Resource HUB *Correspondents*

Current Tools to Combat Malaria -
with Special Focus on *Plasmodium knowlesi* workshop

Complete series

MESA Correspondents bring you cutting-edge coverage from the <u>Current Tools to Combat Malaria-with Special Focus on Plasmodium knowlesi workshop</u>.

March 4 – 8, 2019
Faculty of Medicine, University of Malaya, Malaysia

MESA

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Background

This workshop was conducted by the Department of Parasitology at the Faculty of Medicine, University of Malaya, from 4-8 March 2019. It consisted of a series of lectures and practical sessions.

Countries in the region are working towards malaria elimination and *Plasmodium knowlesi* malaria is the predominant species affecting humans in Malaysia. All countries in Southeast Asia have reported cases of *P. knowlesi*. The Department of Parasitology of the University of Malaya is at the forefront of knowlesi malaria research and strives to share knowledge with other institutes and is always sensitive to global needs. Thus, participants attending this workshop gained valuable information and techniques that they can bring back and implement in their respective institutes and universities. It also served as a platform to interact with various research institutes and universities within the country as well as the region to foster collaboration on *P. knowlesi*.

The workshop had 20 participants from eight countries which are as follows: Bangladesh, Cambodia, China, Indonesia, Malaysia, Philippines, Singapore and Thailand.

The University of Malaya received support from APMEN and the MESA Alliance, hosted by ISGlobal.

Introduction written by Dr Indra Vythilingam

Day 1: 4th March 2019

Opening Ceremony

The Opening Ceremony of this workshop was presided over by **Prof Dr Indra Vythilingam**, the Chairman of the workshop, and officiated by **Prof Dr Ng Chirk Jenn**, the Deputy Dean of Research, Faculty of Medicine, University of Malaya. This workshop was attended by 20 delegates from eight countries, namely Bangladesh, Cambodia, China, Indonesia, Malaysia, Philippines, Singapore and Thailand.

Session 1. Introduction and history of *Plasmodium knowlesi*

A morning session led by **Prof Dr Fong Mun Yik**, introduced the history of *P. knowlesi*. *Plasmodium knowlesi* was discovered in 1927 in its natural simian host, the long-tailed macaque *Macaca fascicularis*. This primate malaria parasite is commonly found in Southeast Asia and the first case of human infection of this species was reported in 1965 in Malaysia. In addition to *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*, *P. knowlesi* is now recognized as the fifth species of *Plasmodium* causing malaria in humans. From time to time, variants and/or strains, or subspecies of *P. knowlesi* have been isolated, but unfortunately, the typical *P. knowlesi* is no longer available for comparative studies. Historically, *P. knowlesi* was used for the treatment of neurosyphilis, however, the infection became so virulent that it had to be terminated with drugs. Dr Fong Mun Yik also pointed out that the morphological similarities between *P. knowlesi* and *P. malariae* necessitate the use of molecular methods for a correct identification.
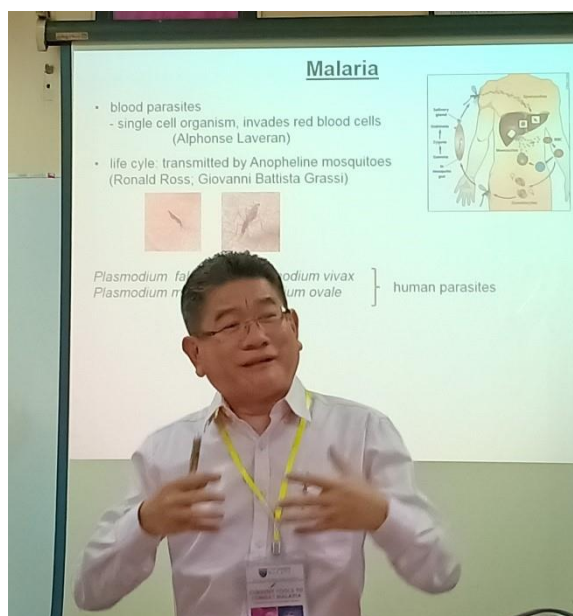

Dr Fong Mun Yik giving his talk on the history of P. knowlesi

Session 2. Public health importance of knowlesi malaria

The public health importance of knowlesi malaria was also lectured by **Dr Fong Mun Yik** in session 2. The transmission of knowlesi malaria in Southeast Asia has been often associated with the natural distribution of mosquitoes of the *Anopheles leucosphyrus* group and the long-tailed macaques. In Southeast Asia, knowlesi malaria is of particular importance in Malaysia, where an increasing trend in the number of cases has been seen. Deforestation and associated environmental changes are the key drivers in *P. knowlesi* transmission. Human behavioural factors with *P. knowlesi* could be targeted in future public health interventions. Dr Fong Mun Yik emphasized that early diagnosis of *P. knowlesi* infection is crucial because the severity is related to its short erythrocytic cycle of 24 hours which may rapidly lead to hyper-parasitaemia. Species diagnosis on admission microscopy was incorrect in 90% of the cases, including 69% misdiagnosed as *P. malariae*, 14% as *P. falciparum*, and 7% as *P. vivax*. To combat this disease, strategies to improve healthcare access in endemic areas; availability of appropriate antimalarials at all facilities; and accurate, sensitive and specific rapid diagnostic tests are the key elements to be taken into consideration. Following the WHO guidelines for malaria elimination strategies, the (1) understanding of parasites, (2) laboratory diagnosis, (3) entomology, and (4) clinical management are the research priorities that should be focused on.

Session 3. Morphological differentiation of *Plasmodium* species

Led by **Prof Dr Rohela Mahmud** and **Dr Amirah Amir**, this session trained the participants on the microscopic identification of the five human malaria species. When malaria is suspected, it must always be confirmed by laboratory diagnosis. However, diagnosis can be difficult when parasitaemia is low and only certain stages such as early trophozoite are present. Notably, *P. knowlesi* shares several morphological characteristics with *P. falciparum* and *P. malariae* which makes an accurate diagnosis by microscopy virtually impossible. Giemsa stained thin and thick blood films remain the standard diagnostic approaches, and each of the five major parasite species has distinguishing characteristics. In this session, the different stages of malaria in red blood cells such as ring, trophozoite, schizont and gametocyte, and their respective morphological characteristics, were demonstrated.
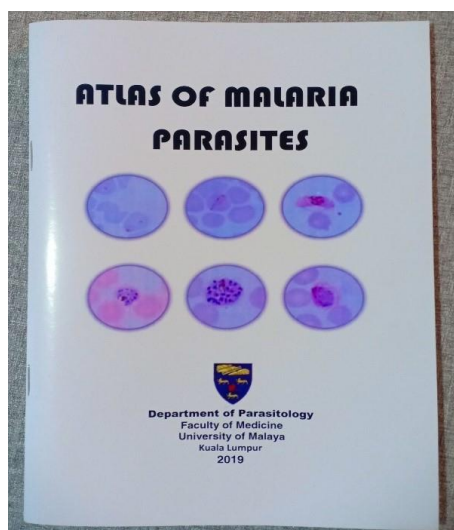
Atlas of malaria parasites published by the Department of Parasitology, Faculty of Medicine, University of Malaya in 2019

**Session 4. Practical: Basic malaria microscopy**

Also led by **Dr Rohela Mahmud** and **Dr Amirah Amir**, this session allowed the participants to identify 20 thin blood films under light microscopes. Different species and stages of malaria parasites were provided in the slides, and the participants were required to identify them accordingly. Several morphological characteristics such as the size of red blood cells (enlarged red blood cells), the presence of double chromatin, observation of multiple infection in a single cell, shapes of the trophozoites, number of merozoites in schizont, and shape of gametocytes are the important keys for species identification.

Dr Amirah Amir (left picture) and Dr Rohela Mahmud (right picture) sharing their experiences in differentiating the five human malaria species based on microscopic technique

Session 5. Insights to vectors of *Plasmodium knowlesi*

This session was led by **Prof Dr Indra Vythilingam** who shared her knowledge on the mosquito vectors of *P. knowlesi* in Malaysia. In 1960s, several species of mosquitoes such as *An. hackeri*, *An. latens*, *An. introlatus* and *An. cracens* were identified as the vectors of *P. knowlesi*. Following the continued efforts in identifying the vectors, *An. dirus* and *An. balabacensis* were incriminated as the vectors of *P. knowlesi* in 2011 and 2015, respectively. Nevertheless, more efforts are yet to be conducted in the identification of other vectors responsible for transmission of the disease in the Southeast Asian region. Changes in the ecological landscape such as deforestation are an important factor responsible for malaria transmission. It has been shown that deforestation coupled with the reduction of malaria cases has led to the emergence of *P. knowlesi*. Dr Vythilingam concluded that strong collaboration among researchers from different parts of the world is warranted to combat this zoonotic malaria which is now occurring in all countries in Southeast Asia, except Timor Leste.
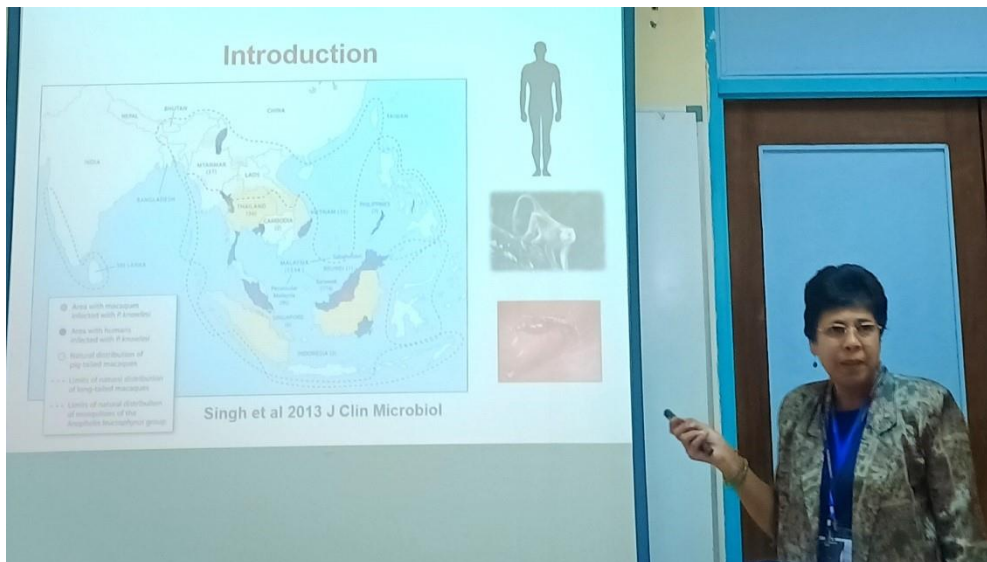
Dr Vythilingam sharing her experiences in identifying the vectors of malaria in Malaysia

Day 2: 5th March 2019

*The organizing committee and participants of the workshop*

The dedicated participants from Bangladesh, Cambodia, China, Indonesia, Malaysia, Philippines, Singapore and Thailand

Session 1. Molecular diagnostic tools for knowlesi malaria

Assoc. Prof Dr Lau Yee Ling gave a lecture on the current molecular diagnostic tools for malaria parasites, including the simian malaria *P. knowlesi*. Specifically, WHO recommends the use of polymerase chain reaction (PCR) technique to confirm *P. knowlesi* cases in the endemic countries.

The existing diagnostic methods primarily comprise three techniques, namely microscopy, rapid diagnostic tests (RDTs), and PCR. Nevertheless, the microscopic technique requires expertise and may produce false positive results, whereas RDTs are not sensitive and cannot differentiate the parasites up to a species level. PCR is the alternative approach because it is potentially rapid; sensitive and specific; and allows species identification, even though training and equipment are required, and it is expensive. There are various PCR methods for diagnosis, including the most widely used nested PCR. The Loop-mediated isothermal amplification (LAMP) has been favourably adopted because it is rapid (less than 1 hour), sensitive and specific, easy to use, affordable and ideal for resource-limited environments.
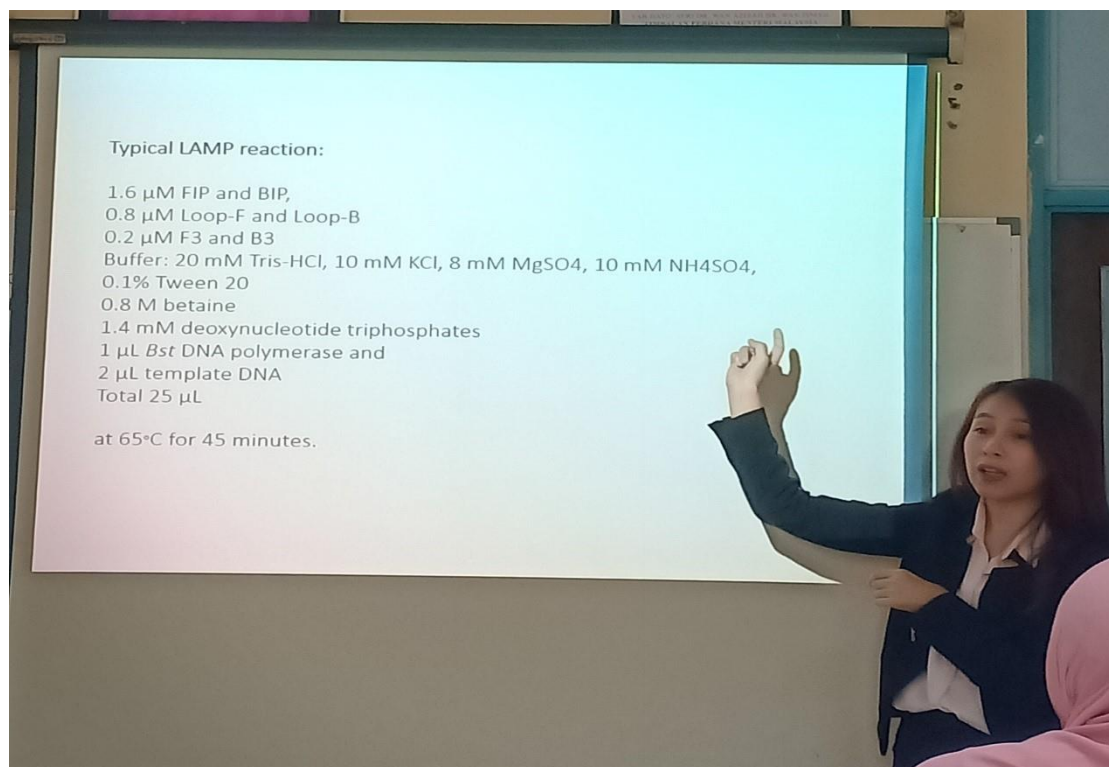
Dr Lau Yee Ling sharing the LAMP protocol with the participants

Session 2. Practical: PCR and LAMP

The practical class for PCR and LAMP was demonstrated by **Dr Jeremy R de Silva** and **Dr Lai Meng Yee**. Participants were given a hands-on experience in performing LAMP assay for *P. knowlesi* and nested PCR assay for all five human malaria parasites.

Practical class for nested PCR and LAMP assay

Session 3. Practical: *Plasmodium knowlesi* culture

The participants were trained by **Dr Cheong Fei Wen** and **Dr Jonathan Liew** for *P. knowlesi* culture. This practical class included medium preparation, synchronisation (separation of malaria life stages), freezing and thawing parasite isolates, and blood purification. This practical provided first-hand knowledge to participants regarding the culture of *P. knowlesi.*

Dr Liew (left) and Dr Fei Wen (right)

Day 3: 6ᵗʰ March 2019

Session 1. Control and intervention of malaria vectors - Ross-Macdonald model

Prof Dr Indra Vythilingam, one of the most renowned medical entomologists in Malaysia, shared her knowledge of the interventions to control malaria vectors in Southeast Asia using the Ross-Macdonald model. An understanding of the relationship between the vectors, their ecology and behaviour, the parasites, and their hosts is of paramount importance when developing and implementing vector control strategies. Accordingly, the aim of this lecture was to introduce the biological features of vector-borne infection, the basic concepts in relation to *Anopheles* and malaria, and discuss the control interventions in relation to *P. knowlesi*. To correlate malaria transmission, human biting rate, proportion of blood meals taken on humans, parous rate of mosquitoes and sporozoite rate are the important parameters to be incorporated in entomological surveillance. She explained that the vectorial capacity of the vectors has been observed to be higher in the forest and farms as compared to the villages.
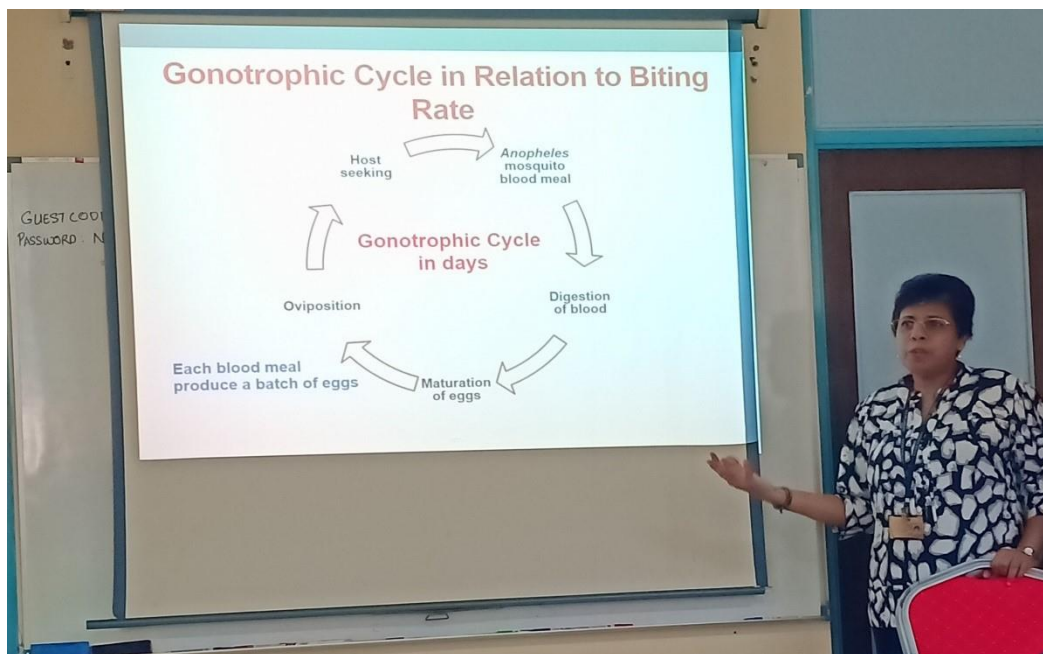
Dr Vythilingam explaining the gonotrophic cycle of Anopheles mosquitoes in relation to their biting rate to the participants

Session 2. Practical: Mosquito identification

The *Anopheles* mosquito identification session was led by **Dr Vythilingam** and assisted by **Dr Jonathan Liew** and **Dr Wong Meng Li**. In the initial session, the participants were taught how to differentiate the sexes of mosquitoes and the basic characters to separate the genus *Anopheles* from other genera. The participants were then guided to identify the subgenera of the *Anopheles* (i.e., *Anopheles* and *Celia*), followed by several species-groups, including the *A. leucosphyrus* group, the important vectors of *P. knowlesi*.

Basic identification of mosquitoes prior to the identification of genera, subgenera, species-groups and species of Anopheles mosquitoes

Session 3. Practical: Mosquito mating and dissection

The participants were also trained by **Dr Vythilingam**, **Dr Liew** and **Dr Wong** on how to force-mate *Anopheles* mosquitoes, followed by lessons in the dissection of mosquitoes for examination of malaria oocytes and sporozoites in the midgut and salivary gland, respectively.

*Knock-downed males of Anopheles dirus to be force-mated with the females*

Malaria-infected Anopheles dirus to be dissected by the participants

Dr Wong (left) and Dr Liew (right) demonstrating the mosquito dissection technique to the participants

Session 4. Practical: Plasmodium knowlesi culture

To continue with the training in *P. knowlesi* culture, the participants were shown the culture medium replenishment technique.
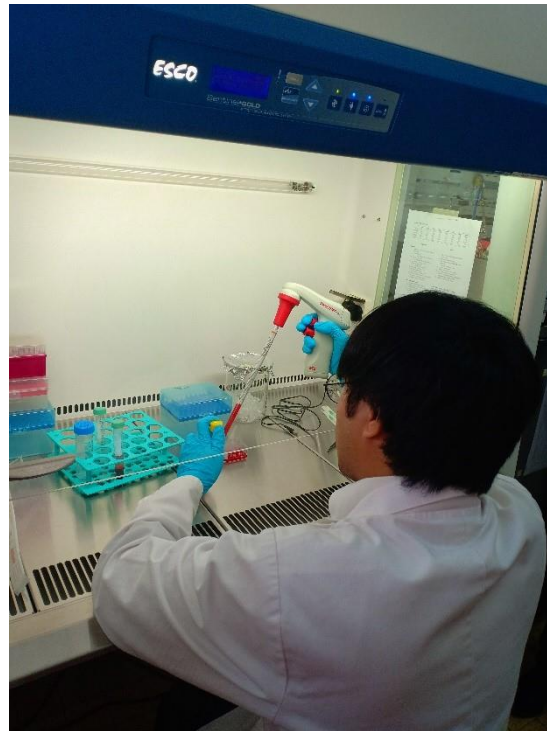
Culture medium replenishment for P. knowlesi performed in a biosafety cabinet

Day 4: 7th March 2019

Session 1. Introduction to geographic information system (GIS)

The geographic information system (GIS) was introduced by **Dr Romano Ngui** in this session. GIS is a powerful set of tools for collecting, storing, retrieving, transforming, and displaying spatial data from the real world. The system can be applied in many fields, including disease epidemiology — one of the objectives of this workshop.

Health professionals can take advantage of all its different utilities, for example, maps can be produced for annual reports or to map outbreaks. Health events can also be linked to socio-economic information. Spatial and temporal analyses can be carried out to examine clustering of cases over time. Importantly, the data can be mapped to aid the cleaning, checking and generation of hypothesis.

Session 2. Spatial and temporal analyses for epidemiology in tropical diseases

This second session was also led by **Dr Romano Ngui** with the aim to provide an overview of spatial-temporal analysis in the context of epidemiological investigations in public health such as infectious diseases and parasitic infections. Transmission of infectious diseases is closely linked to the concept of spatial and temporal proximity. The licenced software ArcGIS, and several public domain software such as GRASS, Autodesk Map2000, Intergraph GeoMedia, Quantum GIS, or DIVA GIS, are some of the available platforms for this spatial-temporal analysis. Furthermore, GIS can provide spatial dimension analysis to epidemiological research, including visualization, exploration, and modelling. Dr Romano Ngui also shared some GIS data on infectious diseases such as chikungunya and soil-transmitted helminthiasis during this session.

Dr Romano Ngui interpreting GIS data

Session 3. Practical: Introduction to GIS, creating a database and spatial visualization

This practical session was conducted at the Department of Geography of the University of Malaya and was led by **Dr Romano Ngui** and **Assoc Prof Dr Aziz Shafie**. A practical introduction to GIS ArcGIS, including the desktop mapping software and the data management software ArcCatalog, was conducted. The real dataset used in this practical class originated from a real study conducted after an outbreak of malaria in Zaire, in the Democratic Republic of the Congo. Data were collected on cases of malaria in villages in the study area, along with population information. With this session, participants covered most of the fundamentals of both GIS and ArcGIS, and were exposed to some of the most relevant information and techniques when producing a GIS map.

The GIS practical class

Day 5: 8th March 2019

Session 1. Phylogenetics and molecular epidemiology of *Plasmodium knowlesi*

Prof Dr Fong Mun Yik shared his research findings on phylogenetics and molecular epidemiology of *P. knowlesi* in Malaysia. Based on the evidence from various genetic markers, two distinct *P. knowlesi* types were found in Borneo and Peninsular Malaysia, which seem a result of having evolved following the sea-level rise at the end of the last ice age (15,000 years ago), which separated Borneo from Peninsular Malaysia. The populations were isolated, along with their monkey and vector hosts. Strikingly, two distinct genetic lineages were also found in the long-tailed macaques which correspond to the populations from Borneo and Peninsular Malaysia, respectively. The incriminated vector species *P. knowlesi* were also found to be different in both regions. Their evolution and distribution were likely shaped by past and recent events and independently became human zoonosis, according to Dr Fong Mun Yik.

Dr Fong Mun Yik emphasized the importance of molecular epidemiology in tracing back the origin of P. knowlesi infection

Session 2. Spatial distribution, epidemiology, and genotyping of *Plasmodium knowlesi* among primates in Peninsular Malaysia

This lecture was given by **Dr Reuben Sunil Kumar Sharma**, veterinarian and veterinary parasitologist from the University Putra Malaysia. In the initial session, Dr Reuben Sunil Kumar Sharma pointed out that forest destruction is a major threat to wildlife in Southeast Asia, which includes the long-tailed macaques. The macaques are being displaced from their natural habitats, being forced to encroach into urban and suburban areas. Subsequently, they have started to colonise and reproduce due to the availability of food resources and shelter. This situation narrows the gap between the human-wildlife interface, and therefore, the zoonotic wildlife diseases are more common due to increased proximity between the infectious agent, host, environment and vector.

The main host of *P. knowlesi*, *Macaca fascicularis*, is the most abundant and widespread non-human primate in a broad range of habitats in Southeast Asia. Its distribution ranges from coastal mangroves to inland hill forest, as well as recreational parks, suburban areas and agricultural areas. A high prevalence of *P. knowlesi*-infected macaques has been observed in Malaysia, but most of the times, these infections are with low parasitaemia, making them an excellent reservoir for this malaria parasite. Therefore, the macaques will be a constant source of human infection as long as the mosquito vector is present. This situation poses a serious challenge to malaria elimination in Malaysia and the Southeast Asian region where the disease is endemic.

Forest destruction is a major threat to wildlife in Southeast Asia, including the long-tailed macaques, according to Dr Reuben Sunil Kumar Sharma

Session 3. The clinical features and management of knowlesi malaria

The participants were delighted to have **Dr Timothy William**, an infectious disease physician from the Gleneagles Kota Kinabalu Hospital, Sabah, as the speaker for the clinical features and management of knowlesi malaria session. Dr William mentioned that distinguishing among *P. falciparum*, *P. vivax* and *P. knowlesi* in places where all three species frequently occur is challenging. Misdiagnosis can potentially lead to inappropriate treatment, including chloroquine therapy for *P. falciparum* and a lack of anti-relapse therapy for *P. vivax*.

Plasmodium knowlesi has been microscopically misidentified as *P. malariae*, *P. falciparum* and *P. vivax* and often associated with case fatalities due to the lack of severe disease recognition and delayed parenteral artesunate. Dr William also shared his knowledge on the drug treatments for the cases of uncomplicated and severe knowlesi malaria. Other supportive therapies such as fluid management, renal replacement therapy, inotropes, respiration ventilation, and blood transfusion were also briefly explained to the participants. The issue of drug resistance was also discussed.

Dr William sharing his experience in treating malaria-infected patients

Session 4. Quiz and end-of-course certificate

At the end of the workshop, the participants took a quiz related to the lectures and practical classes that they attended in this 5-day workshop. A certificate was given to each participant upon completion of the workshop.

Gifts were given to the top three winners of the quiz contest

Closing ceremony

The closing ceremony of the "Current Tools to Combat Malaria-with Special Focus on *Plasmodium knowlesi*" workshop was officiated by the Head of Department of Parasitology, **Prof Dr Suresh Kumar A/L P Govind**. He congratulated the organizing committee members and participants on such a successful event and stressed that collaboration should be initiated, being the way forward to reaching greater heights.

The end of the 5-day workshop. See you again!

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