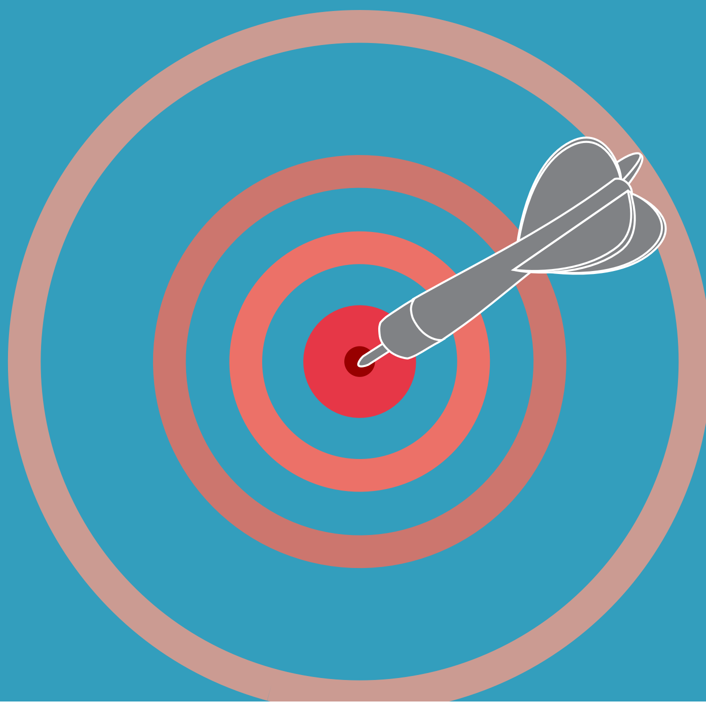
Technical consultation on control of zoonotic malaria

Meeting report, Geneva, Switzerland,
5–7 November 2024



World Health Organization

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ISBN 978-92-4-011085-4 (electronic version)
ISBN 978-92-4-011086-1 (print version)

Suggested citation. Technical consultation on control of zoonotic malaria: meeting report, Geneva, Switzerland, 5-7 November 2024. Geneva: World Health Organization; 2025. Licence: CC BY-NC-SA 3.0 IGO.

Cataloguing-in-Publication (CIP) data. CIP data are available at https://iris.who.int/.

Contents

Abbreviations

ACT	artemisinin-based combination therapy
AL	artemether-lumefantrine
ASMQ	artesunate-mefloquine
DP	dihydroartemisinin-piperaquine
LLIN	long-lasting insecticidal net
LSM	larval source management
NHP	non-human primate
PCR	polymerase chain reaction
PvLDH	Plasmodium vivax lactate dehydrogenase
RCT	randomized controlled trial
WHO	World Health Organization

Executive summary

Since the discovery of numerous human *Plasmodium knowlesi* cases in Malaysian Borneo in 2004, this zoonotic malaria has emerged as a significant public health concern, particularly across the WHO regions of South-East Asia and Western Pacific. In 2023, the World Health Organization (WHO) received reports of 3290 cases of *P. knowlesi* globally, marking an increase of almost 19% from 2022, with Malaysia accounting for more than 87% of these cases. Imported cases have increasingly been reported worldwide, including in the WHO regions of Europe, the Americas and the Western Pacific. However, the true disease burden remains unknown due to diagnostic limitations. Microscopy cannot reliably distinguish *P. knowlesi* from other malaria species, and rapid point-of-care diagnostics are non-specific and have previously had poor sensitivity, meaning that accurate diagnosis requires costly polymerase chain reaction testing. Currently, there are no WHO guidelines or proven interventions for *P. knowlesi* control.

The WHO Global Malaria Programme convened a technical consultation in Geneva from 5 to 7 November 2024. The meeting brought together national malaria programme managers from affected countries, members of the Malaria Policy Advisory Group and the Technical Advisory Group on Malaria Elimination and Certification, research experts and WHO regional advisers. Sessions covered global and country updates, clinical aspects, vector control, reservoirs, environmental drivers, control strategies, and research and development needs.

There is limited evidence of sustained human–mosquito–human transmission of *P. knowlesi.* While some interventions such as personal protection, including chemoprophylaxis and wearing topical repellents, can be implemented immediately, these alone cannot address the increasing mosquito–macaque–human transmission through which the majority of cases are likely to occur. Research priorities were established, focusing on developing species-specific diagnostics, improving surveillance for risk mapping, and developing vector control and macaque management strategies that can decrease the interaction between macaque and human populations.

The emergence of *P. knowlesi* poses unique challenges to malaria elimination, particularly for countries approaching or having achieved elimination of the "human malaria" species (*P. falciparum, P. vivax, P. malariae, P. ovale*). Currently, countries where transmission of "human malaria" has been interrupted but *P. knowlesi* cases persist cannot be officially certified by WHO as malaria-free. In response to this challenge, the Global Malaria Programme has established a subgroup on zoonotic malaria within the Technical Advisory Group on Malaria Elimination and Certification to develop procedures and requirements for certification of malaria elimination in such countries.

The consultation concluded that coordinated efforts are urgently needed as the burden of *P. knowlesi* increases. There was consensus that successful control will require intersectoral collaboration across health, forestry, agriculture and private sectors, along with strong community engagement. Next steps include implementing the research priorities to inform the development of specific WHO guidelines for *P. knowlesi* control. To resolve the challenge of certifying countries where the four "human malaria" species have been eliminated but where other species continue to be transmitted, the Technical Advisory Group on Malaria Elimination and Certification subgroup on *P. knowlesi* will continue its work to finalize deliberations on *P. knowlesi* issues related to certification.

1. Background and rationale

Since large numbers of human cases of *Plasmodium knowlesi* malaria were reported in Malaysian Borneo in 2004 *(1)*, *P. knowlesi* has emerged as a public health issue of concern, especially in certain countries in the World Health Organization (WHO) regions of South-East Asia and the Western Pacific: Indonesia, Malaysia and Thailand. Globally, there were 3290 cases of *P. knowlesi* infection reported in 2023, marking an increase of 18.9% from 2768 cases in 2022.

Malaysia continues to account for the most *P. knowlesi* cases, contributing 87.4% of the global total cases in 2023, followed by Thailand (7.8%), Indonesia (4.4%) and Cambodia (0.3%). In Malaysia, cases increased by 14.9% – from 2505 in 2022 to 2879 in 2023 – with nearly all of them (99.4%) classified as indigenous. Similarly, Cambodia, Indonesia and Thailand saw significant increases in *P. knowlesi* cases between 2021 and 2023. Cambodia had its first reported cases in 2022 and recorded 11 cases in 2023. Indonesia reported an increase from five cases in 2021 to 170 in 2023. Thailand saw an increase from 71 cases in 2021 to 258 cases in 2023. In 2023, the only country to report deaths from *P. knowlesi* was Malaysia, where all 14 indigenous malaria deaths were attributed to *P. knowlesi (2)*. Research has shown that the numbers of imported cases of *P. knowlesi* are increasing, with cases reported in Sweden, Finland, United States of America, Spain, Kingdom of the Netherlands, Australia, New Zealand, Japan, Germany, Türkiye, Italy, Sri Lanka, Brunei Darussalam, Poland, China, France and the Philippines *(3)*.

However, the true burden of *P. knowlesi* and other zoonotic malaria is unknown due to diagnostic limitations. Similarities in the morphology of *P. knowlesi*, *P. falciparum* and *P. malariae* make confirmatory diagnosis by microscopy impossible, and available rapid diagnostic tests (RDTs) are not species-specific to *P. knowlesi*. Currently, the only methods that can be used to accurately diagnose *P. knowlesi* are molecular, mainly polymerase chain reaction (PCR), which are expensive and not available at the point of care in affected countries.

The emergence and increase in burden and transmission of *P. knowlesi* in the WHO regions of South-East Asia and Western Pacific poses unique challenges to malaria elimination. This is especially pertinent for countries that have eliminated or are close to eliminating the "human malaria" species *P. falciparum, P. vivax, P. malariae* and *P. ovale*. Until now, certification has been awarded exclusively to countries where the "human malaria" species have been eliminated. However, the criteria for certification were developed and refined before the emergence of significant *P. knowlesi* transmission to humans. In light of this emerging challenge, WHO convened two advisory groups, which concluded that countries that have eliminated transmission of the four main "human malaria" species but continue to report indigenous transmission of other *Plasmodium* species might be granted certification if the risk of infection to humans is assessed as negligible *(4)*. Following discussions on *P. knowlesi* at the fifth meeting of the Technical Advisory Group on Malaria Elimination and Certification *(5)*, a subgroup on zoonotic malaria was established with the aim of developing the procedures and requirements for certification of countries that have achieved elimination of the four "human malaria" species but transmission of zoonotic malaria continues.

There are currently no WHO guidelines for control of *P. knowlesi*, nor proven control interventions apart from case management. In addition, there are significant gaps in surveillance capabilities and therefore uncertainty around the true burden of disease. It is considered a priority to reduce zoonotic spillover to humans as rapidly and thoroughly as possible to contain morbidity and mortality and to reduce the risk of human-to-human transmission on a larger scale. This will require research and development of control, prevention and surveillance tools, as well as funding, innovative strategies, government commitment and intersectoral collaboration.

A technical consultation was convened by the WHO Global Malaria Programme on the control of zoonotic malaria, in Geneva on 5–7 November 2024. Participants included national malaria programme managers from Indonesia, Malaysia, the Philippines and Thailand, members of the Malaria Policy Advisory Group and the Technical Advisory Group on Elimination and Certification, research experts, WHO regional advisers and Global Malaria Programme staff (Annex 1). The meeting was organized into five sessions (Annex 2):

- Overview of the situation with zoonotic malaria
- Diagnosis and treatment of *P. knowlesi*
- Zoonotic malaria vectors and applied vector control practices
- Certification of malaria elimination in countries with zoonotic malaria
- Control of zoonotic malaria and research needs.

The objectives of the technical consultation were to:

- review the situation with *P. knowlesi* malaria in affected countries and the challenges countries are facing to control zoonotic malaria;
- identify practices and interventions applied by countries that have shown to be effective in controlling *P. knowlesi* malaria and that could be further developed to improve their effectiveness;
- define research questions that need to be addressed to develop effective strategies for controlling zoonotic malaria in order to achieve the "negligible risk" threshold and ensure zero deaths;
- identify the areas where WHO guidance and support are needed.

2. Overview of the situation with zoonotic malaria

2.1 Global overview of the situation with zoonotic malaria in the world and actions taken by WHO to address the issue

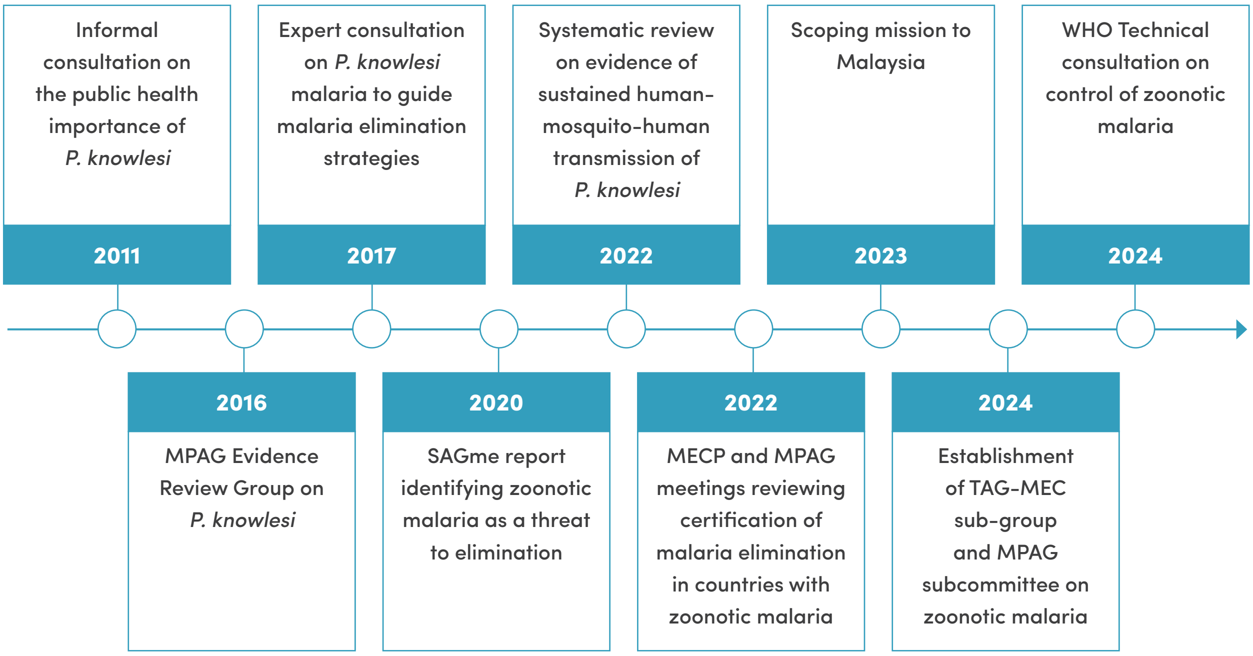
Dr Elkhan Gasimov provided an overview of the emergence of *P. knowlesi* as a public health problem since its identification in 2004 *(1).* He highlighted *P. knowlesi*'s key features, including its vectors belonging to the *Leucosphyrus* group of *Anopheles,* its clinical spectrum from asymptomatic cases to severe malaria and fatalities, and its primary natural reservoir hosts, i.e. the long-tailed (*Macaca fascicularis*) and pig-tailed (*Macaca nemestrina*) macaques.

Dr Gasimov explained *P. knowlesi*'s transmission dynamics, emphasizing the environmental drivers and populations that are most at risk, such as farmers, plantation workers and those involved in forest activities. Current evidence suggests that human–mosquito–human transmission is extremely rare and not sustained.

Updates on *P. knowlesi* cases were shared, along with data from Cambodia, Indonesia, Malaysia, the Philippines and Thailand. According to data provided by the European Centre for Disease Prevention and Control, from 2012 to 2023, 24 *P. knowlesi* cases were imported into Europe, detected in France, Germany, Spain and the United Kingdom of Great Britain and Northern Ireland, highlighting the global relevance of this challenge.

Dr Gasimov summarized WHO's work on *P. knowlesi* to date (Fig. 1) since the first consultation with Malaysia where concerns were raised about the impact of *P. knowlesi* on elimination goals. Key milestones include: i) a Strategic Advisory Group on Malaria Eradication report in April 2020 identifying zoonotic malaria as a threat to eradication that should be monitored and managed; ii) a 2022 systematic review of the literature showing limited evidence of human-to-human transmission chains *(6)*; iii) reviews by the Malaria Elimination Certification Panel and Malaria Policy Advisory Group concluding that "certification might be granted to countries where cases of other *Plasmodium* species are reported if the risk to humans is assessed as negligible" *(7)*; and iv) the establishment of the Technical Advisory Group on Malaria Elimination and Certification subgroup and Malaria Policy Advisory Group subcommittee working on zoonotic malaria.

Fig. 1. WHO's work to date on *P. knowlesi*



MPAG: Malaria Policy Advisory Group; SAGme: Strategic Advisory Group on Malaria Eradication; MECP: Malaria Elimination Certification Panel; TAG-MEC: Technical Advisory Group on Malaria Elimination and Certification

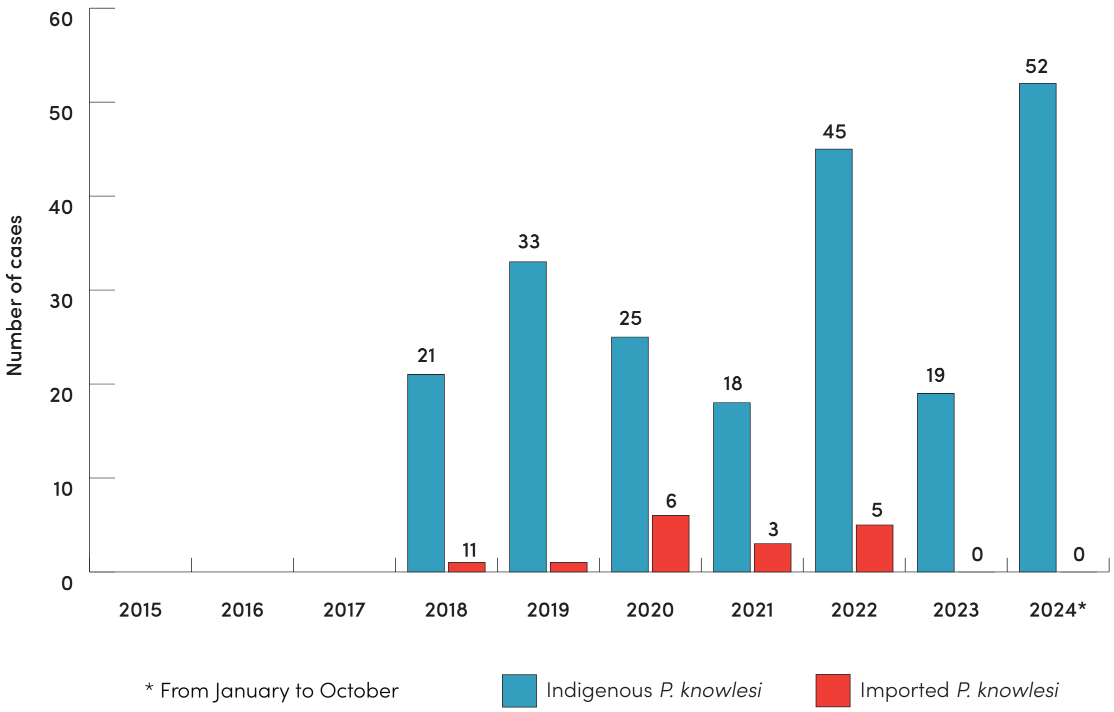
2.2 Updates on zoonotic malaria from national malaria programme managers

2.2.1 Indonesia

Dr Hellen Dewi Prameswari outlined Indonesia's strategy to eliminate "human malaria" species by 2030, while strengthening One Health collaboration to prevent re-establishment of transmission. She reported fluctuations in the number of *P. knowlesi* cases since 2018 (Fig. 2), with the highest number of cases (52) reported in 2024 (January–October). This increase was attributed to a change in case definition in 2024. *P. knowlesi* represents 0.04% of reported cases, with *P. falciparum* and *P. vivax* accounting for most infections. The majority of *P. knowlesi* cases have occurred in Aceh and North Sumatra, predominantly in men aged 15–64 years, including farmers, forest workers, informal forest labourers, police and military personnel.

Dr Dewi Prameswari highlighted that the major *P. knowlesi* vectors in Indonesia are *An. cracens, An. introlatus, An. balabacensis* and *An. leucosphyrus,* which are associated with forest habitats (forest fringe, deep forest and mixed shrub vegetation). Surveillance for *P. knowlesi* in Indonesia includes case definitions for suspected zoonotic malaria, which becomes probable zoonotic malaria upon positive microscopy results. Species-specific case confirmation is by PCR. Uncomplicated *P. knowlesi* malaria is treated with an artemisinin-based combination therapy (ACT), dihydroartemisinin-piperaquine (DP), for three days without primaquine; severe *P. knowlesi* malaria is treated with intravenous artesunate. Case notification, investigation and epidemiological surveillance in Indonesia are the same as for “human malaria” cases.

Fig. 2. Number of confirmed cases of *P. knowlesi* in Indonesia



Currently, there are no specific interventions for controlling zoonotic malaria in Indonesia. Vector control is by long-lasting insecticidal nets (LLINs) distributed to communities living in forest fringe areas and to forest goers in highly endemic areas. Dr Dewi Prameswari identified that the challenges in Indonesia include limited diagnostic capacity, under-reporting of cases, limited surveillance of vector and reservoir species, lack of zoonotic vector control, insufficient awareness among health professionals, lack of focal points for regional networks of *P. knowlesi* surveillance, and lack of funding for zoonotic malaria research activities. She emphasized the need for WHO support in developing diagnostic guidelines, improving capacity in surveillance and vector control, funding research and fostering cross-border collaboration with Malaysia and other countries on *P. knowlesi* surveillance.

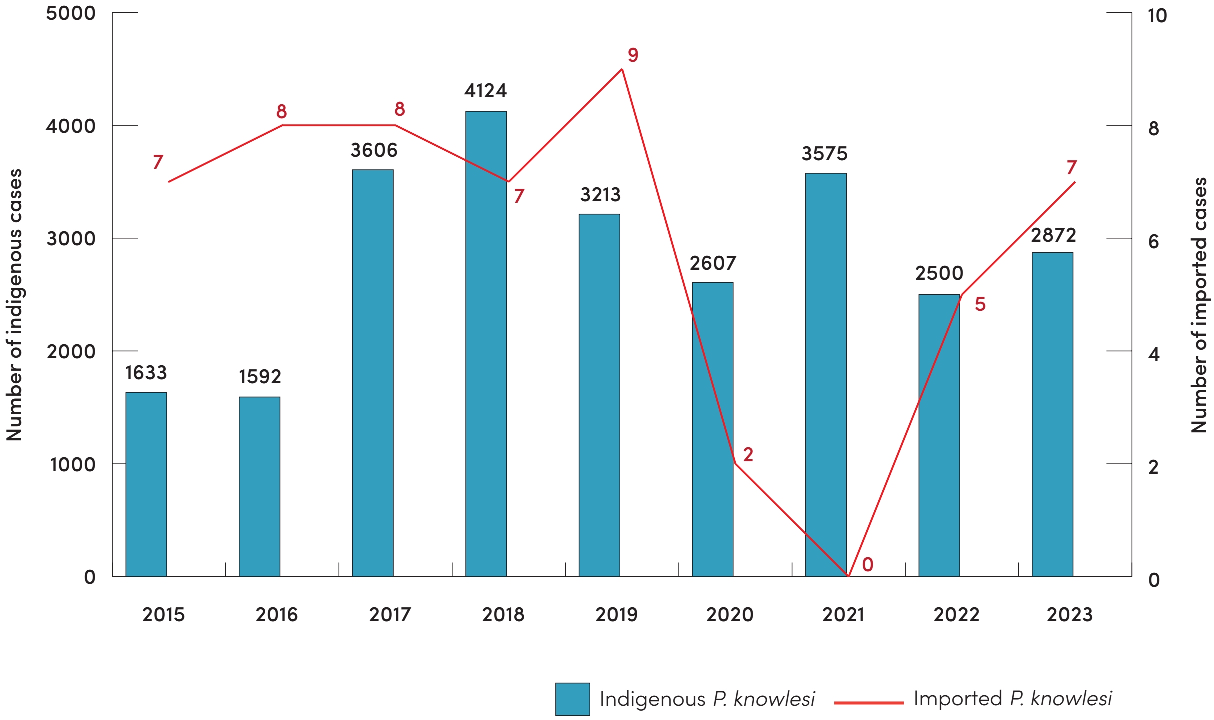
2.2.2 Malaysia

Dr Jenn Zhueng Tam detailed Malaysia’s progress towards eliminating “human malaria” species and the challenges posed by zoonotic malaria. Malaysia has had zero indigenous “human malaria” cases since 2018. The majority of imported or introduced “human malaria” cases are *P. vivax*. Zoonotic malaria, primarily *P. knowlesi*, accounts for 79% of malaria cases, with 2872 indigenous *P. knowlesi* cases reported in 2023 (Fig. 3). Areas of high incidence include the states of Sabah and Sarawak in Malaysian Borneo, and Kelantan in Peninsular Malaysia. The epidemiology of *P. knowlesi* mirrors that in Indonesia, with most cases occurring in men aged 20–49 years, particularly small-scale farmers, plantation workers, self-employed individuals, and those living close to forest fringes.

The major vectors are *An. cracens*, *An. introlatus*, *An. balabacensis* and *An. latens*, which are all exophagic, exophilic and simio-anthropogenic. Surveillance for *P. knowlesi* in Malaysia includes case definitions for suspected zoonotic malaria, which becomes probable zoonotic malaria upon positive microscopy results. Case confirmation is by PCR. Uncomplicated *P. knowlesi* malaria is treated with an ACT, artesunate-mefloquine

(ASMQ), for three days without primaquine. Severe *P. knowlesi* malaria is treated with intravenous artesunate. All malaria cases in Malaysia are hospitalized. Case investigations are completed within three days of notification, with emphasis on travel history, macaque sightings, environmental setting of the patient's residence, and determination of the source or locality of infection.

Fig. 3. Number of confirmed cases of *P. knowlesi* in Malaysia



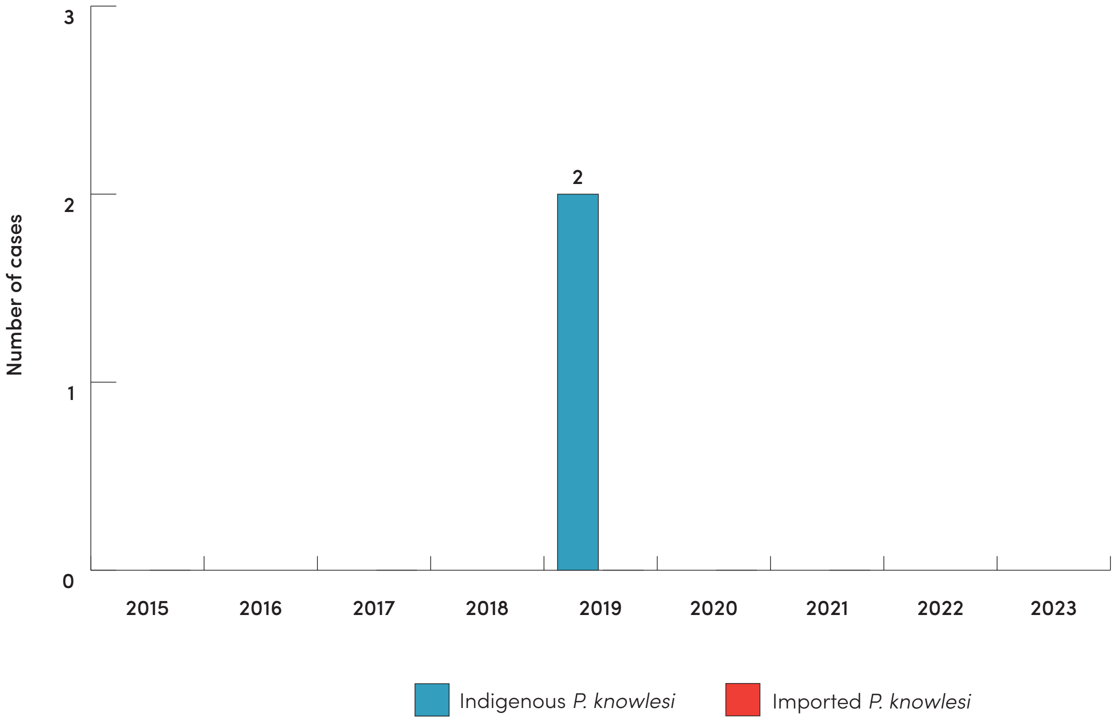
Dr Tam attributed Malaysia's success in "human malaria" elimination to strong government commitment, an integrated health care system, a well funded malaria control programme, community volunteers and public–private collaboration. The formation of the National Technical Zoonotic Malaria Committee within Malaysia's Ministry of Health has allowed for intersectoral collaboration at the zoonotic malaria programme level. Zoonotic malaria, however, faces challenges such as limited research on vector control, cases living in remote areas with little access to health care, the economic burden of hospitalization for people with malaria, and diagnostic issues. Support from WHO and other partners is required for establishing a WHO Collaborating Centre for zoonotic malaria to prioritize research and development on vector control, RDT diagnostics, mapping of *P. knowlesi* prevalence in reservoirs and research on other non-zoonotic malaria species (*P. cynomolgi*, *P. inui*, *P. coatneyi*, etc.)

2.2.3 Philippines

Dr Liezl A. Fernandez outlined progress in the Philippines towards its goal of malaria elimination by 2030. The country is taking a subnational approach to elimination, aiming to declare 81 out of 82 provinces malaria-free by 2027. Currently, 72 provinces are malaria-free, nine have zero indigenous cases, and one, Palawan, remains active. Most confirmed cases (84%) are *P. falciparum*, with 6193 indigenous cases reported in 2023. The burden of *P. knowlesi* is low, with only two confirmed cases in the past five years – both PCR-confirmed cases in Palawan in 2019 (Fig. 4). Additional *P. knowlesi* cases have been identified in prevalence surveys from 2006 to 2018 in Palawan, Tawi-Tawi and Sultan Kudarat. Risk factors for zoonotic malaria include forest fragmentation from logging, increased macaque–human contact, and at-risk occupations including farming, mining and forestry.

The major zoonotic malaria vectors in the Philippines are *An. flavirostris* and *An. balabacensis.* The Philippines does not have a definition for a suspected *P. knowlesi* case. All *P. malariae* cases are subject to PCR confirmation. There are no defined epidemiological surveillance methods for zoonotic malaria cases. Treatment for all species and mixed infections is with AL.

Fig. 4. Number of confirmed cases of *P. knowlesi* in the Philippines



There are no interventions for zoonotic malaria in the Philippines, and vector control methods are aimed at "human malaria" species, for example LLINs and indoor residual spraying. Challenges include under-recording of *P. knowlesi* cases, limited knowledge of vector species, limited epidemiological surveillance data on both macaques and humans, rapid turnover of health care workers, and diagnostic and quality assurance limitations. Dr Fernandez emphasized the need for WHO support in policy development for zoonotic vector control, capacity-building for diagnosis and treatment of severe *P. knowlesi,* improvement of testing procedures, surveillance for the prevalence of zoonotic malaria, and intersectoral collaboration.

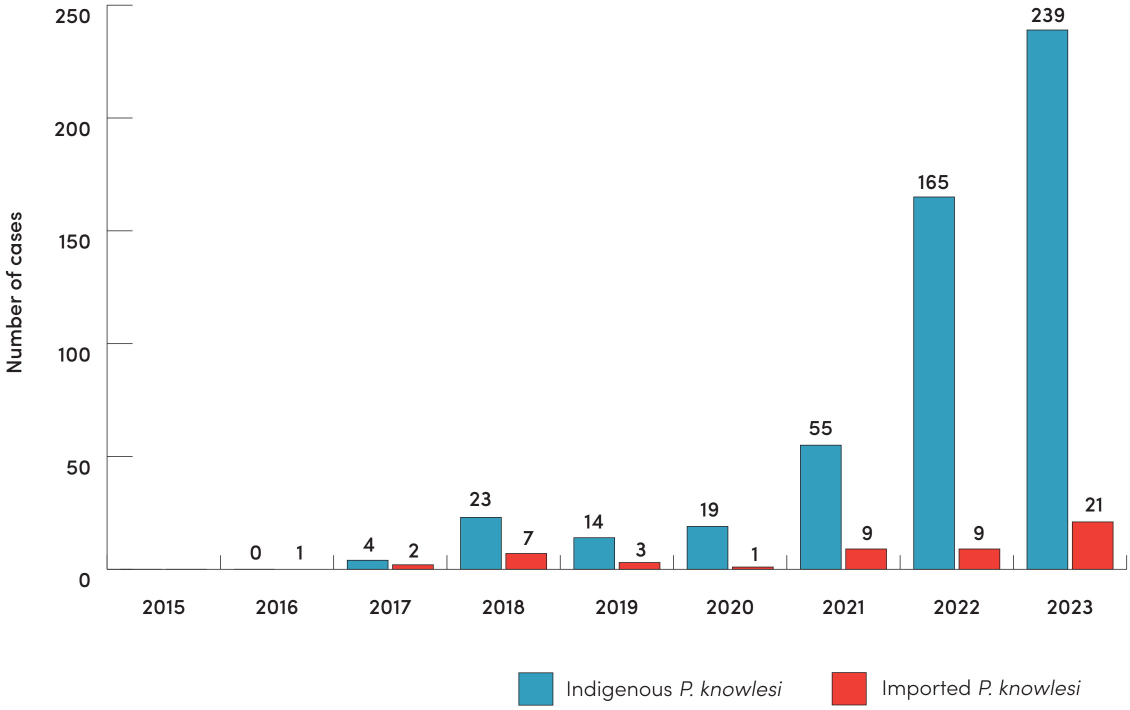
2.2.4 Thailand

Dr Prayuth Sudathip outlined Thailand's progress towards eliminating malaria by 2024, highlighting challenges due to cross-border movement with Myanmar, which has led to a rise in imported and indigenous cases. The country is taking a subnational approach to malaria elimination, with 49 provinces verified as malaria-free. *P. vivax* accounts for 95.7% of "human malaria" cases, with 8913 cases reported in 2023, an increase from 2022. The first *P. knowlesi* case was reported in 2018, with cases rising since 2020 and reaching 239 in 2023 (Fig. 5). The majority of cases occur in southern Thailand, close to the borders with Myanmar and Cambodia.

High-risk groups include men over 25 years of age, occupations including farmers, military personnel and forest workers, and tourists. Factors contributing to the increase in cases include increased forest exposure, eco-tourism, movement of populations from non-endemic into endemic areas, land use changes and improved malaria surveillance as the country nears elimination of the "human malaria" species. The primary vector *An. dirus* is anthropophilic and exophilic, and has been reported throughout Thailand. Its main habitats are forests, orchards and tree plantations.

Thailand does not have a definition for a suspected *P. knowlesi* case. Diagnosis of cases is by microscopy and RDT, followed by confirmation by PCR. Thailand has no specific epidemiological surveillance methods for zoonotic malaria cases, but cases are added to the zoonotic malaria focus database. All cases of *P. vivax* malaria confirmed by microscopy and RDT are treated with chloroquine and single-dose radical cure with tafenoquine after excluding glucose-6-phosphate dehydrogenase (G6PD) deficiency by testing. Upon PCR confirmation of a *P. knowlesi* case, a three-day course of chloroquine is used for treatment.

Fig. 5. Number of confirmed cases of *P. knowlesi* in Thailand



The National Malaria Strategic Plan does not include any interventions specifically for control of zoonotic malaria. Routine measures taken in high-risk populations are active case detection and vector control, including LLINs, indoor residual spraying, personal protection and health education. Surveillance occurs in collaboration with the Department of National Parks, Wildlife and Plant Conservation. Dr Sudathip identified a number of challenges, including highly mobile populations, limited data on reservoirs, lack of species-specific RDTs and lack of interventions targeting zoonotic malaria. The key areas where WHO and other partner support is needed are in developing species-specific RDTs and point-of-care tools, developing control interventions, and supporting research on zoonotic malaria epidemiology and surveillance.

2.3 Zoonotic malaria of concern: Pan American Health Organization

Dr Maria de la Paz Ade began her presentation by providing information on the distribution of "human malaria" species in the Americas. Of the total 505 610 malaria cases reported in 2023, *P. vivax* accounted for over 70% of cases, *P. falciparum* for over 20% and *P. malariae* for less than 1%. She introduced the zoonotic malaria species *P. simium* and *P. brasilianum,* noting diagnostic challenges due to their similarity to *P. vivax* and *P. malariae*, respectively. *P. simium* can be identified by PCR, while no differential diagnosis exists for *P. brasilianum*. The spatial distribution of *P. brasilianum* in non-human primates (NHPs) spans the Bolivarian Republic of Venezuela, Brazil, Colombia, Costa Rica, French Guiana (France), Panama and Peru.

Dr Ade presented some of the available evidence on zoonotic malaria in Brazil, shared by the Brazilian national counterparts working in this area for this specific meeting. Surveillance studies have found 12% of surveyed NHPs positive for *P. simium*, *P. brasilianum*, *P. vivax* or *P. malariae*. In the Mata Atlântica forest near Rio de Janeiro, *P. simium* and *P. brasilianum* cases have been reported in humans as accidental hosts, with transmission primarily occurring between NHPs and forest vectors. An outbreak of 49 human *P. simium* cases in 2015–2016 was linked to *An. cruzii*, which inhabits both the forest canopy and ground level *(8)*. Brazil's response includes developing methods for differential diagnosis, providing health education to forest-adjacent communities and conducting mathematical modelling to understand zoonotic malaria transmission dynamics.

Key questions and challenges remain around the required levels of surveillance of NHPs, the role of One Health, and the development of methods for differential diagnosis. Dr Ade suggested the implementation of a special surveillance protocol response to *P. malariae* cases associated with exposure in the forest, due to the likelihood of *P. brasilianum* being incorrectly diagnosed as *P. malariae* in the Americas.

2.4 Spatial distribution and epidemiological risk factors for zoonotic *Plasmodium* infection among NHPs in WHO regions of South-East Asia and Western Pacific

Dr Reuben Sharma discussed *P. knowlesi* reservoir hosts, describing six naturally infected NHP species in the WHO regions of South-East Asia and Western Pacific. The primary host is the long-tailed macaque, which is an asymptomatic carrier with low-grade parasitaemia, making it an efficient reservoir. The long-tailed macaques' abundance and adaptability to human habitats after being displaced from their natural habitats make them a dominant wildlife reservoir host for zoonotic pathogens.

Dr Sharma presented results from sampling efforts across the two WHO regions, noting that the majority had been focused on long-tailed macaques in Peninsular Malaysia, with limited studies elsewhere. High prevalence of *P. knowlesi* in NHPs was reported, particularly in Borneo, Peninsular Malaysia, the Philippines and Singapore. In a case study in Peninsular Malaysia, over 90% of long-tailed macaques sampled tested positive for *P. knowlesi*, *P. cynomolgi*, *P. inui* or *P. coatneyi*. The prevalence of *P. knowlesi* and *P. cynomolgi* infections was highest among adult males, with infections equally likely across urban, suburban, forest and plantation habitats.

NHP habitat loss is a major contributor to zoonotic malaria transmission, necessitating control strategies that target ecotones between forest and urban areas. Dr Sharma emphasized the need for further research on NHP reservoir species across Southeast Asia countries, the impact of land use changes, urban transmission dynamics and vectors, and enhanced surveillance through international collaboration.

2.5 Discussion points from this session

The discussion explored the complexities of addressing zoonotic malaria transmission. The distinction between peri-domestic and forest transmission was highlighted, emphasizing the need for targeted, context-specific interventions. Further research into micro-epidemiology and tailored guidelines for diverse settings were advocated for, especially taking into account the changing epidemiology of "human malaria" species. The opportunity to incorporate vector and reservoir distribution data along with climate and environmental data through Earth Observation into integrated risk maps for targeted control was discussed.

Chemoprophylaxis for high-risk groups was proposed, with insights shared from studies in Indonesia and Malaysia.

Challenges in macaque sampling, their potential as a reservoir and the risks of urban transmission were discussed. "Push and pull" interventions to manage human–macaque overlap, and oral baiting of macaques with hormonal contraceptives, treatment and vaccines were proposed.

3. Diagnosis and treatment of *P. knowlesi*

3.1 Clinical spectrum of *P. knowlesi* malaria and current point-of-care diagnostics

Professor Nicholas Anstey presented an overview of the clinical spectrum of *P. knowlesi* malaria and its diagnostic challenges. He highlighted the clinical range – from asymptomatic infections to severe malaria (which occurs in 6–9% of clinical cases) and death. The clinical presentation of severe *P. knowlesi* malaria mirrors that of severe *P. falciparum* in adults but does not cause coma. Infections can, but rarely do, cause very high parasitaemia; instead, they usually cause severe disease at a lower parasitaemia, with a lower fever threshold than for *P. falciparum* and *P. vivax*. The median parasitaemia in severe *P. knowlesi* cases is lower than it is in severe *P. falciparum* cases. Parasitaemia is a key predictor of severe disease, with levels greater than 15 000 µL increasing the risk of severe disease 16-fold.

Pregnant individuals face a lower risk of infection but higher likelihood of severe disease. Infection during pregnancy has caused maternal death and adverse neonatal outcomes.

Misdiagnosis remains a critical challenge. A review of deaths from *P. knowlesi* malaria in Sabah, Malaysian Borneo, from 2015 to 2017 found that 90% involved initial microscopic misidentification, with 77% of cases being misdiagnosed as *P. malariae (9)*. Professor Anstey made a case for microscopists in *P. knowlesi*-endemic areas where *P. malariae* is rare to take local epidemiology into account and report all "*P. malariae*" cases as *P. knowlesi*. This approach has been implemented in Sabah.

Recent developments in RDTs have led to improved sensitivity, especially with *P. vivax* lactate dehydrogenase (*Pv*LDH) RDTs. However, tests are not species-specific, particularly between *P. vivax* and *P. knowlesi*. RDT target antigens include histidine-rich protein 2 (HRP2), *Plasmodium* lactate dehydrogenase and aldolase, but none of these older RDTs are sufficient for detecting *P. knowlesi*. Some newer *Pv*LDH RDTs now have adequate sensitivity to detect *P. knowlesi*. Species-specific RDTs for *P. knowlesi* will be very important for timely diagnosis and treatment, as well as for understanding the true burden, particularly in co-endemic settings.

Professor Anstey recommended revisions to the *WHO guidelines for malaria (10)* and the WHO *Management of severe malaria: a practical handbook (11)* to:

- incorporate the 2014 WHO pragmatic clinical diagnostic criteria for severe malaria in *P. knowlesi*, including reporting cases microscopically diagnosed *P. malariae* as *P. knowlesi* in South-East Asia and Western Pacific; and
- for the pragmatic clinical diagnostic criterion for use of intravenous artesunate, reduce the existing 2014 WHO parasitaemia threshold from 20 000/µL to ≥ 15 000/µL for *P. knowlesi* and "*P. malariae*" in South-East Asia and Western Pacific regions.

3.2 Molecular diagnostics and new tools

Professor Lau Yee Ling provided an overview of diagnostic methods for *P. knowlesi*, highlighting the need for affordable and rapid or point-of-care tests with adequate sensitivity that are species-specific.

Molecular diagnostic methods offer improved accuracy and lower limits of detection. Conventional PCR methods (single-step, multiplex and hexaplex), nested and hemi-nested PCR, real-time PCR, quantitative PCR and reverse transcription PCR were reviewed and are summarized in Table 1. Most of these methods require specialized equipment and are relatively expensive as diagnostic tests. Real-time PCR, which quantifies parasites and detects results in real time, is comparatively the most expensive molecular method.

Innovative isothermal amplification methods, which do not require a PCR machine, were discussed. Recombinase polymerase amplification is rapid and sensitive but expensive, while loop-mediated isothermal amplification is cost-effective and may be suitable for some resource-limited rural settings due to minimal equipment needs.

Table 1. Summary of molecular diagnostics available for *P. knowlesi* identification, their limit of detection and the time taken to process samples

Diagnostic test	Limit of detection (range) p/μL	Time taken
RDT	100 – 1000	15 – 30 min
Microscopy	5 – 100	40 – 90 min
Nested PCR	0.1 – 10	6 – 8 hours
Reverse transcription PCR	0.1 – 20	2 – 3 hours
Recombinase polymerase amplification	1 – 10	12 – 20 min
Loop-mediated isothermal amplification	0.075 – 3	30 – 60 min

Professor Lau recommended developing protocols that reduce contamination risks, developing and validating molecular tests for point-of-care use, and integrating these tools into existing diagnostic workflows to enhance *P. knowlesi* detection and management.

3.3 Diagnostics used for national disease burden surveillance

Professor Jenarun Jelip highlighted the critical role of establishing a clear testing policy to accurately reflect *P. knowlesi* disease burden. Malaysia's testing policy was rolled out in a stepwise manner. Prior to 2011, PCR was limited to quality assurance; however, in 2011, it became mandatory for confirming *P. malariae* and *P. knowlesi* cases, and was extended to all malaria cases in Sabah by 2015. In 2017, genomic analysis was introduced for case classification under the malaria elimination scheme.

Light microscopy remains the gold standard for diagnosis of malaria. Malaysia has a network of five public health laboratories with 48 WHO-certified and 52 nationally certified malaria microscopists. Collaborations with the WHO Regional Office for the Western Pacific, UK NEQAS and the Research Institute for Tropical Medicine in the Philippines ensure rigorous external quality assurance for both microscopy and molecular diagnostics. Despite reduced "human malaria" testing since 2008, the costs for quantitative PCR have risen due to the testing policy and the increasing number of zoonotic malaria cases.

Professor Jelip emphasized developing rapid point-of-care tests and building molecular diagnostic capacity as priorities for advancing national surveillance.

3.4 Diagnostics for community surveillance

Professor Chris Drakeley began by discussing how the low prevalence (< 1%) of *P. knowlesi* in human populations, its limited geographical range and limitations in traditional diagnostics complicate efforts to capture infection dynamics at a population level.

Professor Drakeley highlighted the utility of serology in identifying *P. knowlesi*-specific antibodies from minimal blood or serum samples. Antibodies persist for longer in the blood than the parasite itself, serving as proxies for exposure over longer time frames. This enables community-level epidemiological evaluations, particularly in low-prevalence settings where active infections are difficult to detect. Serological analysis would also permit assessment of the absence of exposure to infection, i.e. where no antibodies are observed in sampled individuals. A 2016 study in Sabah showed that seropositivity was associated with forest activity, male gender, contact with macaques and older age, and negatively associated with increased elevation, higher socioeconomic status and use of insecticides *(12)*. Recent developments in refining *P. knowlesi* serology were presented, including combining multiple antigen responses and assessing cross-reactivity with the "human malaria" species.

Professor Drakeley proposed methods for integrating serology into *P. knowlesi* surveillance. Strategies could include targeting high-risk groups, combining PCR and active case detection with serology at sentinel sites, and periodic convenience sampling in groups such as plantation workers. These sites could also host vector and primatology surveys, acting as sentinel sites. He emphasized the need to leverage mathematical models and statistical analyses to optimize targeting and data interpretation, while incorporating serological assays into surveillance systems to harness their unique ability to capture species-specific exposure to parasites over a longer time frame.

3.5 Treatment of uncomplicated and severe *P. knowlesi*

Professor Anstey outlined current treatment recommendations for *P. knowlesi* malaria, highlighting the absence of drug resistance due to its zoonotic nature. While all antimalarials have been clinically effective, only ACTs (AL, ASMQ) and chloroquine have been evaluated in randomized controlled trials (RCTs). For uncomplicated malaria, ACTs (AL, ASMQ, DP) are the first-line treatment, with chloroquine as the second-line treatment. For severe malaria, intravenous artesunate is the recommended treatment.

Evidence from RCTs comparing ACTs and chloroquine supports the use of ACTs as the first-line treatment. While both treatments are 100% efficacious, ACTs offer advantages, including reduced anaemia, faster parasite and fever clearance *(13)*, and economic benefits through shorter hospital stays. Importantly, ACTs treat chloroquine-resistant *P. falciparum* and *P. vivax*, which could be misidentified as *P. knowlesi (9)*. Based on this high-certainty evidence, Professor Anstey proposed that the next update of the *WHO guidelines for malaria (10)* separate *P. knowlesi* from other non-falciparum species and recommend ACTs as the first-line treatment.

Although no RCTs have assessed intravenous artesunate for treatment of severe *P. knowlesi*, clinical evidence has demonstrated its efficacy, with a 0% case fatality rate in a large Sabah study and a six-fold reduction in the overall *P. knowlesi* case fatality rates after statewide implementation *(14, 15)*. In addition, one RCT showed that adjunctive paracetamol improved outcomes in severe *P. knowlesi*, reducing fever, acute kidney injury and proteinuria. Professor Anstey advocated for adjunctive therapy with three days of regularly dosed paracetamol in severe *P. knowlesi* cases to be added to the *WHO guidelines for malaria (10)*.

3.6 Discussion points from this session

Experts emphasized that WHO can provide support for *P. knowlesi* diagnosis. WHO should prioritize systematic literature reviews to inform recommendations and the inclusion of *P. knowlesi* in WHO prequalification mechanisms for RDT procurement. External quality assurance programmes were also highlighted as critical for maintaining countries' diagnostic capacity.

The timing and cost of establishing *P. knowlesi* surveillance systems were discussed, with Malaysia's stepwise approach serving as a model, balancing epidemiology and cost considerations.

Experts underscored the value of microscopy, particularly for identifying high parasite densities. Experts agreed with the recommendations to lower the threshold for intravenous artesunate treatment from 20 000/µL to 15 000/µL and to report suspected *P. malariae* cases in endemic areas as *P. knowlesi*. This emphasizes the importance of educating microscopists and clinicians on local epidemiology to improve diagnostic accuracy and treatment outcomes. The role of molecular diagnostics, bolstered by coronavirus disease (COVID-19) infrastructure, was highlighted for zoonotic malaria confirmation.

Surveillance in asymptomatic carriers was highlighted as important for monitoring potential human–mosquito–human transmission adaptations and broader epidemiological changes. Chemoprevention using chloroquine was proposed for high-risk groups in the states of Sabah and Sarawak, given its safety and the absence of chloroquine-resistant *Plasmodium* species. Experts also advocated for the inclusion of *P. knowlesi* in trials for novel antimalarial drugs.

4. Zoonotic malaria vectors and applied vector control practices

4.1 Overview of the bionomics of zoonotic malaria vectors and future control strategies

Professor Indra Vythilingam described research on *P. knowlesi* vectors in Malaysia since 2004. Studies have incriminated *An. latens*, *An. balabacensis* and *An. cracens* as the primary vectors, showing higher vectorial capacity in forest habitats and in Malaysian Borneo than in Peninsular Malaysia. Studies have revealed high parity rates (60–70%) in vectors, indicating failures in current vector control strategies. Macaque-baited trapping studies have found that vectors were most attracted to reservoirs at midnight when *P. knowlesi* gametocytes peak. Haplotype analysis has shown close genetic links among *P. knowlesi* parasites isolated from mosquito vectors, macaques and humans, indicating active zoonotic transmission. Historical and recent data on the distribution of the *Leucosphyrus* group show significant overlap between predicted vector occurrence and *P. knowlesi* cases; however, more work is required to understand the distribution of this vector group.

Key challenges to understanding *P. knowlesi* vector bionomics include morphological species identification, emphasizing the need for advanced diagnostic tools such as multiplex PCR assays. Professor Vythilingam also made a strong case for maintaining and developing skilled entomologists across the WHO regions of South-East Asia and Western Pacific. Dissecting mosquitoes to determine parity is a crucial step in calculating vectorial capacity for transmission studies, and the ability to demonstrate the presence of sporozoites in the salivary glands of mosquitoes is required for vector incrimination.

Professor Vythilingam called for screening of high-risk populations for non-*P. knowlesi* zoonotic malarias. Macaques, mosquitoes and humans have been found to be infected with *P. cynomolgi, P. inui and P. coatneyi* in Southeast Asia, and therefore continued spillover of these parasites is a risk.

Effective vector control strategies remain elusive due to the exophagic and exophilic behaviours of zoonotic malaria vectors. Potential tools include toxic sugar baiting, insecticide-treated clothing, spatial repellents and larval control, but further research is needed.

4.2 Current vector control strategies

Dr William Hawley provided an overview of vector control strategies for *P. knowlesi* and highlighted gaps in interventions across the regions of South-East Asia and Western Pacific. There has been minimal implementation of interventions in Cambodia, Indonesia, Myanmar and Thailand, and limited trials in Malaysia.

Dr Hawley highlighted the unique challenge with zoonotic malaria: unlike with "human malaria", zoonotic malaria requires interventions that target both NHPs and vectors. Two transmission pathways that could be targeted were outlined: mosquito–human and macaque–mosquito–human.

For mosquito–human transmission, hammock nets and forest packs with repellents have shown promising results in forest habitats. Outdoor residual spraying using deltamethrin formulations may reduce village-level risks, but operational challenges and insufficient evidence limit its use. Spatial repellents could also be useful, especially for huts where forest goers rest overnight. Larval source management (LSM) remains challenging due to natural, diffuse habitats, but could benefit from local knowledge for mapping and community-based interventions. Emerging tools such as drones could assist in habitat mapping and larvicide application.

To address macaque–mosquito–human transmission, potential interventions are mass drug administration with endocticides (e.g. ivermectin) in macaque populations, macaque population control via contraceptives and strategies to reduce human–macaque overlap, including community education and land use regulations; these should always take local cultures and beliefs into account. Dr Hawley emphasized that different cultures place different values on NHP populations, which impacts the NHPs' behaviours towards humans. Behavioural modifications, such as feeding stations to bait macaques away from human habitats, could reduce their role as a reservoir.

Integrated approaches combining human repellents with vector-targeting tools and macaque strategies may offer promising avenues for zoonotic malaria control.

4.3 Discussion points from this session

Dr Seth Irish provided a summary of the vector control session before opening the floor to discussion. He noted the important aspects of zoonotic malaria vector biology that had been discussed and the gaps in knowledge that persist, including incrimination of vector species, detection of mosquito resting and larval sites, biting times and seasonality. He outlined the potential control mechanisms that had been discussed and the challenges of assessing these tools in the context of *P. knowlesi*. These challenges may be addressed by learning from control of other vector-borne diseases such as West Nile virus and leishmaniasis. In addition, Dr Irish listed some enabling factors of control, including mapping of high-risk areas for chemoprophylaxis and larval sites, participatory research, and research into NHP host movement and range.

Key concerns were raised during the discussion regarding the accurate identification of vector species, with experts emphasizing the importance of dissection for sporozoite detection in mosquito salivary glands, rather than whole-mosquito PCR testing, which could lead to false vector incrimination.

While theoretical support exists for various vector control strategies, the group noted the critical gap in implementation evidence. Participants discussed LSM as a potential intervention, particularly in transitional landscapes, and suggested a targeted approach considering human cases and macaque populations. Community engagement was highlighted as crucial for maximizing the effectiveness of LSM. Novel approaches were discussed, including autodissemination of pyriproxyfen.

The potential of modelling to enhance *P. knowlesi* control challenges was emphasized, particularly for optimizing integrated strategies combining multiple vector, human and reservoir interventions in high-risk areas and populations.

5. Certification of malaria elimination in countries with zoonotic malaria

5.1 Challenges in certifying countries with zoonotic malaria and actions taken by WHO, and draft of the procedures for verification of elimination of non-zoonotic malaria in countries affected by zoonotic malaria

Dr Gasimov presented an overview of the WHO certification process for malaria elimination and addressed the challenges posed by the emergence of zoonotic malaria. He outlined that WHO certification requires evidence of zero indigenous malaria cases for at least three consecutive years and demonstration of a functional programme to prevent re-establishment of transmission throughout the country.

The presentation highlighted the complexities faced in countries where transmission of "human malaria" species has been interrupted, but *P. knowlesi* cases persist. Currently, such countries cannot be officially certified by WHO as malaria-free, which potentially creates a disincentive for maintaining efforts to eliminate non-zoonotic malaria. While zoonotic malaria may never be eliminated, the elimination of "human malaria" still represents a huge achievement. Detailed information was provided on the Terms of Reference and work of the Technical Advisory Group on Malaria Elimination and Certification subgroup on zoonotic malaria, which was established following discussions on *P. knowlesi* at the fifth meeting of the Advisory Group *(5)*.

The Technical Advisory Group on Malaria Elimination and Certification is the only entity that is mandated to advise WHO on the update of the certification criteria and operating procedures. During its fifth meeting held in Cairo, Egypt, on 27–28 November 2023, the committee acknowledged the challenges of tackling the transmission of *P. knowlesi (5)*. The Group confirmed that, according to the current WHO elimination guidance and definitions, certification of malaria elimination cannot be granted to a country where several thousands of local cases are reported annually, as the risk cannot be considered negligible. However, the Group concluded that official recognition could be considered to acknowledge Malaysia's achievement of interrupting transmission of the four main "human malaria" species, if this is verified.

The option of formally recognizing "human malaria" elimination, proposing the term "attestation" instead of "certification", was presented for discussion. This process would maintain the same rigorous standards as certification but would not result in addition to the WHO official register of malaria-free countries and territories. The criteria and procedures for attestation were outlined, emphasizing that it would acknowledge achievement, while clearly distinguishing it from malaria-free certification.

5. 2 Discussion points from this session

The participants acknowledged that existing certification frameworks and terminology were not designed to address zoonotic malaria and lacked clarity and consistency in this context. They highlighted the need to revise definitions and key terms such as "human malaria", "elimination" and "eradication" in WHO official texts, taking zoonotic malaria into account.

Participants discussed the concept of a "negligible risk" threshold for zoonotic malaria elimination, below which a country could potentially be certified as having eliminated malaria, provided that all "human malaria" species had been successfully eliminated. The need to define such a threshold was highlighted, as this would give countries with zoonotic malaria transmission a target to achieve, which is something that is currently lacking. An approach using a threshold based on rates or prevalence, rather than absolute case numbers, was discussed. The importance of the diagnostics being used was emphasized. Participants suggested learning from the certification processes for other diseases such as lymphatic filariasis and taking a subnational approach.

Participants acknowledged that, while countries with zoonotic malaria should prioritize reduction of morbidity and mortality from malaria regardless of certification status, the lack of clear targets makes it difficult to plan for and reach "the last mile". A key concern was the potential disincentivization of maintaining efforts to eliminate "human malaria" species in the absence of formal recognition. Moreover, countries could feel penalized for their efforts to identify zoonotic malaria. To address these issues, participants proposed a "dual certification" process whereby separate certification categories for "human" and zoonotic malaria would be developed, acknowledging the distinct challenges posed by natural reservoirs.

Participants stressed the importance of sustained funding for national malaria programmes to maintain surveillance and control of zoonotic malaria, even as they approach elimination of "human malaria". Participants emphasized that zoonotic malaria requires long-term strategies and continued cooperation between affected countries and WHO.

6. Control of zoonotic malaria and research needs

6.1 Malaria Policy Advisory Group subcommittee on control of zoonotic malaria: plans

Professor Drakeley introduced the work of the Malaria Policy Advisory Group subcommittee on control of zoonotic malaria and its plans. The subcommittee's initial focus is on reviewing existing options for control and their likely success through literature reviews and discussions with national malaria programmes. The group aims to provide feedback on the possible impact of control strategies and how these may help countries to reach the threshold of "negligible risk". The subcommittee notes that certification needs to reflect contemporary challenges. This is especially important with zoonotic malaria posing a potential issue in many South-East Asian and South American countries, many of which are at an advanced stage of the elimination process.

6.2 Assessing evidence on non-zoonotic *P. knowlesi* transmission

Professor Drakeley presented evidence on non-zoonotic (human–mosquito–human) transmission of *P. knowlesi*.

A recent systematic review indicated that, while such transmission is biologically possible, there is limited evidence of its widespread occurrence in endemic areas *(6)*. Laboratory studies have supported the biological feasibility of non-zoonotic transmission. In 1968, a full *P. knowlesi* human–mosquito–human transmission cycle was demonstrated under controlled laboratory conditions *(16)*. More recent research has shown low rates of human–*An. dirus* mosquito transmission of *P. knowlesi* via membrane feeding *(17)*.

Research on *P. knowlesi* infection dynamics in macaques has revealed strong synchronization of *P. knowlesi* with circadian rhythms; gametocytes peak in peripheral blood at night, which aligns with mosquito biting and macaque roosting times.

An analysis of more than 30 000 malaria records from Malaysian surveillance data examined the individual case reproductive numbers for *P. knowlesi*, with the aim of evaluating the probability of human–mosquito–human transmission from spatiotemporal patterns of reported *P. knowlesi* cases. The findings suggested that spatiotemporal patterns were consistent with infections from a large unobserved reservoir, likely macaques, rather than from undetected human–mosquito–human transmission. This was supported by the simultaneous analysis of *P. falciparum* and *P. vivax* cases, which showed a strong association with a human reservoir of infection. Genetic linkage studies could further validate these conclusions *(18)*.

6.3 Land use changes and the emergence of zoonotic malaria

Dr Kimberly Fornace presented the evidence linking *P. knowlesi* cases to land cover changes, particularly forest fragmentation. Integrating diverse data sources from Malaysian Borneo, the research explored multiple components of *P. knowlesi* transmission dynamics, including reservoir macaque behaviour, vector ecology, human space use and deforestation. Studies have consistently found increased risk of exposure and infection at forest fringes.

Dr Fornace highlighted the significant challenges associated with the emergence of zoonotic malaria, which is occurring amid unprecedented levels of environmental change, declining immunity to “human malaria” species, and a lack of proven interventions to reduce or stop transmission. She advocated for innovative approaches such as leveraging Earth Observation tools and other technologies to integrate health and environmental data, which can allow for more targeted surveillance and vector control measures.

Addressing the underlying drivers of spillover could help to reduce transmission of zoonotic malaria. Habitat modification to reduce human–macaque overlap, and vector control could be implemented in high-risk areas. Targeting reservoir macaques could also prevent spillover. Oral baiting of macaques for vaccination or treatment (a method effective in controlling echinococcosis and rabies) offers potential, but requires product development for vaccines and efficacy trials.

Dr Fornace also highlighted co-benefits of interventions such as forest restoration, with early studies showing reductions in vector abundance and improvements in carbon sequestration.

Dr Fornace emphasized using *P. knowlesi* ecology to guide environmentally targeted control measures. Forest edges are high-risk areas, and Earth Observation tools enable real-time habitat monitoring for interventions such as chemoprophylaxis and vector control in high-risk populations, such as logging camps. Research priorities should include integrating surveillance with environmental and wildlife monitoring, land management to reduce zoonotic malaria risks, spatially targeted treatment of high-risk populations, interventions to reduce prevalence of pathogen in wildlife reservoirs, and vector control.

6.4 *P. knowlesi* deep dive: a research landscaping review

Dr Nana Aba Williams presented work landscaping the research on *P. knowlesi*. The "deep dive" created a database containing updated information on completed and ongoing projects and research portfolios of countries and institutions, with the aim of identifying key questions under evaluation. The landscaping review identified 69 projects, of which 12 were active. Overall, the research areas with the most projects were parasite genetic diversity and basic science. Transmission, risk of infection, vaccines, clinical outcomes and diagnostics were also popular research areas. No active projects on vector control strategies were identified. Active research was mostly taking place in Malaysia, with some studies in Cambodia, Indonesia and Thailand.

6.5 Project BITE: evaluating bite prevention tools for malaria elimination

Professor Allison Tatarsky presented ongoing research in the Greater Mekong subregion assessing mosquito bite prevention tools to support malaria elimination. The research is focused on forest vectors that transmit "human malaria" species and have similar bionomics to *P. knowlesi* vectors.

The study is evaluating interventions including volatile pyrethroid spatial repellents, insecticide-treated clothing and topical repellents. Semi-field and field trials in Cambodia tested transfluthrin-based volatile pyrethroid spatial repellents, etofenprox-treated clothing with picaridin, and combined interventions. Results showed substantial reductions in *Anopheles* landings, with protective efficacy ranging from 61% to 95%. Mathematical modelling highlighted significant reductions in vectorial capacity, although this waned over time *(19)*. This study identified promising tools for targeted deployment in high-risk populations and areas.

6.6 Options for control and supporting research

Professor Drakeley provided an update on recent advances in understanding the basic biology of *P. knowlesi,* with relevance to control. The adaptation of *P. knowlesi* to culture in human erythrocytes has enabled studies of its drug susceptibility *(20)*. This work will offer valuable insights for developing chemoprevention strategies for high-risk populations and allow for *P. knowlesi* to be tested against newer compounds and drugs that are developed.

Studies have shown that *P. knowlesi* lacks redundancy in human cell invasion, taking longer to invade human cells compared to macaque cells, and is overall less efficient in this process *(21, 22)*. These findings indicate that *P. knowlesi* remains an opportunistic infection in humans, with as yet limited adaptation to human hosts.

A controlled human malaria infection study *(23)* testing a potential *P. vivax* vaccine demonstrated a response that may be cross-protective against *P. knowlesi*, highlighting opportunities to leverage *P. vivax* development platforms for *P. knowlesi* vaccine development. These findings underscore the potential to adapt existing tools and technologies to address *P. knowlesi* control more effectively.

6.7 Developing a research and development agenda

Professor Drakeley presented the occupational or activity-based risk factors for human cases of *P. knowlesi* in Malaysia *(24)*. Most (50–80%) infections are in groups such as agricultural workers, plantation workers, and those involved in logging and other forest-related work or recreation. These groups could potentially be targeted using chemoprophylaxis or, in the longer term, a vaccine. Targeted personal or spatial vector control could also be used. For the remaining (20–50%) cases, the priority should be to determine the environments in which exposure is occurring and develop control strategies such as NHP baiting, environmental management and vector control to reduce human–macaque overlap.

The increase in the burden and transmission of *P. knowlesi* requires a coordinated effort to improve surveillance, prevention and control capabilities, as options are currently limited. Community education is the only intervention that is currently being deployed to control zoonotic malaria specifically. Chemoprophylaxis of high-risk groups as a preventive intervention was widely discussed; however, while this intervention would reduce the disease burden, it would do little to stop zoonotic transmission of malaria, as human–mosquito–human transmission is unlikely. Therefore, there is a strong need to develop tools and combine strategies to stop the spillover of *P. knowlesi* from NHP populations to humans.

Professor Drakeley presented the research priorities from each country representative, shown in Fig. 6. Most priority areas were suggested by more than one country.

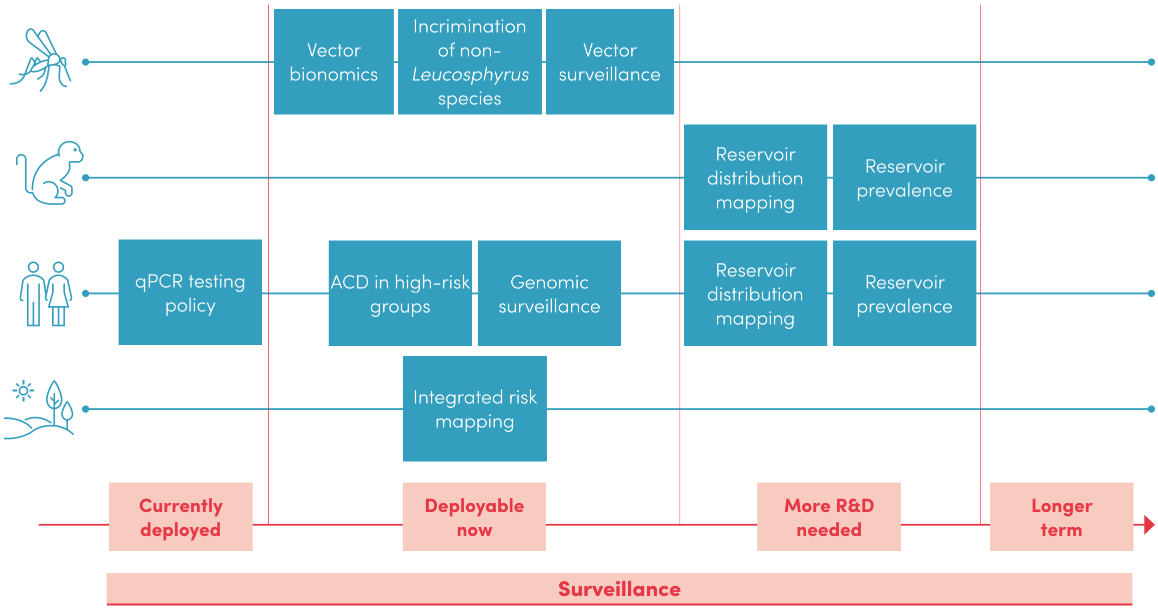
Fig. 6. Research priorities suggested by country representatives (Indonesia, Malaysia, the Philippines and Thailand)

Research priorities from country representatives				
Diagnostic methods	**Epidemiological surveillance**	**Reservoir populations**	**Parasite characteristics**	**Vector control**
• Development of rapid/ point-of-care *P. knowlesi* diagnostic	• Sentinel sites for humans and reservoir surveillance • Potential for human-mosquito-human transmission • Define foci of zoonotic malaria Prevalence monitoring and surveillance in humans and reservoirs	• Definition of reservoir hosts • Mapping of *P. knowlesi* prevalence in macaque populations	• *P. knowlesi* virulence • *P. knowlesi* genome sequencing • Research into non-*P. knowlesi* zoonotic malarias	• Vector control for zoonotic malaria vectors • Community-based risk reduction programmes • Personal protection • Up-to-date maps of the distribution and density of *P. knowlesi* vectors

Due to the multifaceted nature of the problem of zoonotic malaria, three research agendas were drafted under the following categories: i) surveillance (Fig. 7); ii) interventions for control (Fig. 8); and iii) basic biology research (Fig. 9). These were further categorized into the components of transmission dynamics: vector, reservoir, human disease and land management. As each research area has different levels of evidence and is at different stages of development, each area was placed along a timeline of availability for deployment, from "deployable now" to "longer term".

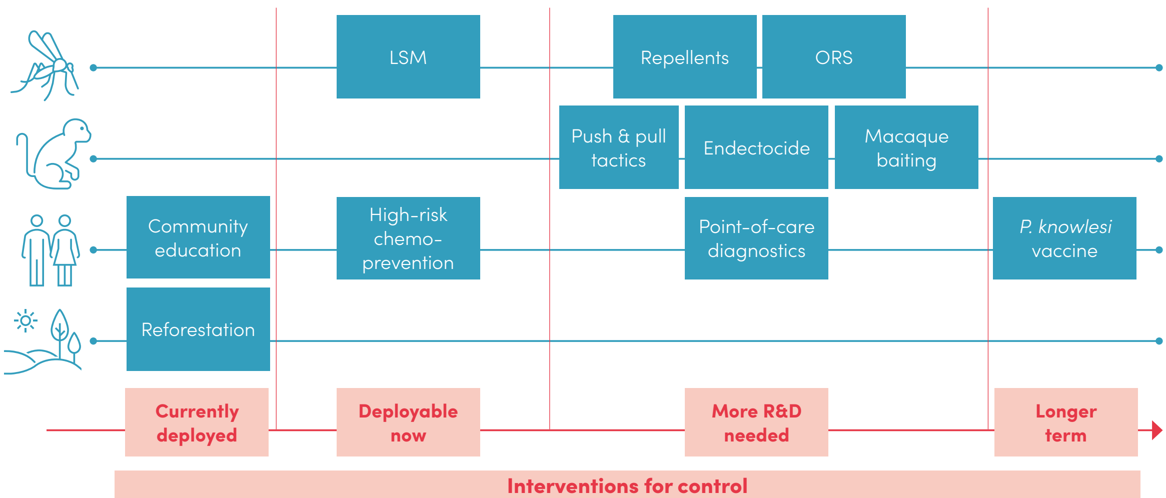
The key call for prioritization in terms of improving surveillance was for a species-specific *P. knowlesi* rapid or point-of-care diagnostic to allow for timely diagnosis and treatment and to improve burden estimates. These efforts would be supported by the inclusion of *P. knowlesi* in the WHO prequalification mechanism for malaria RDTs. The feasibility of country strategies to include molecular methods in testing policies, similar to Malaysia, or at the point of care should also be assessed. Increased surveillance of the density, distribution and infection prevalence in reservoir NHPs and incriminated vectors should also be prioritized. This will allow for comprehensive risk mapping to target control strategies that are suitable for zoonotic malaria. Developing *P. knowlesi*-specific serological diagnostics and incorporating them into surveillance strategies will require more research and can provide significant benefits for surveillance in terms of the time frame of exposure that they represent, especially given that infections are still rare. Active case detection in high-risk groups can address gaps in the knowledge on the asymptomatic burden of *P. knowlesi* and monitor for changes in transmission dynamics.

Fig. 7. Schematic of surveillance interventions along a timeline of availability



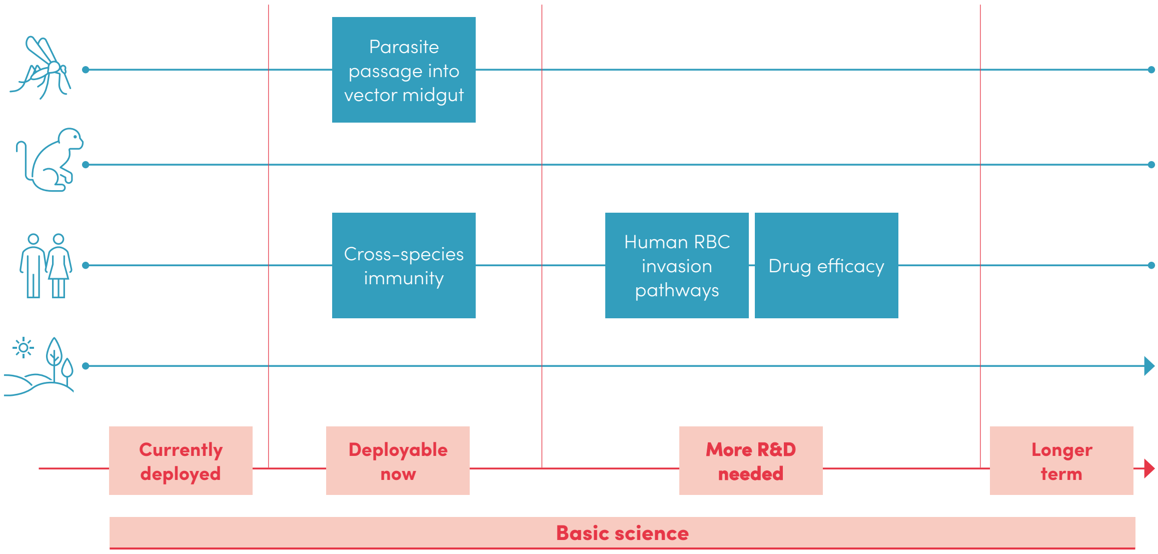
For control interventions to interrupt zoonotic transmission, macaques and vectors must be targeted to prevent transmission to humans. Research and development efforts should prioritize repellents, delivery of LSM and outdoor residual spraying. Once developed and evaluated, these interventions could be delivered in a targeted manner, coupled with chemoprophylaxis and community education. "Push and pull" tactics that simultaneously make human habitats less attractive to macaques and make natural habitats more attractive should be developed; using food sources may be efficacious. Macaque baiting with oral contraceptives or treatment, and longer term potentially with vaccines, was widely discussed but will require more research and development. The development of vaccines that protect against *P. knowlesi*, potentially a *P. vivax* vaccine that provides cross-protection against *P. knowlesi,* may be possible in the longer term.

Fig. 8. Schematic of control interventions along a timeline of availability



Research into the basic biology of the *P. knowlesi* parasite provides the building blocks for control and surveillance interventions. Understanding the mosquito-stage parasite progression from midgut into salivary glands is important for correctly incriminating vector species and can elucidate questions around vectorial capacity and transmission. As *P. knowlesi* is emerging amid a backdrop of declining prevalence of "human malaria", it will be important to understand the immunological implications of transmission in populations with lower levels of malaria exposure and protection. Monitoring efficacy in current and novel drugs will be important to ensure proper treatment and effective chemoprophylaxis interventions. Finally, continued research on *P. knowlesi* human cell invasion will enable monitoring for any potential adaptations that may favour non-zoonotic transmission and will inform vaccine development.

Fig. 9. Schematic of basic biology research along a timeline of availability



7. Conclusions

This technical consultation reviewed the situation with *P. knowlesi* and other zoonotic malaria species in affected areas, and the challenges countries are facing to control them.

Zoonotic malaria is a complex issue, and the key challenges faced were elucidated throughout this meeting. First, there are no proven control interventions for zoonotic malaria. National malaria programmes in countries affected by zoonotic malaria have no guidelines for practices or interventions to follow, making it difficult to control the disease. Second, the lack of a species-specific rapid or point-of-care diagnostic and the requirement of PCR for confirmation of *P. knowlesi* and other zoonotic malaria cases make it logistically difficult and expensive to estimate the true burden of disease. Third, knowledge of the density, distribution and prevalence of *P. knowlesi* and other zoonotic malaria species in NHP reservoirs, as well as in incriminated vectors, across South-East Asia and Western Pacific regions is lacking. This gap makes it difficult to develop comprehensive risk maps to target interventions. These problems are occurring against a backdrop of high levels of environmental degradation, deforestation and fragmentation, which are associated with the emergence of *P. knowlesi* and other zoonotic malaria species. In addition, as prevalence of "human malaria" continues to decline across Southeast Asia, it is unclear how changing levels of human immunity may impact the dynamics of zoonotic malaria. The latter statement is true also for other parts of the malaria-endemic world. Therefore, countries with potential risk areas should be vigilant in detecting zoonotic malaria.

Zoonotic malaria poses a unique challenge to the certification of countries for malaria elimination. Alternative certification options for countries where the transmission of "human malaria" has ended but zoonotic malaria cases remain were also discussed. Attendees suggested that the language in key definitions and official texts regarding WHO certification for malaria elimination and global malaria eradication could be revised to take into account the emergence of zoonotic malaria.

The increasing burden and transmission of *P. knowlesi* calls for coordinated efforts to enhance surveillance, prevention and control strategies, as current interventions are limited. While chemoprophylaxis for high-risk groups was widely discussed as a promising intervention to protect people from getting infected, no matter where the reservoir of infection is, this approach would not do much to control the disease, as it would not affect the main reservoir of *P. knowlesi*. Research priorities were developed and include species-specific *P. knowlesi* diagnostics to improve timely treatment and burden estimates. Increased surveillance of reservoir NHPs and vector populations will be important to improve risk mapping. Effective control interventions must target macaques and vectors and prevent overlap with human populations. It will also be important to prioritize the development of repellents, LSM and outdoor residual spraying, as well as oral baiting and "push and pull" tactics for macaques. These tools, coupled with environmental management, chemoprophylaxis and human education, could substantially decrease zoonotic transmission, especially if targeted at high-risk areas and populations.

Zoonotic malaria is a multifaceted problem spanning multiple sectors, including health, forestry, private sector and agriculture. Intersectoral collaboration and engagement with the communities most impacted will be paramount to successful control.

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Annex 1. List of participants

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All temporary advisors and experts invited to attend the above meeting submitted their declarations of interest, which were assessed by staff members of the WHO Global Malaria Programme. Based on this assessment and considering the nature of the meeting (with no policy recommendations and no product assessments anticipated), all the temporary advisors were permitted to participate fully in the discussions and deliberations of the meeting.

Annex 2. Agenda

Day 1 – Tuesday, 5 November 2024		
Opening session		
Moderator: Elkhan Gasimov, Head of Elimination Unit, WHO Global Malaria Programme		
09:00–09:30	• Opening remarks from Director, Global Malaria Programme • Self-introduction of participants	Daniel Ngamije Madandi
09:30–09:45	• Declarations of conflict of interest • Background and meeting objectives	Elkhan Gasimov
Session 1: Overview of the situation with zoonotic malaria		
Co-chairs: Allan Schapira, Jenn Zhueng Tam		
09:45–10:30	Global overview of the situation with zoonotic malaria in the world and actions taken by WHO to address the issue	Elkhan Gasimov
11:00–11:45	Situation with zoonotic malaria in Indonesina	Hellen Dewi Prameswari
11:45–12:30	Situation with zoonotic malaria in Malaysia	Jenn Zhueng Tam
13:30–14:15	Situation with zoonotic malaria in the Philippines	Liezl A. Fernandez
14:15–15:30	Situation with zoonotic malaria in Thailand	Prayuth Sudathip
16:00–16:30	Zoonotic malaria of concern: Pan American Health Organization	Maria de la Paz Ade
16:30–17:30	Spatial distribution and epidemiological risk factors for zoonotic *Plasmodium* infection among NHPs in South-East Asia	Reuben Sharma
17:30–17:40	Wrap-up of the day	Isabel Byrne

Day 2 – Wednesday, 6 November 2024		
Session 2: Diagnosis and treatment of *Plasmodium knowlesi*		
Co-chairs: Nicholas Anstey, Jenarun Jelip		
09:00–09:30	Clinical spectrum and point-of-care diagnosis of *P. knowlesi* malaria	Nicholas Anstey
09:30–09:55	Molecular diagnostics and new tools	Lau Yee Ling
09:55–10:15	Diagnostics used for national malaria disease burden surveillance	Jenarun Jelip
10:15–10:35	Diagnostics for community surveillance of *P. knowlesi*	Chris Drakeley
11:00–11:45	Moderated discussions on diagnosis practices applied in countries and challenges the countries are facing	Nicholas Anstey
11:45–12:15	Treatment of uncomplicated and severe *P. knowlesi*	Nicholas Anstey
12:15–13:00	Moderated discussions on treatment practices applied in countries and challenges the countries are facing	Nicholas Anstey

Session 3: Zoonotic malaria vectors and applied vector control practices		
Co-chairs: Indra Vythilingam, Prayuth Sudathip		
14:00–14:40	Overview of bionomics of zoonotic malaria vectors and future control strategies	Indra Vythilingam
14:40–15:30	Current vector control strategies	William Hawley
16:00–16:50	Moderated discussions • Incrimination of vectors • Sentinel sites and mapping of vector distribution • Vector control tools and strategies for intervention, including larval control • Land use changes in relation to vector distribution	Indra Vythilingam
16:50–17:00	Conclusions of the session	Seth Irish
17:00–17:15	Wrap-up of the day	Isabel Byrne

Day 3 – Thursday, 7 November 2024		
Session 4: Certification of malaria elimination in countries with zoonotic malaria		
Co-chairs: Pedro Alonso, Kamini Mendis		
09:00–10:15	Challenges in certifying countries with zoonotic malaria and actions taken by WHO	Elkhan Gasimov
10:15–11:00	Draft of the procedures for verification of elimination of non-zoonotic malaria in countries affected by zoonotic malaria	Elkhan Gasimov
11:30–12:00	Moderated discussions	Larry Slutsker
Session 5: Control of zoonotic malaria and research needs		
Co-chairs: Chris Drakeley, Hellen Dewi Prameswari		
12:00–12:10	Malaria Policy Advisory Group subcommittee on control of zoonotic malaria: plans	Chris Drakeley
12:10–12:30	Assessing evidence on non-zoonotic *P. knowlesi* transmission	Chris Drakeley
13:30–14:30	• Land use changes and the emergence of zoonotic malaria • *P. knowlesi* deep dive: a research landscaping review • Project BITE: evaluating bite prevention tools for malaria elimination	Kimberly Fornace Nana Aba Williams Allison Tatarsky
14:30–15:30	*P. knowlesi*: options for control and supporting research	Chris Drakeley
16:00–16:15	Wrap-up	Isabel Byrne
16:15–16:30	Closing remarks	Elkhan Gasimov

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