as a major obstacle to reaching the 2030 malaria elimination goals in the region. Farquhar explained that the vivax serology partnership (VISPA) is collaborating with stakeholders to support National Malaria Control Programs (NMCPs) in implementing *P. vivax* serological testing and treatment

(PvSeroTAT). Interviews and focus group discussions are being conducted to assess the feasibility and readiness of health systems for PvSeroTAT in two scenarios: diagnosing relapse risk to guide radical cure and population-based screening for recent infections. Farquhar shared initial insights from surveys conducted after the 2023 APMEN vivax working group meeting, which aimed to identify barriers to serologically guided treatment at both the health system and community levels. VISPA has been implemented in several countries, including Laos, Cambodia, the Philippines, and Latin American nations. She underlined that the findings will help inform the communication toolkit for NMCPs to enhance engagement with PvSeroTAT strategies.

This report is brought to you by the MESA Correspondents Syaiful Rizal, Nurul Izza Zakaria, Muhammad Hafizu Sulaiman and Sam Jian Hung. Senior editorial support has been facilitated by Prof. Indra Vythilingam and Prof. Balbir Singh.

Day 4: Sunday, 22nd September 2024

Symposium P 12 - Eliminating Malaria in the Greater Mekong Subregion: How Can Success Be Ensured?

Sovannaroth Siv (National Center for Parasitology, Entomology and Malaria Control, Cambodia) explained the elimination approach in Cambodia, using the Last Mile elimination program to accelerate the elimination of *Plasmodium falciparum* in areas where remaining cases were focalized. The Last Mile intervention package includes the recruitment and training of village malaria workers (VMWs) and mobile malaria workers (MMWs), the provision of additional long-lasting insecticide-treated nets (LLINs) and long-lasting insecticide-treated hammock nets (LLIHNs), and conducting weekly fever screenings. It also provides intermittent preventive treatment for those going into the forest and targeted drug administration (TDA). Siv emphasized that by incorporating the Last Mile package, early detection of cases increased from 92% in 2021 to 100% in 2023. Additionally, the program ensured sufficient personal protection with 100% coverage using LLINs or LLIHNs, and 84.5% of participants completed two rounds of TDA within a one-month interval.

Chanaki Amaratunga (Mahidol Oxford Tropical Medicine Research Unit – MORU, Thailand) shared the development of the Triple Artemisinin-based Combination Therapies (TACT) project, called DeTACT, which was conducted at eight sites in Africa and two sites in Asia. Artemisinin-based combination therapy (ACT), the first-line treatment for malaria, has shown resistance in the Southeast Asia (SEA) region. The global spread of multidrug-resistant parasites could derail malaria elimination efforts and reverse the gains made in malaria control. The DeTACT project focuses on drug development, clinical trials, mathematical modeling, bioethics, market positioning, and communication and engagement. Preliminary results indicated that TACTs, specifically artemether-lumefantrine with amodiaquine (AL+AQ) and artesunate-mefloquine with piperaquine (ASMQ+PPQ), were well tolerated and safe to use. In Cambodia, randomized, partially blinded, placebo-controlled clinical trials demonstrated that AL+AQ is more effective than AL alone. A mathematical model also suggested that TACTs can delay artemisinin resistance and reduce treatment failure. Amaratunga emphasized the need for early engagement with the National Malaria Control Program (NMCP), to be crucial to ensuring a smooth transition to TACTs.

Frank Smithuis (Myanmar Oxford Clinical Research Unit, Myanmar) discussed the role of community health workers (CHWs) in malaria elimination in Myanmar. Access to healthcare services for these communities is extremely challenging. Most villagers only have access to untrained informal healthcare providers who do not offer malaria tests. Patients with undifferentiated fever receive a variety of inappropriate treatments. To overcome this issue, community health workers (CHWs) were trained, resulting in early detection and treatment of malaria, which led to reduced transmission. However, the malaria-CHW network is not cost-effective and is becoming less popular over time. Improvements were made by transitioning to an integrated CHW model that incorporates other disease management programs, such as tuberculosis, malnutrition, and non-communicable diseases. Integrating malaria into broader healthcare packages can improve the chances of eliminating malaria, as well as enhance overall health and save lives. This approach also helps to build community trust.

Cindy Chu (Lao-Oxford Mahosot Hospital Wellcome Trust Research Unit, Lao People's Democratic Republic) proposed the Southeast Asia Dose Optimization of Tafenoquine (SEADOT) trial to determine whether a 50% increase in the current tafenoquine dose (300 mg) in SEA can provide better radical cure efficacy in 4 months. The study proposed to start recruiting at consultation time for malaria diagnosis. Then, screening and consent along with

randomization in the study clinic was done and this marked day 0. Follow up in the study clinic was every week for the first month, and after that each month until the fourth. The challenges presented were several, the small number of individuals with malaria infections and the need of balancing multiple studies. Also, the cost per individual enrolled was very high as it included transportation and infrastructure at distal health delivery. The staff workload also posed a challenge along with the epidemiological changes in *P. vivax* showing different results. From there, Chu underlined the importance of funding, human resources and health systems for increasing the chances for success in research. Engagement in health systems, national programs, utilizing human resources from different global partners were identified as also crucial in driving quality research output.

Md Abul Faiz (Mahidol Oxford Tropical Medicine Research Unit – MORU, Thailand) addressed current malaria control and elimination tools evaluated and implemented in the Greater Mekong Subregion (GMS) such as targeted Mass Drug Administration (MDA), ITNs, vector control, among others. It was seen that targeted MDA could only achieve a transient reduction in malaria followed by resurgence. Therefore, it was hypothesized that the addition of mass R21/MatrixM (R21/MM) vaccination to current malaria elimination tools might provide additional protection against longer lasting interruptions. From this, a factorial, cluster-randomized controlled trial in Bangladesh is being prepared which will allocate 100 villages between four study arms (25 villages each): vaccines, MDA, combined vaccines, and MDA, or controls receiving the standard of care. This project, proposed to start in 2025, will have MDA and vaccine interventions given over 3 months, with a booster vaccination at 12 months. Passive case detection and prevalence surveys will be used to evaluate effectiveness over two years against *P. falciparum* infections. To make this an effective reality, multi-faceted community engagement was emphasized to enhance coverage and acceptability. Faiz then explained that novel communication tools such as posters, flipcharts, and short videos, will be created taking into consideration the multi-ethnic communities and vulnerable populations such as migrants and illiterate groups.

Malaria – Diagnostics 2

Dylan Pillai (University of Calgary, Canada) presented on active case detection (ACD) and treatment of malaria in pregnancy using Loop-Mediated Isothermal Amplification (LAMP) technology. Pillai hinted on worldwide over-exposure of malaria in pregnancy with over 30 million pregnant women at risk. Currently, intermittent preventive treatment (IPT) does not cover 1st trimester and is hindered by drug resistance and significantly poor compliance among pregnant women. They evaluated a promising molecular approach of LAMP that can screen and treat malaria in pregnancy at all trimester stages among over 2000 pregnant women in Ethiopia. The 3-year study demonstrated high sensitivity of LAMP in detecting asymptomatic malaria cases. They interestingly observed significantly greater weight gain in babies born under the LAMP method compared to the standard of care method. The study concluded the effectiveness and feasibility of molecular diagnostic tools such as lamps in malaria control among pregnant women in Ethiopia.

Phoutnalong Vilay (Ministry of Health, Lao People's Democratic Republic) presented an application of the LAMP method in Lao PDR to accelerate malaria elimination in the country. *P. falciparum* and *P. vivax* are major parasites of human malaria in Lao PDR, a country with the goal of achieving malaria elimination by 2030. *P. vivax* cases in the country continue to rise which are related to hidden hypnozoites and low-density infections that go undetected using conventional diagnostics. As such Villey together with his team proposed to implement the LAMP method to detect asymptomatic cases in 25 health facilities in endemic areas due to its advantages of sensitivity, specification, cost-effectiveness, storage flexibility, and quick

result processing time. The project trained researchers and health care workers from the endemic regions on LAMP for an effective operation and also aimed to provide new malaria guidelines that will incorporate LAMP in control interventions in the country.

Benoit Malleret (National University of Singapore, Singapore) presented a study that investigated the effect of methylene blue (MB) treatment on cerebral malaria in *P. coatneyi*-infected macaques. MB was originally synthesized for use as a textile dye. However, it has been reported to be used for the treatment of malaria in the 19th century and its effectiveness against even chloroquine resistant *Plasmodium* has been reported in recent years. The study administered MB as intravenous injection into six *Plasmodium* infected macaques. Their study revealed MB to be effective against cerebral malaria with an advantageous proactive effect that starts in the brain stem. Based on their findings, Malleret also revealed the potential of neutrophils in cerebral malaria pathogenesis and projects the use of macaques as models for severe malaria studies due to observed similarity of *P. coatneyi* to human *P. falciparum*.

Geoffrey McFadden (University of Melbourne, Australia) presented on the development of a simple kit to kill malaria parasites in mosquitoes. They fed mosquitoes by incorporating the drugs with sucrose and methylene blue to monitor feeding in the mosquitoes. Results showed that mosquitoes fed with the antimalarial atovaquone produced fewer sporozoites compared to other antimalarial drugs and the control group (no treatment). T111, a compound from atovaquone, seems to be responsible for this. He therefore presented T111 as a promising compound for malaria control in mosquitoes, having an interesting ability to eliminate sporozoites in *Anopheles* mosquitoes within one day. He further attributed the effect of T111 to its ability to act as an acridone inhibitor of cytochrome *b* in the parasite. Therefore, the kit incorporated T111 and sucrose onto a fabric for mosquitoes to feed with the objective to stop *Plasmodium* development in mosquitoes.

Alexander Maier (Australia National University, Australia) presented on harnessing cholesterol uptake in malaria parasites for therapeutic application. Malaria parasites rely on cholesterol for various activities including the host's organ inversion. However, the parasite does not produce cholesterol and hence relies on cholesterol produced in host cells other than the red blood cells. Maier sees an opportunity to use cholesterol as a weapon to deliver drugs that will effectively fight malaria parasites. His findings revealed that administering a conjugate of cholesterol and primaquine (Chol-PQ) increased the effectiveness of primaquine against asexual stages of *Plasmodium*. In addition, the combination was found to be less toxic to host cells and was also found to induce the uptake of new drugs and increase the efficacy of antimalarial drugs. Not only on malaria, the method was found to be effective against other non-malaria parasites.

Mohd Adilin Bin Yaacob (Institute for Medical Research, Malaysia) presented an in-house multiplex assay that can be used for the identification of *P. knowlesi* and *P. cynomolgi*. He hinted on zoonotic malaria as a threat to the elimination in the region. Zoonotic malaria transmission is increasing in the region compared to a decade ago with the increase in transmission of simian malaria and its associated undiagnosed cases. Using computational analysis, optimization of reaction, and multiplex environment, they developed and tested the performance of their multiplex assay. The assay successfully detected single infections with 100% specificity with minimal probe interference commonly seen in most Multiplex qPCR. However, the assay performance was reduced in quadruple infection cases, which they attributed to variation in parasitemia levels in the *Plasmodium* species investigated. He concluded that the assay provided a reliable and sensitive method for detecting mono-infections involving *P. knowlesi* and *P. cynomolgi*. However, further optimization of the assay is required for the detection of mixed infections.

Sophie Collier (The University of Melbourne, Australia) explained a study on organellar inheritance in *P. berghei*. Mitochondria and plastids are unique organelles, with uniparental (maternal) inheritance predominating in most eukaryotes, although other patterns of inheritance also exist. To investigate organellar inheritance in a sex-specific manner, Collier developed single-sex *P. berghei* lines with fluorescently tagged apicoplasts and mitochondria. She reported that using a combination of lattice light-sheet and expansion microscopy, they observed that the mitochondrion and apicoplast are excluded from newly formed male microgametes during exflagellation. By contrast, female gametocytes possess elongated, perinuclear positioned apicoplasts and an expanded mesh-like mitochondrial network that encapsulates or cradles the nucleus throughout. Upon screening 1.9 million sporozoites across seven crosses, they identified a single male leakage event, thus demonstrating for the first time that drug resistance encoded by the mitochondrial genome of malaria parasites can, very infrequently, be inherited from the male parent. Overall, this work will better inform future therapeutic strategies targeting these organelles.

Taofic Bouwe (Ryukyus University, Japan) presented a proactive case detection intervention for asymptomatic malaria infections in a district in the Lao People's Democratic Republic. Using a cross-sectional community-based survey they investigated the prevalence and factors associated with asymptomatic malaria infections involving over 600 participants in the region. Their study found prevalence of asymptomatic malaria of 1.8% out of 2% overall malaria prevalence from the study subjects. They interestingly found age to be a factor associated with asymptomatic cases with adults in their 40s having significantly higher asymptomatic malaria. Bouwe concluded that acquired immunity through repeated exposure in adults to be a contributing factor for the observed results. Bouwe also addressed protection measures and risk behaviour, noting that working at night in the forest and not using bed nets could increase the risk of being bitten by mosquitoes that spread malaria.

Zhaoqing Yang (Kunming Medical University, China) presented a study that shed light on the concerning trend of imported malaria cases in China with the aim to deepen understanding of the imported malaria cases and inform effective prevention and control measures in the country. Over the past decade, over 20,000 imported malaria cases were reported in the country, accounting for 98.71% of all malaria cases in the country, with *P. falciparum* as the major parasite responsible for the reported cases. She explained that the imported malaria cases were largely from Africa and with few from Asian countries. The trend according to Yang requires an urgent need for enhanced surveillance and management strategies, improved diagnostic capabilities, and more effective treatment protocols to address the imported cases.

Plenary 7 - Does prior exposure to vaccines or parasitic infection promote non-specific protection against unrelated pathogens?

Alan Sher (National Institutes of Health – NIH, United States) stated that childhood vaccination with Bacillus Calmette-Guérin (BCG) and helminth infections were hypothesized to provide microbial stimuli that might be conferring non-specific protection against COVID in resource limited countries/regions. From this, two different routes of BCG vaccination, subcutaneous (sc) and intravenous (iv), were delivered to mouse models, which were then infected with the SCV2 alpha variant of CoV-2. They found that the conventional cutaneous route provided no significant protection against subsequent SCV2 infection in mouse models but iv vaccination dramatically reduced the peak viral loads in the "mild" mouse model of SCV2 infection and protected against lethal disease in infected K18-hACE2 mice. However, the iv BCG vaccine (conventionally intradermal) is not clinically approved in practice. This study revealed potential new mechanisms of innate resistance to SCV2 for iv vaccination models.

Firstly, the protection against SCV2 induced by iv BCG vaccination was dependent on both T lymphocytes and the cytokine IFN-γ. Besides, IFN-γ itself was found to be sufficient in limiting viral load and preventing disease in SCV2 infection models. Finally, one proposed mechanism was that strong sustained local IFN-γ response was induced by iv BCG, which remodeled the pulmonary innate response and primed myeloid and epithelioid cells for enhanced control of pathogen challenge. Thus, IFN-γ priming of pulmonary epithelial cells enabled early control of SCV2 viral load. Prior *N. brasiliensis* (helminth) infections were found to partially protect mice against SARS-COV2 infection and disease. In contrast to iv BCG vaccinations, the effects of prior worm infection were late acting in sc (>7 days). The induced resistance was dependent on CD8+ T cells, simultaneously dependent on alveolar macrophages with alternatively activated M2 markers.

Symposium P27 (20952 & 20972) - Innovative Vector Control (SIT, Boosted SIT, ITT Wolbachia): Where Do We Stand About Their Efficacy Against the Vectors and Pathogens They Transmit?

Lu Deng (National Environment Agency, Singapore) presented a solution that provides high-throughput mosquito sex separation technology, within the context of the sterile male mosquito release approach. He discussed the limitations of existing sex separation methods used in Incompatible Insect Technology (IIT) which hinder the effectiveness of large-scale releases of sterile male mosquitoes. To address these limitations, the team under the Project Wolbachia Singapore developed a novel technology designed to optimize larval rearing conditions, thereby increasing the production of male over female mosquitoes for mass release. The internationally patented technology provides accurate 1st insta larvae counting, optimal larval rearing density, optimal larval food, accurate feeding regime, and environmental control. The technology tested in a study involving over 40,000 households was found to effectively reduce 90% of the *Aedes* population. According to Deng, this breakthrough will significantly scale up the IIT strategy, improving the effectiveness of efforts to control *Aedes* mosquito populations. This advancement represents a critical step forward in the fight against mosquito-borne diseases, not only in China but in other countries fighting vector-borne diseases. Deng mentioned that the technology has already been adopted by 16 countries around the world.

Josiane Etang (University of Bertoua, Cameroon) discussed a community-oriented approach to managing mosquito vectors and insecticide resistance. Vector-borne diseases account for 17% of the global burden of communicable diseases. Etang systematically reviewed 53 articles and presented her findings on mosquito-borne disease burdens in urban and rural Cameroon from 2002 to 2021, revealing a high prevalence of malaria in urban areas and increases in *Anopheles* species and *Aedes aegypti*. She outlined Kdr mutation, 1014F and 1014S as common resistance markers found among mosquitoes in the country. Interventions included distributing insecticide-treated bed nets (ITNs), improving environmental management, eliminating aquatic plants, managing water levels, and removing breeding containers. Key challenges identified were a lack of community awareness, insufficient coordination between health sectors, gaps in healthcare and community health workers, lack of education, and limited financial support. Potential new vector control tools include insecticides with various modes of action, spatial repellents, vector traps, and integrated approaches combining vector control with medicine and vaccines.

This report is brought to you by the MESA Correspondents Syaiful Rizal, Nurul Izza Zakaria, Muhammad Hafizu Sulaiman and Sam Jian Hung. Senior editorial support has been facilitated by Prof. Indra Vythilingam and Prof. Balbir Singh.

Day 5: Monday, 23rd September 2024

Malaria - Epidemiology 2

Yee Ling Lau (University Malaya, Malaysia) explained new diagnostic options to detect malaria in Southeast Asia and beyond. In 2022 based on WHO information, there were an estimated 5.2 million malaria cases and 90,000 deaths in South East Asia. Existing diagnostic methods including microscopy, rapid diagnostic tests (RDTs), and nested PCR have disadvantages. Lau talked about a new method very sensitive and specific, and cheaper than the existing ones called loop-mediated isothermal amplification (LAMP) which is based on the amplification of the DNA. Some advantages of this technique are that it requires a short time, does not need a thermocycler, is cost effective, and can detect 0.001% parasitemia. Based on a clinical evaluation in the Kapit Hospital in Sarawak from April to September 2022, it had 100% sensitivity and 97.8% specificity.

Tam Thanh Le (Ministry of Health, Vietnam) (Ministry of Health, Vietnam) analyzed malaria trends in Central Vietnam from 2018 to 2022 using a Bayesian approach. Malaria remains a major public health issue worldwide. Le collected data on malaria cases, environment, climate, and population from 2018-2022, and used zero-inflated Poisson regression with a Bayesian framework for spatio-temporal modeling. The results showed a decrease in malaria incidence over this period. The modeling also showed the risk of *Plasmodium falciparum* increased fivefold with a 1-unit rise in the normalized difference vegetation index (NDVI) and by 8% for every 1ºC rise in maximum temperature (TMAX) with a 6-month lag. A 1 mm increase in precipitation with a 6-month lag reduced risk by 1%. For *P. vivax*, a 1-unit NDVI increase at a 1-month lag raised risk fourfold, with an additional 6% and 3% risk increase for each 1ºC rise in daytime land surface temperature and TMAX at a 6-month and 4-month lag, respectively. Spatial analysis showed higher malaria risk in the Central Highlands and southeast regions of Central Vietnam. These findings are important for planning strategies to eliminate malaria in Vietnam.

Win Han Oo (Burnet Institute, Australia) discussed a personal protection package aimed at reducing malaria transmission among forest-going mobile and mobile and migrant populations (MMPs) in Cambodia and Lao PDR. This package was tested using a cluster-randomized controlled trial with a nested mixed methods study. He mentioned that countries in the Greater Mekong Subregion (GMS) were committed to eliminating malaria by 2030, but current vector control interventions were insufficient, especially in preventing early outdoor biting by dominant vector species. The trial, conducted in 488 villages, distributed a personal protection package, including long-lasting insecticidal hammock nets, picaridin insect repellent, and health communication pamphlets, to MMPs. He noted that knowledge, attitudes, and personal protection practices among MMPs improved by the end of the trial compared to the baseline. Furthermore, MMPs expressed a willingness to continue using the package due to its convenience in their workplace. Overall, he concluded that the personal protection package was effective in reducing malaria prevalence among MMPs, acceptable to both the MMPs and health stakeholders, and could be considered for national and regional scale-up to support regional malaria elimination efforts.

Wilson Tavares (University of Maryland, United States) described the population structure of *P. falciparum* in southwestern Africa, using data from Angola. He explained that malaria is a major cause of mortality and morbidity in Angola, with over 9 million cases and more than 13,000 deaths in 2022. Tavares collected dried blood spot samples between January and April 2022, after which DNA was extracted and sequenced using the Illumina NovaSeq 6000. He reported that the results indicated low but significant genetic differentiation. Tavares noted

that clinical isolates from provinces with unstable transmission exhibited very low complexity, and population analysis revealed low but significant genetic differentiation. He further mentioned that these results, combined with additional samples from Angola, would be used for a detailed study of migration within the country.

Zulkarnain Md Iris (Universiti Kebangsaan Malaysia, Malaysia) stressed the need for new and improved screening tools and strategies for detection and management of very low-density parasitemia in the field. Therefore, the objective was to evaluate malaria prevalence by integrating molecular and serological measures among hard-to-reach indigenous Orang Asli communities. Samples of 1954 individuals who appeared healthy showed no malaria parasites found with microscopy, but molecular testing revealed 7 cases of *P. knowlesi* mono-infection (0.4%), with no human malaria detected. This highlighted the importance of molecular detection among the hard-to-reach Orang Asli population in Malaysia. Besides there were higher proportions of *P. knowlesi* observed in the 19-40 age group of Orang Asli communities. The Basic Local Alignment Search Tool (BLAST)search of cox3 sequences of *Plasmodium* spp. also revealed identity ranges from 99.31% to 99.77%, showing high sequence similarity that reflects common ancestry to other *P. knowlesi* isolates. It was also found that humoral antibodies against *P. falciparum* antigens were higher than *P. vivax* antigens since *P. falciparum* circulates at higher parasite density that induces higher antibody levels, making it more detectable.

Sophie Zaloumis (University of Melbourne, Australia) created a mock study to predict the artemisinin resistance status of *P. falciparum* malaria isolates utilizing *in vivo* transcription data. The simulation was based on published transcriptomic data and tracking resistance to artemisinin collaboration (TRAC). The predictors were simulated *in vivo* transcriptomics data from a multivariate normal distribution with 1043 participants and an abundance of 5061 mRNA transcripts. The outcome was a slow clearing *P. falciparum* infection expressed as parasite clearance half life. This resulted in training data that fit well with machine learning methods (low bias). The test achieved a min of ~0.101 error at the largest training set size of 835. The gap between training and test curves was small at the largest training set signifying low variance. In conclusion, a minimum data set of 1043 (training set, 835 and test set, 208) was required to develop a prediction model that can achieve a balanced error rate of 0.101 for the prediction of slow clearing *P. falciparum* infections using in vivo transcriptomics data.

Rita Reyburn (World Health Organization, Lao People's Democratic Republic) reported a decline in malaria cases from 2018 to 2022. However, persistent localized transmission hotspots within high-risk populations remained a challenge. Interventions were introduced in target villages which include political advocacy, community engagement, village census, top up of long-lasting insecticidal nets, targeted drug administration, and monthly intermittent preventive treatment for forest goers. After implementation, the target districts reported 81% fewer cases of *P. falciparum* in 2022 and 47% fewer cases of *P. falciparum*, compared to 2019-2020. A time series analysis was done to assess the impact of these strategies from 2019 to 2024. For the *P. falciparum* and *P. vivax* results, the model demonstrated no significant immediate effect, showing a sustained decline in cases after implementation of 10% and 12% respectively. The declines were also due to strong routine interventions and the depletion of the parasite reservoir in the source communities within the district.

Frantisek Stejskal (Charles University, Czech Republic) spoke on malaria imported to the Czech Republic, specifically in the University Hospital Bulovka. With a total of 260 patients with malaria from 2006 to 2024, it represented 55.6% of all cases imported to the Czech Republic. *P. falciparum* was the dominant species at 77.7% followed by *P. vivax* (16.5%), *P. ovale* (3.5%) and *P. malariae* (2.3%). The origin of patients with malaria were mostly from

Africa (75%), Southeast Asia (15.7%) followed by South Asia (4%), Latin America (4%), and the airport (<1%). Severe falciparum malaria was reported in 46 cases with a male:female ratio of 3.2:1 at median age of 44 years old which contributed to 4 deaths. The highest complications for patients with severe malaria were hyperparasitaemia and jaundice. It was observed that the laboratory parameters for patients with severe falciparum malaria who died showed higher levels of white blood cell counts, hemoglobin levels, C reactive protein, and lactate levels.

Symposium P19 - Japanese innovations for the global malaria elimination: Zero malaria campaign by 2030

Shigeyuki Kano (National Center for Global Health and Medicine, Japan) addressed the global issues of malaria elimination, to which Japan can contribute. The Okinawa Infectious Diseases Initiative and the Global Fund were introduced in 2002 as a result of the G8 Kyushu-Okinawa Summit. In 2014, Asia-Pacific leaders committed to achieving a malaria-free region by 2030 at the East Asian Summit, a goal also adopted by the African Union in 2016. Kano addressed several challenges to malaria elimination, such as drug resistance, asymptomatic reservoirs, infections caused by *P. knowlesi*, and the presence of *P. vivax* hypnozoites. He also highlighted strategies for malaria elimination, including vector control for prevention, case management, chemotherapeutic interventions, new diagnostic tools or vaccines, surveillance, monitoring and evaluation, and the strengthening of health systems for resilient, sustainable health and universal health coverage. These strategies were developed in depth by the following speakers. He then briefly introduced two organizations involved in malaria elimination in Japan: Malaria No More Japan (MNMJ) and the National Center for Global Health and Medicine (NCGM).

Ray Nishimoto (Koei Chemical Co. Ltd., Japan) provided an overview of integrated vector management by discussing the history of Sumitomo Chemical products recommended by the World Health Organization (WHO). The company produces insecticide-treated nets (ITNs), larvicides, space sprays, and indoor residual sprays. Insecticide resistance in mosquitoes has spread widely in the African and Asia-Pacific regions. WHO has recommended seven classes of insecticides: organochlorines, organophosphates, carbamates, pyrethroids, pyrroles, neonics, and meta-diamides. The private sector faces challenges such as a small market with fierce competition, expensive R&D and registration costs. Nishimoto highlighted that continuous innovation is essential to eliminate malaria, and improved tools and approaches are needed to manage resistance. Therefore, product development partnerships have become the driving force in developing new tools. With political will and regulatory harmonization, the Asia-Pacific region has the opportunity to demonstrate global leadership in eliminating malaria.

Aya Konishi (Sysmex Corporation, Japan) introduced the XN-31, an automated hematology analyzer. This analyzer can provide qualitative results for malaria-infected red blood cells, including counting and percentage, automatically within one minute. It offers a sensitivity of 20 parasites/μL and requires only a low level of expertise. On the contrary, utilizing flow cytometry techniques, it produces results in a scattergram pattern. In a study conducted in Thailand to assess the performance of the XN-31 in detecting both symptomatic and asymptomatic malaria infections, the results indicated that in symptomatic patients, the sensitivity of the XN-31 was comparable to microscopy and superior to rapid diagnostic tests. Thus, the instrument can support early diagnosis and treatment of malaria.

Keiko Watanabe (Eiken Chemical Co. Limited, Japan) discussed a simple and rapid diagnostic method for malaria elimination. Loop-mediated isothermal amplification (LAMP) addresses

many of the problems associated with commonly used diagnostic methods such as RDTs, microscopy, and PCR. Among the advantages of this molecular method is its ability to detect asymptomatic cases in patients with low parasitemia that go undetected by other methods. The LAMP Test Kit comes with free maintenance, an 18-month shelf life, a five-year life expectancy, and flexible storage conditions. Watanabe further discussed the operating procedure and reported the performance of the test kit. She concluded that LAMP is a new and promising tool for the diagnosis and surveillance of malaria in elimination settings.

Kazuhiko Yano (National Center for Global Health and Medicine, Japan) presented a promising antigen that can be used in malaria vaccine development and also serve as a target for monoclonal antibody drug development. Enolase is an enzyme that acts as an antigen in malaria infections. The antigen has been reported to be easily recognized by antibodies in malaria patients. Patients' sera were used in their study to extract and screen proteins for the specific enolase antigen, 47 kD. Rabbits were immunized with the synthesized form of the antigen, leading to the production of polyclonal antibodies. The antibodies were found to significantly inhibit *Plasmodium falciparum* in vivo. To validate effectiveness, Yano introduced the synthesized antigen AD22 into mice and monkeys. All animals produced antibodies that reduced parasitemia levels and delayed disease severity. The study also developed a human monoclonal antibody targeting AD22, with potential for use in the treatment of drug-resistant malaria. He regarded this finding as an innovative contribution to the fight against malaria.

Symposium P18 - Ivermectin and Malaria EliminationMalaria – Molecular Biology

Kevin Kobylinsky (Mahidol Oxford Tropical Medicine Research Unit – MORU, Thailand) presented the use of ivermectin in the fight against malaria. Ivermectin is an antiparasitic drug that has been successfully used to control diseases such as onchocerciasis and lymphatic filariasis, among others. Surprisingly, ivermectin was found to have a lethal effect on malaria vectors. He shed light on the lethal effect of ivermectin on *Anopheles* mosquitoes feeding from long-lasting ivermectin-injected cattle and buffalo. Their study found that ivermectin caused mortality in mosquitoes for over 70 days post-treatment in cattle. In buffalo, however, some mosquitoes resisted the treatment; thus, he recommended further studies to determine the ideal dosage of long-lasting ivermectin.

Joel Tarning (Oxford University, United Kingdom) presented a study that investigated ivermectin's pharmacometric properties and its lethal effect on mosquitoes. Their previous study observed differences in mosquito mortality, in vitro and in vivo. As such, they used in vitro and clinical samples to isolate, characterize, and measure ivermectin metabolites. The study also investigated ivermectin's lethality on mosquitoes and drug interactions in human volunteers. Three novel ivermectin metabolites were isolated and synthesized which were found to have a similar mortality effect on mosquitoes as ivermectin. Additionally, the study modeled pharmacometrics and pharmacodynamics to predict drug dosing scenarios for ivermectin administration in treatment and mass drug administration.

Achaporn Yipsirimetee (Mahidol Oxford Tropical Medicine Research Unit – MORU, Thailand) discussed the activity of ivermectin against the asexual and sexual stages of *Plasmodium falciparum* and its interactions with antimalarial drugs. Ivermectin is a complementary malaria vector control agent that reduces *Anopheles* mosquito survivorship, subsequently decreasing malaria incidence and prevalence. Ivermectin acts on the mosquito, liver, and blood stages of *Plasmodium*. A study on the asexual and sexual blood stages of artemisinin-sensitive and artemisinin-resistant *P. falciparum* showed no difference in half-maximal inhibitory concentration (IC50) values between the two. Male gametocytes were more susceptible to ivermectin than female gametocytes. In vitro, drug interaction tests between ivermectin and

eight antimalarial drugs resulted in no interactions. Thus, Yipsirimetee concluded that ivermectin was unlikely to interfere with the antimalarial activity of commonly used antimalarial drugs.

Wang Nguitragool (Mahidol University, Thailand) presented a cluster-randomized trial of mass ivermectin administration in Surat Thani, Thailand, from 2017 to 2023. Surat Thani was selected due to low migration, the presence of *Plasmodium falciparum* and *P. vivax* in the area, and its homogeneous ecology. A baseline survey was conducted in 2019, and mass drug administrations (MDAs) of ivermectin began in 2022. This trial aimed to evaluate the impact of repeated ivermectin MDAs on malaria transmission. Ten treatment and ten control clusters were formed, with each cluster containing 300 participants. Three rounds of MDAs were performed. Adverse events such as dizziness, drowsiness, and diarrhea were reported among the participants, leading to a decrease in participation in the second and third rounds of MDAs. No impact was observed in the trial, which might have been due to the relapse of *P. vivax*, the low prevalence of malaria, migration of participants and mosquitoes, as well as low treatment coverage, which was only 60% to 70%.

Malaria – Epidemiology 3

Chua Hock Hin (Sarawak General Hospital, Malaysia) addressed that the malaria cases in Sarawak were mostly zoonotic in origin from *P. knowlesi*, followed by imported human malaria of either *P. vivax* or *P. falciparum*. The study objective was to assess the epidemiology and risk factors for malaria deaths from 2014 to 2023 in Sarawak. A total of 27 deaths were reviewed, where 92.6% of them were local natives, median age of 63 years old and male:female ratio of 10:17. Fever was reported as the most common symptom (85%). A big obstacle was the mean time from patient symptom onset to first presentation to health care which was ~ 5.3 days while to malaria diagnosis was ~ 5.5 days. This contributed to some patients experiencing death which took less than 3 days (from presentation to death) due to prolonged time before first presentation and diagnosis. The mean malaria parasite count was 157,879/uL of blood which was high. The treatment that was given to all the patients was IV artesunate, with one exception of oral chloroquine.

Odai Sichanthongthip (Centre for Malariology, Parasitology and Medical Entomology – CMPE, Lao People's Democratic Republic) conducted a surveillance assessment in Lao PDR with the WHO Malaria Surveillance Assessment Toolkit, about 1) performance, 2) context and infrastructure, 3) technical and process and 4) behavior to provide a set of evidence-based recommendations to identify surveillance issues and bottlenecks. Sites that were selected for data collection were provinces with burden reduction districts, only elimination districts, and with no reported malaria cases in the previous 5 years. The overview result of the surveillance assessment showed 1) performance at 76% which recommended transitioning towards a single data storage system, 2) context and infrastructure at 70% with collection of case investigation and travel history, expansion of malaria surveillance with Mobile Malaria Workers, 3) technical and process at 67%, with new dashboards and validation rules to be entered in data storage system to avoid transcription errors and 4) behavior was at 70%, with supervision form included in the data storage and refresher trainings. The new surveillance system was updated based on these recommendations for 2024.

Hyun-Il Shin (Korea Disease Control and Prevention Agency, Republic of Korea) performed molecular analysis using four genes to determine patient cases and a mdr-1 gene to find drug resistance for *P. vivax* malaria patients in the Republic of Korea. The four protein subtypes found were PvMSP-1, PvAMA- 1, PvCSP, and PvDBP. In 2023, out of the 14 recurrence cases, 1 case of recurrence and 13 cases of relapses were found. Out of the 33 suspected cluster

cases, 13 cases were confirmed to have the same genotype between patients. Through cluster analysis identified with the same genotype, it was possible to identify areas where patients and vectors should be intensively managed. Besides, no drug resistance mutation (PvMDR y976F) was found. Although no mutation in the resistance gene to chloroquine has been observed, there is still a need for continuous molecular epidemiological surveillance annually.

Nisa Fauziah (Universitas Padjadjaran, Indonesia) addressed how Indonesia still faces challenges in imported malaria cases, which originated from endemic areas like Eastern Indonesia. The study focused on malaria surveillance data in West Java, focusing on specific challenges encountered for improved insights and strategies. From 2019 to 2023, a total of 2013 cases were reported, where six were indigenous from the Pangandaran regency, and the rest were imported. Pangadaran is a coastal area with high population mobility and tourist activities, making it an ideal breeding ground for malaria vectors. Furthermore, Cimahi City and Depok City gave the highest number of malaria cases with all cases being imported, because they serve as major military bases, often deploying soldiers to malaria-endemic regions. Men had higher cases than women, because of their higher involvement in outdoor occupations. The age group of 15-64 years old was also highly infected with malaria because of their activity and mobile segment. Besides, soldiers and police officers had the highest risk among other occupations due to the deployment nature of their work to malaria-endemic areas.

Fedri Rinawan (Universitas Padjadjaran, Indonesia) stressed that host migration and cases from work travel or tourism could potentially cause local transmission. The relationship between malaria and natural environmental risk factors (NERFs) was investigated in this study. The malaria data came from the malaria information system (e-SISMAL) from 2019 to 2021. The distribution mapping of the estimated regression coefficients and the standard deviation of the residuals was then analyzed using Ordinary Least Square (OLS) in ArcGIS Pro. Results showed the Koenkers test of p-value 0.055620, with stationarity ($p>0.05$), which implies stable relationships in the spatial context and good linear models for the predictions. Additionally, the Jacque-Bera test result (p-value 0.000000) indicated a non-normal residual. Malaria was explained by 4.03% (R2) of the NERFs. Almost all NERFs contributed significantly. From the analysis results, household and personal factors were suggested as the main parts of the prevention. Rinawan provided a recommendation that emphasized advocacy to the government regarding the risk areas (collaborative surveillance) to prepare for future malaria cases.

Henry Surendra (Monash University, Indonesia) discussed the application of serological surveillance to assess malaria transmission in areas with varying endemicity across Indonesia. He explained that to achieve elimination, it is becoming increasingly important to identify and target transmission foci. Surendra proposed using serological assessments to estimate malaria transmission intensity in three endemic settings in Indonesia, utilizing both ELISA and Luminex MAGPIX. He reported varying results across these settings. According to him, this method allows for the analysis of community-based serological data to confirm malaria elimination and to identify clusters with high exposure in areas that have reported zero cases over the last three consecutive years, health facility-based serological surveillance can predict receptive areas at risk of malaria and seropositivity to Etramp5.Ag1 in children is a potential marker of recent *P. falciparum* exposure in the population.

Nur Faeza Abu Kassim (Universiti Sains Malaysia, Malaysia) spoke about the application of alginate-gelatin hydrogel beads (AGHB) in controlling mosquito populations within high-risk building areas. She explained that mosquito-borne diseases have been a serious global issue

for the past 50 years, causing 390 million infections per year in humans. According to her, communities in high-risk buildings are more vulnerable to these diseases, and traditional mosquito control methods have become less effective. Kassim conducted a study in a rural area near a forest with dense vegetation, which provided a strategic environment for the *Aedes* mosquito population. She used hydrogel sugar bait technology, which offers several advantages, and followed three steps: pre-treatment, treatment, and post-treatment. Data analysis, performed using the Kruskal-Wallis H test, revealed a statistically significant difference between indoor and outdoor environments. Kassim concluded that AGHBs were more effective indoors, particularly against the targeted species *Aedes aegypti*.

Olawale Quazim Junaid (Universiti Malaya, Malaysia) discussed the prevalence and risk factors of non-febrile malaria co-infection with hepatitis B virus (HBV) antigen among pregnant women seeking antenatal care in Damaturu, northeast Nigeria. He noted that in 2022, malaria accounted for an estimated 608,000 deaths worldwide, with 96% of these deaths occurring in Africa, and that it can cause prenatal mortality during pregnancy. According to Junaid, blood samples were collected and processed using hematology analysis, HBV antigen tests, thin and thick film microscopy, and rapid diagnostic tests (RDTs), while volunteers also completed questionnaires. The results revealed that 129 (33.6%) of the women tested positive for malaria, 25 (6.5%) for HBV, and 20 (5.2%) had co-infection. Junaid reported that pregnant women over the age of 38 showed the highest prevalence of malaria at 43.5%, HBV at 13.0%, and co-infection at 13.0%. He concluded that while the high prevalence of malaria among pregnant women could be fatal, the low prevalence of HBV infection and co-infection with malaria could still negatively impact pregnancy outcomes.

Eddy Octavio Martinez Avendaño (Universidad Mayor de San Andrés, Bolivia) described the situation of *P. falciparum* in Bolivia, from its imminent elimination to its reintroduction and rapid spread. He explained that the Amazon is the most malaria-endemic region of the Americas, and northern Bolivia, being part of this region, accounts for over 90% of malaria cases, primarily due to *P. vivax* and *P. falciparum*. According to Avendaño, *P. falciparum* was reintroduced in 2019 by an infected individual from Brazil, which led to local transmission and a subsequent rise in cases. He highlighted that the exclusion of support from collaborators (volunteers) in the diagnosis and treatment of cases, along with socio-political conflicts related to the 2019 government change and the COVID-19 pandemic, may have compromised achievements in malaria control. Avendaño reported that, currently, about 90% of malaria cases are concentrated in just nine Amazonian municipalities. However, he emphasized that the reintroduction and reestablishment of *P. falciparum* transmission serves as a reminder that malaria surveillance requires significant effort, even when cases are sporadic or reporting remains at zero.

Keynote 2 - Antimalarial Drugs: Past, Present and Future

Sir Nicholas White (Mahidol Oxford Tropical Medicine Research Unit – MORU, Thailand) explained the antimalarial drugs: past, present, and future. In 2022, there were an estimated 249 million malaria cases and 608,000 deaths. White dwelled into the history of antimalarial drug discovery addressing ongoing challenges in controlling malaria. He emphasized that despite current efforts, malaria mortality is still not accurately estimated and called for more funding and new tools but questioned how effectively they are being utilized. The history of plant-based treatments, starting with quinolines from the cinchona tree, was discussed along with William Henry Perkin's attempt to synthesize quinine, which failed but led to the discovery of a dye instead. Charles Ledger later found an abundance of quinine-producing plants in the Peruvian forest and sold seeds to the Dutch, who dominated 90% of the market, benefiting global supply. White noted that methyl chloroquine and quinidine were more effective but

toxic, leading to the widespread use of chloroquine. When resistance emerged, the focus shifted back to quinine, and later to artemisinin, discovered through Project 523 in China. Artemisinin, particularly artesunate, proved highly potent, with artesunate reducing malaria mortality by one-third compared to quinine. When ACT (artemisinin-based combination therapy) was introduced, questions remained about proper dosage and patient suitability. White raised concerns about artemisinin resistance and concluded that combining existing medications could be the way forward in combating malaria.

Closing Ceremony

The 21st International Conference of Tropical Medicine and Malaria 2024 (ICTMM 2024), held in Kuching, Malaysia, from 19-23 September 2024, concluded with a closing ceremony where notable individuals—including leading experts in malaria research, poster presenters, and contributors to both ICTMM 2024 and malaria research—along with esteemed institutions, were honored for their exceptional contributions. **Prof. Dr. Siti Nursheena Mohd Zain**, Organizing Chair of ICTMM 2024, expressed her appreciation to sponsors, partners, and the Malaysian Government for their support in making the event a success. She stressed the importance of collective efforts to speed up the fight against malaria. **Dr. Lucas Low Van Lun**, President of the Malaysian Society of Parasitology and Tropical Medicine (MSPTM), used the occasion to thank everyone involved in organizing ICTMM 2024 and acknowledged the contributions of all the participating scientists. He also mentioned that MSPTM will hold the 4th Asia Pacific Rickettsial Conference (APRC4) from 29 September to 1 October 2025 in Penang, Malaysia. The conference was officially closed by **Prof. Dr Malcolm Jones**, President of the International Federation for Tropical Medicine (IFTM), who also announced that ICTMM 2028 will take place in Liverpool, United Kingdom.

This report is brought to you by the MESA Correspondents Syaiful Rizal, Nurul Izza Zakaria, Muhammad Hafizu Sulaiman and Sam Jian Hung. Senior editorial support has been facilitated by Prof. Indra Vythilingam and Prof. Balbir Singh.

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