

## Informal Consultation on the Public Health Importance of *Plasmodium knowlesi*



Kuching, Sarawak, Malaysia  
22–24 February 2011



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Ministry Of Health, Malaysia

**Informal Consultation on the Public Health Implications  
of Malaria in Humans due to *Plasmodium knowlesi***



22–24 February 2011, Universiti Malaysia Sarawak, Kuching, Sarawak, Malaysia

English only

**REPORT**

**INFORMAL CONSULTATION ON THE PUBLIC HEALTH IMPORTANCE OF  
*PLASMODIUM KNOWLESI***

Convened by:

WORLD HEALTH ORGANIZATION  
REGIONAL OFFICE FOR THE WESTERN PACIFIC

Kota Samarahan, Sarawak, Malaysia  
22- 24 February 2011

## NOTE

The views expressed in this report are those of the participants of the Informal Consultation on the Public Health Importance of *Plasmodium Knowlesi* and do not necessarily reflect the policies of the Organization.

**Keywords:** *Plasmodium knowlesi*, *Plasmodium malariae*, nested PCR, vector, primate malaria, macaques

Cover photo by Anthony Sebastian.

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OBSERVERS AND SECRETARIAT

## LIST OF ACRONYMS

ACT	Artemisinin-based combination therapy
DNA	Deoxyribonucleic acid
FIND	Foundation for Innovative New Diagnostics
G6PD	Glucose-6 phosphate dehydrogenase
HIS	Health information system
LAMP	Loop-mediated isothermal amplification
MRC	Malaysia Research Center
PCR	Polymerase chain reaction
Pf	<i>Plasmodium falciparum</i>
Pk	<i>Plasmodium knowlesi</i>
pLDH	Plasmodium lactate dehydrogenase
Pm	<i>Plasmodium malariae</i>
Pv	<i>Plasmodium vivax</i>
QEH	Queen Elizabeth Hospital, Kota Kinabalu
RDT	Rapid diagnostic test
RNA	Ribonucleic acid
SOP	Standard operating procedure
UNIMAS	University Malaysia Sarawak
WHO	World Health Organization

## SUMMARY

*Plasmodium knowlesi*, a malaria parasite prevalent in certain species of monkeys, is a zoonosis which also can affect humans. In 1965, the first case was reported of a human infection with *P. knowlesi* acquired in Malaysia. Since then, there have been cases reported in many other countries in South-East Asia, with a significant number of them in a few countries.

It is a public health problem limited to population groups that live, work in or visit forested areas.

This Informal Consultation on the Public Health Importance of *Plasmodium knowlesi* examined the current status of *P. knowlesi* infections in humans in terms of geographic distribution, clinical manifestations, methods of diagnosis, treatment and control.

The following recommendations were made:

- (1) To estimate the burden of *P. knowlesi* in humans, the incidence of *P. knowlesi* should be estimated through well-designed studies in areas where macaques and vectors have been shown to exist and where transmission of *P. knowlesi* to humans has been demonstrated.
- (2) As diagnosis of *P. knowlesi* based on microscopy is not definitely possible, in designated areas with known human cases of *P. knowlesi* microscopy results recorded as *P. malariae* should be reported as *P. malariae/P. knowlesi* in order to guide case management.
- (3) As polymerase chain reaction (PCR) is the only validated diagnostic method available to confirm *P. knowlesi*, standard operating procedures (SOPs) for PCR for *P. knowlesi* and all other human malaria parasite species should be worked out together with quality assurance systems and should be adhered to. The procedures should be coordinated by WHO.
- (4) National reference laboratories for *P. knowlesi* should be established as part of a regional network that includes a regional reference laboratory.
- (5) *P. knowlesi* should be included in the development of new diagnostic methodologies, especially easy-to-use and rapid ones. In the meantime, existing dual pLDH malaria rapid diagnostic tests that have been prequalified by WHO-FIND should be evaluated for their ability to detect *P. knowlesi*. FIND is the Foundation for Innovative New Diagnostics.
- (6) Clinicians and laboratory staff should be aware of the existence of *P. knowlesi* in the area and the possibility of atypical and severe clinical presentations to ensure rapid and effective *P. knowlesi* diagnosis and case management and to prevent deaths.
- (7) *P. knowlesi* symptoms, clinical course and management should be included in national malaria treatment guidelines, on reporting forms and in health staff training in all South-East Asian countries.
- (8) While chloroquine has been fully efficacious for *P. knowlesi* treatment, in countries where *P. falciparum* is prevalent, all suspected or doubtful *P. malariae/P. knowlesi* cases should be treated as *P. falciparum* according to national guidelines. They should be monitored closely because uncomplicated *P. knowlesi* cases rapidly can lead to severe disease with a high case fatality rate.

- (9) Severe *P. knowlesi* cases should be treated like severe falciparum malaria according to national guidelines.
- (10) All severe malaria cases and deaths in hospitals should be reviewed routinely and documented according to a standard format, including confirmation of malaria by PCR that includes all species, including *P. knowlesi*.
- (11) Personal protection measures (such as insecticide-treated mosquito nets, protective clothing and repellents) and/or chemoprophylaxis together with health promotion should be implemented for populations at risk.
- (12) Countries should establish a comprehensive strategy to control *P. knowlesi* consisting of rapid diagnosis, appropriate timely treatment, personal protection, surveillance and health information targeting populations at-risk and health staff as well as operational research.
- (13) Control measures for *P. knowlesi* should be considered as an opportunity to strengthen comprehensively prevention and control of all malarias in vulnerable groups as well as the health system.
- (14) Countries that have achieved malaria elimination or are close to elimination should be vigilant with regard to *P. knowlesi* and formulate a strategy to deal with it.
- (15) Surveillance should be continued to detect human-to-human transmission of *P. knowlesi*. If it is confirmed and *P. knowlesi* becomes the fifth human malaria parasite, it then would be inconsistent with malaria elimination.
- (16) Funds should be mobilized to carry out key research in places with known human cases of *P. knowlesi*.
- (17) Further consultation about *P. knowlesi* should be organized in two years to review new scientific evidence and follow up on progress made in the implementation of the above recommendations.



## 1. INTRODUCTION

*Plasmodium knowlesi* is a malaria parasite prevalent in certain species of monkeys but it also affects humans. In 1965, the first case of a human infection with *P. knowlesi* acquired in peninsular Malaysia was reported and since then it has been reported in several other countries in South East Asia, with a significant number of cases in a few countries.

*P. knowlesi* has long been a subject of basic research. It was used as a simian model for the study of the pathology of human malaria, and its full genome has recently been published. However, a large knowledge gap exists in regard to its epidemiology, clinical manifestations in humans, its public health implications and effect on malaria control and elimination, and appropriate strategies for prevention and control.

The informal consultation was convened to bring together researchers, epidemiologists, public health experts and representatives of the ministries of health of countries with confirmed or possible human *P. knowlesi* infections to discuss public health implications of *P. knowlesi* malaria in the Region and define the way forward.

The objectives of the consultation were:

- (1) to review available knowledge and share unpublished data and experiences in order to identify the public health significance of *P. knowlesi* infection in humans;
- (2) to make recommendations on appropriate diagnostic, case management, prevention and control strategies for *P. knowlesi* infection in humans, and possible implications for malaria elimination; and
- (3) to define a research agenda for diagnosis, case management, prevention and control of *P. knowlesi* infection in humans.

## 2. PROCEEDINGS

WHO Regional Office for the Western Pacific's Informal Consultation on the Public Health Implications of Malaria in Humans due to *Plasmodium knowlesi* was held on 22-24 February 2011, at the University of Malaysia Sarawak, Kota Samarahan, Sarawak. The Deputy Vice Chancellor of the University Malaysia Sarawak, gave the first welcome remarks. Dr Eva Maria Christopher outlines the background, objectives, and expected outcomes of the consultation. The consultation was official opened by the Deputy Director General of Health Malaysia, Dr Lokman Hakim Sulaiman.

The agenda of the meeting is provided in Annex 1, the list of meeting participants, temporary advisers, observers and secretariat is provided in Annex 2.

## 2.1 Technical Session 1

### Review of available knowledge of *P. knowlesi* infections in humans

#### 2.1.1 Historical aspects and overview of the epidemiology of *P. knowlesi* in South East Asia - Balbir Singh

*P. knowlesi* is widely distributed in South East Asia. Infections are acquired in forested areas inhabited by macaques where humans live or work. At highest risk are farmers, hunters, logging camp workers, army personnel and travelers to forested areas. There are a number of mosquito vectors identified as being responsible for transmission of *P. knowlesi* to humans but all are members of the *Anopheles leucosphyrus* group that are found throughout the South East Asian region. The disease in humans is often fatal but can be fully treated using existing drugs including chloroquine and artemisinin and its combinations.

*P. knowlesi* was first isolated from a macaque imported into India from Singapore. The first human case was reported in 1965 by Chin et al., of an American who had acquired the infection while working in Peninsular Malaysia [1]. In 1971, a presumptive human case was reported in peninsular Malaysia [2]. More recently, interest in human *P. knowlesi* was triggered by the description of a large focus of human cases in the Kapit Division of Sarawak, Malaysian Borneo [3]. Between 2000 and 2010, 2,229 malaria cases in Sarawak [including 36 cases from 1996 were investigated by PCR, out of which 879 or virtually every case previously diagnosed by microscopy as *P. malariae* were actually *P. knowlesi* [4, 5]. Only six *P. malariae* were found and all six were imported.

The natural reservoir hosts and source of human infections in Sarawak, Malaysian Borneo are the long-tailed macaque (*Macaca fascicularis*) and pig-tailed macaque (*Macaca nemestrina*), and in Peninsular Malaysia the banded leaf monkey (*Presbytis melalophus*) has also been identified as a natural host. In Kapit, Sarawak, one study found 71 (86.6%) of 82 long-tailed macaques and 13 (50%) of 26 pig-tailed macaques were positive for *P. knowlesi* [6]. See Figure 1.

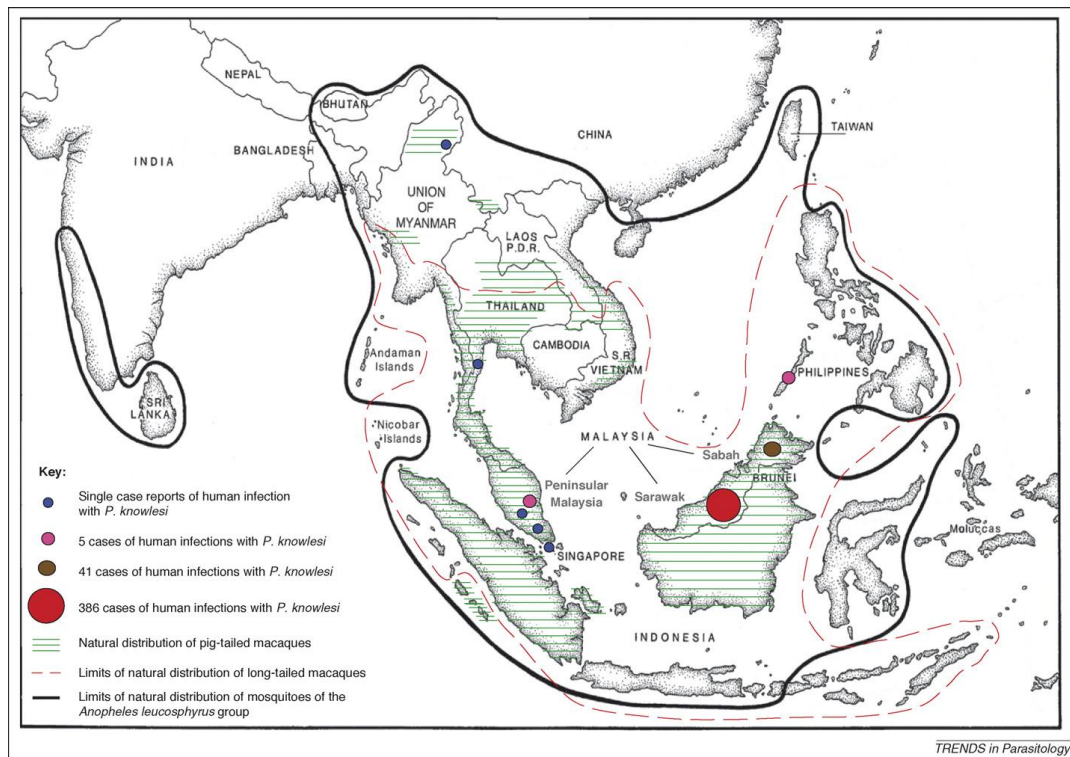
The vector responsible for monkey-to-human infection in Sarawak is *Anopheles latens*. It feeds outdoors after dusk on the blood of humans and monkeys living in close proximity to the jungle. [7].

Monkey-to-human transmission has probably been occurring since ancient times [6]. In Sarawak, Malaysian Borneo, transmission is characterized by low incidence of parasites in humans. Mainly adults are infected and no clustering is seen in longhouse communities, indicating that people staying or working in the jungle and jungle fringe are at highest risk. Human-to-human transmission of *P. knowlesi* can occur under experimental conditions, and although it has not been demonstrated under natural conditions, it may be taking place and it may take place in the future.

Because it is not possible to microscopically differentiate *P. malariae* and *P. knowlesi* in Sarawak, all cases diagnosed as *P. malariae* should be reported as *P. knowlesi*. Human cases show a wide spectrum of disease. Most are characterized as chronic and symptomatic but some cases can be severe leading to death. Parasitemia of *knowlesi* malaria cases varies: 19% of 107 cases in the Kapit study had more than 5,000 parasites per  $\mu\text{l}$  of blood and 30.8% had less than 500 parasites per  $\mu\text{l}$  of blood [5].

Cases can be successfully treated with a combination of chloroquine and primaquine. In the original Kapit study there were no deaths [3]. Deaths have, however, been confirmed. One from Brunei (Singh, unpublished data), three from Sabah ([5] and Singh, unpublished data) and 13 from Sarawak (Singh, unpublished data) [8].

Figure 1. Number and distribution of reported human infections with *P. knowlesi*, the limits of natural distribution of two species of macaques and the limits of natural distribution of mosquitoes of the *An. Leucosphyrus* group



Source: Trends in Parasitology. 2008

### 2.1.2 *P. knowlesi* in human challenge studies and syphilis therapy – Kevin Baird

Historically thousands of humans were infected with *P. knowlesi* as treatment of tertiary syphilis. In 1932 Knowles and Das Gupta [9] infected three individuals that cleared their infections without therapy. Nicol described the therapy in 1935 [10]. Chopra and Das Gupta reported treating two syphilis patients in 1936 [11]. Ciuca treated hundreds of patients in Romania [12]. *P. knowlesi* was considered to be more effective than *P. vivax* for treating syphilis because it produced higher and more frequent fevers because of its 24 hour cycle, and the fact that it was readily available from monkeys. *Vivax* malaria had an 8% risk of causing death while most *P. knowlesi* infections self cleared. One drawback was that patients often became immune to the parasite and therefore were immune to therapy.

A number of different strains were used for syphilis treatment. Five strains of *P. knowlesi* are archived in the United States.

### 2.1.3 Clinical features and pathophysiology of knowlesi malaria – Janet Cox-Singh

A series of clinical studies conducted in the hospital at Kapit, Sarawak, Malaysian Borneo provided a clinical description for *P. knowlesi* including uncomplicated and complicated/fatal infections [5, 8].

*P. knowlesi* morphology is the same as *P. malariae* but the similarity ends there because *P. knowlesi* exhibits a much greater capacity for mortality with infection than *P. malariae* in South East Asia. *P. knowlesi* has the shortest asexual replication cycle of all on the Plasmodium species infecting humans and non-human primates. Furthermore all red blood cells are permissive to

invasion by *P. knowlesi* merozoites. Therefore parasitemia in *P. knowlesi* infections increase daily and is not restricted by erythrocyte age. Parasitemias can reach >500,000 parasites/ul within 3-5 days following the onset of symptoms [4]. Parasitemia is associated with severe *P. knowlesi* malaria and we suggest that in patients infected with this potentially rapidly replicating species of Plasmodium that parasitemias >50,000 parasites/ul be treated urgently and managed in a high dependency unit until the parasitemia is controlled. In contrast *P. malariae* has a 72 hour erythrocytic cycle, and therefore parasitemia increases every third day and parasitemias remain low because red cell invasion is restricted to very old red blood cells. [13]

Results of a prospective study showed that patients with uncomplicated cases of *P. knowlesi* had overall lower parasitemias, relatively higher serum creatinine, were not clinically anemic (<7.1g/l) but had significantly lower platelet levels than comparable groups of patients with *P. falciparum* or *P. vivax*. [5]

Clinical studies in Sarawak, Malaysian Borneo, indicate that at least 10% of patients with *P. knowlesi* malaria develop severe disease as classified by the WHO with approximately 1% fatality. [5] Therefore accurate reporting of *P. knowlesi* infections is important to ensure that clinicians, particularly in the South East Asian region, treat knowlesi infections with the same urgency required for *P. falciparum*. Knowlesi malaria should be suspected in patients with a parasite morphology of *P. malariae*, parasitemias >5,000/ul, platelet counts <150,000/ $\mu$ L and a recent history of time spent in the jungle or forested areas of South East Asia [14].

#### 2.1.4 Treatment of knowlesi malaria and studies on antimalarial drug sensitivity of *P. knowlesi* - Cyrus Daneshvar

A prospective clinical study was carried out looking at patients that presented at Kapit District Hospital, Sarawak, Malaysia from 2006-2009[5]. Patients recruited into the study were older than 15 years, had no reported usage of anti-malarial therapy within 14 days of presentation, had parasitemias less than 100,000/ $\mu$ I of blood, were not pregnant and had a single species *P. knowlesi* infection defined by PCR. Blood was collected on filter paper daily during admission and thereafter on days 7, 14, 21 and 28. At each visit a clinical review was done and blood films made. Parasite DNA was extracted from blood spots collected on filter paper and analyzed by nested PCR to assess for parasite recrudescence and re-infection. Patients were treated with 25mg/kg of chloroquine orally over 48 hours. Two 15 mg doses of primaquine were given on days 1 and 2 of admission.

Out of 145 admissions with non-falciparum malaria, 111 were due to *P. knowlesi* and 96 met the study criteria. Out of those 73 agreed to partake in the 28 day in vivo study and 60 completed the study.

The study showed that early effective treatment is important. The median [interquartile] time to become negative by PCR was 3 [2-3] days. All patients were PCR negative at 7, 14, 21 and 28 days. No patients were readmitted during the 2 year period of the study.

The results showed that chloroquine appears to be a very effective anti-malarial in uncomplicated knowlesi malaria infections. [15] Patients rapidly improved symptomatically and the treatment was well tolerated. There was no evidence of resistance/treatment failure. This study did not examine the optimal treatment for severe cases, and given the difficulties in rapidly excluding falciparum infections (which would be resistant to chloroquine), standard malaria guidelines should be followed – which in most countries would include an artemisinin derivative.

2.1.5 Laboratory diagnosis of *P. knowlesi* - *Shamilah Hisam and Balbir Singh*

*P. malariae* and *P. knowlesi* cannot be distinguished microscopically using thick films. There are some minor differences (see below) that may be seen in thin films but these are usually not enough to be able make a definitive diagnosis [16].

Table 1. Morphological Characteristics of *P. knowlesi* and *P. malariae*

<i>P. knowlesi</i>	<i>P. malariae</i>
Mature schizonts have up to 16 merozoites	Schizonts contains 6-12 merzoites
Late trophozoites <u>sometimes</u> have amoeboid cytoplasm	Late trophozoites have compact cytoplasm

Thin films are sometimes made but are often not fixed and stained. There are also limitations because of the poor quality of blood films, poor staining, the short examination time and lack of experience on the part microscopists that limit their use for diagnosing *P. knowlesi*.

Even in properly made and stained thin films, the early ring forms look like *P. falciparum* and all the other stages look like *P. malariae*. Different forms are often seen in same patient. By looking at a single parasite or even multiple parasites on a thin blood film, *P. malariae* cannot usually be differentiated from *P. malariae* or in some cases *P. falciparum*.

Given that most microscopists only use thick films for diagnosis, in areas with known knowlesi microscopists should report everything that looks like *P. malariae* as Pm/Pk.

The two available alternatives are PCR and RDTs.

Nested PCR is currently the definitive method for diagnosing *P. knowlesi* [3-5]. The nested PCR developed at UNIMAS has been the key to our current knowledge about the geographic distribution and possible public health impact of *P. knowlesi*. To do the PCR properly a good laboratory setup is needed with at least three separate rooms.

There are no commercially available RDTs specifically for *P. knowlesi* and those RDTs that have been evaluated and detect *P. knowlesi* are not sensitive enough to pick up infections with less than 500 parasites per  $\mu\text{l}$  of blood [17-19]. In the Kapit study for example, 30.8% of *P. knowlesi* positive by PCR had less than 500 parasites per  $\mu\text{l}$  blood, so they would have been missed with an RDT [5].

Based on current studies, diagnosis of *P. knowlesi* should be based on the following criteria:

- (1) Where *P. knowlesi* infections of humans are known to occur and *P. malariae* cases are minimal, every case that microscopically looks like *P. malariae* has to be assumed to be *P. knowlesi* and reported as such.
- (2) Nested PCR assays and real-time PCR assays are the standard for correct identification but both require stringent lab set-ups and careful analysis of results.
- (3) PCR is not suitable for routine diagnosis because of the time taken to obtain results and cost but can be justified for confirmation, determination of parasite density and overall quality control.

- (4) Available RDTs that have been evaluated have shown low sensitivity. More RDTs need to be evaluated.

For Sarawak, Malaysian Borneo, the recommendations based on the results of recent studies are:

- (1) All cases diagnosed as *P. malariae* by microscopy, unless there is a history of recent travel overseas are assumed to be *P. knowlesi* and treated accordingly. There is no need to wait for confirmation by PCR.
- (2) For Peninsular Malaysia and Sabah, Malaysian Borneo, more data are needed but based on current data all cases diagnosed by microscopy as *P. malariae* should be reported as Pm/Pk.
- (3) If it looks like *P. malariae* by microscopy, and the patient presents with thrombocytopenia, parasitemia greater than 10 000 parasites/µl blood and a travel history to forested areas in South East Asia then the case should assumed to be *P. knowlesi* and treated accordingly. There is no need to wait for confirmation by PCR.

#### 2.1.6 Entomological aspects of *P. knowlesi* transmission - *Indra Vythilingam*

Results of entomological studies in Kapit, Sarawak demonstrated that *An. latens* was the primary vector for *P. knowlesi* [7, 20]. It bites both monkeys and man (simio-anthropophagic). *An. latens* is also the vector of human malaria in Sarawak. In this study, other simian malaria parasites (*P. inui*, *P. coatneyi*, and *P. fieldi*) were also present in *An. latens*. The monkey-to-human biting ratio was found to be 1:1.3. *An. latens* was mainly exophagic. In farms and forested areas the peak biting time was between 19:00 to 20:00. The entomological inoculation rate ranged from 0.69 to 1.4.

An *cracens* was found to be the vector based on four mosquitoes that were found positive for *P. knowlesi* in Kuala Lipis (Pahang- peninsular Malaysia) *An. cracens* was exophagic and does not enter houses to bite. The peak biting time was between 19:00 to 21:00. The monkey-to-man biting ratio was 1:2 [20].

In a similar study carried out in Sabah *An. balabacensis* was implicated as the primary vector of *P. knowlesi*. It is also the primary vector for human species of malaria in Sabah.

In Viet Nam the vector is *An. dirus* [21].

Vector studies need to be conducted in the region where knowlesi malaria is occurring in humans. Malaria is a zoonosis and existing vector control measures will not be able to control the vectors [22]. This is important for malaria elimination to be successful.

#### 2.1.7 *P. knowlesi* in macaques - *Joost Philippa*

*P. knowlesi* naturally infects populations of macaque, of which there are 22 species and numerous sub-species worldwide. The natural reservoir is currently mainly associated with three non-human primate species: namely the long-tailed macaque (*M. fascicularis*); the pig-tailed macaque (*M. nemestrina*); as well as mitred leaf monkeys (*Presbytis melalophus*). Several known mosquito vectors of *P. knowlesi* are attracted to both humans and non-human primates, which could lead to non-human primate-to-human transmission.

Macaque species show a clear variation in response to infection. Experimental infection of Rhesus macaques (*M. mulatta*) with *P. knowlesi* causes severe malaria, and has a comparable

phylogeny and host-parasite relationships to human malaria parasites. Infections in Rhesus macaque models are characterized by anemia, hemolysis and kidney dysfunction so they have been used to study the pathology of severe and cerebral malaria [22, 23]. In its natural hosts, the long-tailed and pig-tailed macaques and mitred leaf monkeys, *P. knowlesi* infection results in a chronic infection with low grade parasitemia and mild transient disease [24, 25].

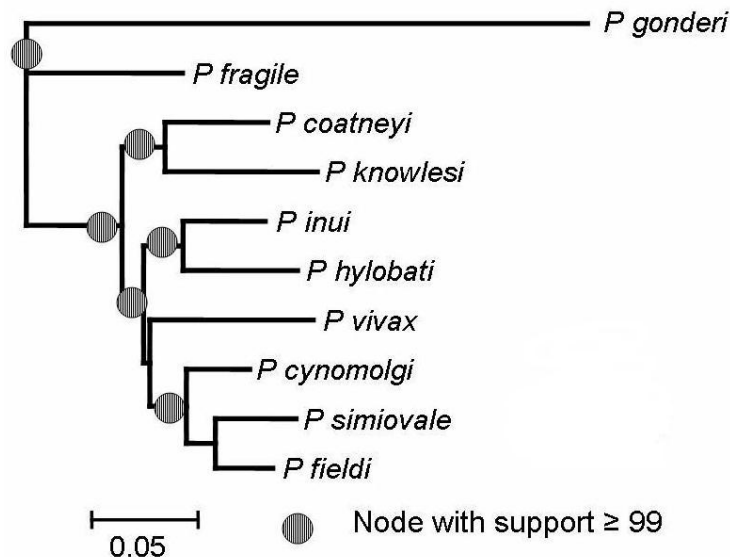
The epidemiology of malaria in macaques or other potential non-human primate host species has not been adequately described in large part due to difficulties in trapping and taking samples from non-human primates in the wild. Published prevalence results generally vary from 0% to 8% but a recent study in Thailand showed that up to 38.1% of macaques tested were positive for *P. knowlesi* [26]. More surveillance needs to be done. Non-invasive sample collection including fecal samples would be one way to overcome the problems associated with trapping and sampling macaques [27, 28], therefore these methods should be validated.

#### 2.1.8 Biology of cross-species among the primate malarias - *John Barnwell*

In 1908 *P. brasilianum* was the first species of Plasmodium identified a Cacajao callus monkey captured in the north east Amazon basin. Since then *P. brasilianum* has been found in many species of neotropical primates in central and south America. It is genetically synonymous with *P. malariae*. In 1939 *P. simium* was identified in a Howler monkey from southern Brazil. This species distribution is confined to the Atlantic forests of southern Brazil in *Alouatta* and *Ateles* species. It also bears genetic identity with *P. vivax*. These primate malaria species probably arose from anthroponotic events in the last 500 years.

In Asia the non-human species of primate malaria parasites found in monkey hosts are *P. knowlesi*, *P. coatneyi*, *P. inui*, *P. fragile*, *P. cynomolgi*, *P. simiovale* and *P. fieldi*. Those found in gibbons are *P. hylobati*, *P. eylesi*, *P. jefferyi*, *P. youngi* and *P. pitheci*, and *P. sylvaticum* that infect orangutans. See Figure 2.

Figure 2. Phylogenetic Relationship of Non-Human Malaria Parasites [29]



In addition to *P. knowlesi* that has been found to infect humans under conditions of natural transmission by forest (Leucosphyrus) mosquitos, *P. cynomolgi* and *P. inui* have also been shown to infect humans under experimental conditions by blood or mosquito transfer, but transmission of these two species has as yet not been found occurring in nature. Presently, the human infections with *P. knowlesi*, and perhaps other simian species, represent zoonotic events.

*P. knowlesi* is characterized by the following: a 24-hour blood stage growth cycle; no Schüffner's stippling in giemsa stained blood smears (only Sinton-Mulligan dots); production of 8 to 12 merozoites; expression of classic variant antigen genes; erythrocyte invasion is Duffy blood group dependent in humans; and nonproduction of liver stage hypnozoites to produce relapses.

*P. cynomolgi* is genetically akin to *P. vivax*. It has a classic relapse mechanism with hypnozoites in the liver, shows Schüffner's stippling, has a 48-hour blood stage cycle that develops 14 to 20 merozoites, and there are no classic variant antigen genes.

*P. inui* is also genetically related to *P. vivax* but phenotypically like *P. malariae*. It has a 72-hour blood stage cycle, produces 10 to 16 merozoites, and Ziemann's dots in the red cell in Giemsa stained blood smears. It causes chronic long-term infections.

## 2.2 Technical Session 2: Sharing of country data and experiences on epidemiology, diagnosis, treatment, prevention and control of *P. knowlesi* in the framework of current national malaria control and elimination strategic plans

### 2.2.1 Knowlesi malaria in humans peninsular Malaysia - *Christina Rundi*

Between 2007 and 2010, a total of 201 cases of *P. knowlesi* were reported in Malaysia. There have been three PCR confirmed deaths due to *P. knowlesi* out of a total of 18 malaria deaths since 2007.

Cases have been predominantly adult males (81% male, 19% female) living or working in forested areas. There have been some cases among urban residents (85% rural, 15% urban) including army personnel but in each case there has been a link to forested areas.

The magnitude of human cases of *P. knowlesi* in peninsular Malaysia is not fully understood.

### 2.2.2 Clinical features and epidemiology of *P. knowlesi* in malaria in humans in Sabah, Malaysia - *Timothy William*

A retrospective study carried out at the Queen Elizabeth Hospital (QEH) in Kota Kinabalu, Sabah, Malaysia showed that *P. knowlesi* is a major cause of severe malaria and mortality at QEH and that *P. knowlesi* accounted for about 24% (78/324) of all cases of malaria at QEH

The study looked at 74 cases diagnosed microscopically as *P. malariae* together with four diagnosed as *P. falciparum/P. vivax*, 56 were confirmed as *P. knowlesi* by PCR, 22 were classified as severe, and 34 as uncomplicated according to WHO criteria. The study looked at symptoms, treatments and outcomes. There was an 11% overall fatality rate but in severe cases the fatality rate was 28%. Of all those who died 64% had acute respiratory distress syndrome, 83% had renal failure, and 67% had shock. Thrombocytopenia was seen in all cases.

Results of the review of treatment outcomes showed that artemisinin combination therapy is effective treatment for uncomplicated knowlesi malaria and artesunate is effective treatment for severe knowlesi malaria. Both are associated with rapid parasite clearance times. Queen Elizabeth Hospital policy is to use ACT and artesunate for treatment of *P. knowlesi*.



The study showed that in Sabah all severe cases microscopically diagnosed as *P. malariae* should be suspected to be *P. knowlesi* and therefore treated the same as *P. falciparum*. Similarly, uncomplicated cases diagnosed treated as *P. malariae* often rapidly become severe and if not treated in time can lead to death. As a result, all physicians in Sabah should be aware of this, and treat and monitor cases accordingly.

#### 2.2.3 Entomological studies of *P. knowlesi* transmission in Sarawak, Malaysia - *Asmat Matusop*

Entomology studies carried out in 2005 and 2006 in Kapit district, Sarawak, Malaysia using human bare leg catches and monkey-baited traps showed that *An. latens* was the vector of *P. knowlesi*. It exhibited exophilic and acrodendrophilic behavior. The man-to monkey biting ratio was 1.8:1. The vector was abundant during two periods: April-June and October-December. Peak biting times in the forest was between 7-8 pm, in farms between 1-2 am outside longhouses between 11 pm-2 am and inside between midnight and 2 am.

#### 2.2.4 Knowlesi malaria in humans in Brunei Darussalam - *Kamaluddin Yassin*

Between 1999 and 2010, 50 cases of *P. knowlesi* were reported. Of these, 46 were Bruneian and four were foreigners. There was one confirmed death in 2002. All cases were adult males and most were exposed in the deep jungle areas of Temburong, Tutong and Belait districts that border Sarawak, Malaysia. The one patient who died was a border patrol officer who worked in those jungle areas. The death was attributed to the fact that he was late in seeking treatment and had other underlying medical conditions. Currently, the Brunei Health Department recommends that anyone entering the jungle areas including soldiers, officers from government agencies such as the Survey Department, Land Department, and Police Rangers strictly adhere to chemoprophylaxis and that on returning they be screened for malaria parasites.

#### 2.2.5 Knowlesi malaria in humans in Singapore - *Ooi Peng Lim*

Singapore noted that with the availability of *P. knowlesi*-specific primers for the detection of simian malaria, increasing numbers of this rare form of malaria were reported in countries in the Region. In April 2007, a cluster of three cases of simian malaria were confirmed among national servicemen training in a forested area at Lim Chu Kang where long-tail macaques have been sighted. Since then, another four locally acquired cases involving military personnel (including one case in 2006 reclassified from *P. malariae*) and ten imported cases have been reported.

In a study of the ecological reservoirs in Singapore, *P. knowlesi* has been found in long-tailed macaques located in one forested training area. Long-tailed macaques are frequently seen in the fringes of the nature reserves and residential areas of Singapore but *P. knowlesi* has not been found in those peri-domestic populations.

It is probable that the national servicemen acquired local *P. knowlesi* infection in their restricted training area whilst the imported cases were exposed during camping and/or trekking trips deep into endemic forested areas around the region. The current assessment is that the risk to public health remains low and there is no need for media statements which might cause undue alarm and discourage tourism. The situation continues to be monitored closely.

#### 2.2.6 Knowlesi malaria in humans in Indonesia - *Elvieda Sariwati*

In 2010 *P. knowlesi* was reported in an Australian who returned from Kalimantan where he worked in a forest area. *P. knowlesi* was also found in four out of 22 slides of suspected simian malaria taken in Kalimantan. Preliminary results from a study done in 2010 showed that out of 251 blood slides positive by microscopy (130 Pf, 110 Pv, 2 Pm and 9 Pf/Pv) six from south and

central Kalimantan originally diagnosed as *P. falciparum* were confirmed by PCR to be *P. knowlesi*. One was a single-species infection of *P. knowlesi* and five were mixed *P. knowlesi* and *P. falciparum*. All cases were successfully treated with artesunate/amodiaquine plus primaquine. Further research is needed to understand the factors affecting the transmission of *P. knowlesi*. There are plans to train health personnel to be aware of *P. knowlesi* and to improve diagnosis including use of PCR. Further research is needed to understand the factors affecting the transmission of *P. knowlesi* including the burden of the disease. There are plans to transfer the knowledge of *P. knowlesi* in training for health personnel and to improve diagnosis including the use of PCR.

#### 2.2.7 Knowlesi malaria in humans in the Philippines - *Jennifer Luchavez*

In 2008, five cases of *P. knowlesi* in humans were reported from Palawan, Philippines [30]. Another four cases were detected since this report, making a total of nine cases of *P. knowlesi* including one mixed (Pf/Pm/Pk). All cases were confirmed by PCR, out of 15 originally diagnosed by microscopy as either *P. falciparum* (2), *P. malariae* (8) or mixed of the two species (5).

All were from forested areas of Palawan where the long-tailed macaque (*Macaca fascicularis philippinensis*) is found and has been reported in the past to be infected with *P. cynomolgi*, *P. inui* and *P. knowlesi*. The results of limited entomology studies so far indicate that only *An. flavirostris* has the possibility of transmitting malaria parasite from monkeys to humans in the areas where the Pk were reported. There are ongoing vector studies to confirm the presence of *P. knowlesi* sporozoites in mosquitos that will definitively identify the vector.

Work is underway to develop simple serological and molecular assays to identify current and previous infections of *P. knowlesi*. This includes: 1) serology (ELISA) to estimate transmission intensity of the various simian species and define potential antigenic targets to discriminate exposure of *P. knowlesi* from other malarias; 2) defining age-specific exposure and infection rates; 3) developing molecular assays (PCR) to identify different species and compare prevalence generated through routine microscopy (or RDTs); and 4) integration of serological and molecular data into GIS maps to identify potential foci of infections, and assess the contribution of the different species, including *P. knowlesi* to the total malaria burden in Palawan.

#### 2.2.8 Knowlesi malaria in humans in Cambodia - *Siv Sovannaroth*

Two cases of *P. knowlesi* were reported in 2010 in Pailin province along the Thailand-Cambodia border. Both cases were males aged 40 and 41 who had spent time in forest areas. One was asymptomatic originally diagnosed by microscopy as *P. falciparum* but the other was symptomatic (fever of 38.5o C) originally negative; both were confirmed as *P. knowlesi* by PCR/sequencing.

Large-scale studies are proposed to describe the epidemiology of *P. knowlesi* in Cambodia focusing on Pailin and Pursat provinces but eventually expanding to other parts of the country where there are still large areas of forest. Studies should include identification of monkey reservoirs, and identification of vectors. Microscopists will be retrained to better recognize and report *P. malariae* as possible Pm/Pk.

#### 2.2.9 Knowlesi malaria in humans in Thailand - *Thammapalo Suwich*

The first human case of *P. knowlesi* was reported in August 2000. A 38-year-old Thai male presented with daily fever, headache intermittent chills, sweating and malaise that lasted for four days. He had worked for a week in a forest near the Thailand-Myanmar border. The Giemsa-stained thin blood film showed 10% young trophozoites, 45% growing trophozoites, 40%

schizonts, and 5% gametocytes that was compatible with a diagnosis of *P. malariae*. PCR showed *P. knowlesi* that was closely related to the W1 and Nuri strains. Subsequently, in 2009 additional cases of *P. knowlesi* were confirmed by PCR: one imported from Gua Musang in Malaysia; two from Yala Province near the Malaysian border and one from Chantaburi near the Thailand-Cambodia border. All were associated with forested areas.

A survey in monkeys done in Narathiwat in southern Thailand found *P. knowlesi* in four different species: *Macaca nemestrina* (pig-tailed macaque); *Macaca fascicularis* (long-tailed macaque); *Macaca arctoides* (stump-tailed macaque); and *Sannopitecus obscurus* (dust-lead monkey). Possible vectors, member of the *Leucosphyrus* group, are abundant in Thailand so the potential for *P. knowlesi* transmission is present. It is proposed that physicians, especially in areas with populations of forest workers, be alerted to the possibility of *P. knowlesi* infections and that health education be given to the workers about the importance of personal protection. A systematic study needs to be done to further define the areas with high risk for human infections.

#### 2.2.10 Knowlesi malaria in humans in Viet Nam - *Ta Thi Tinh*

The first three cases of *P. knowlesi* were detected by a survey done in Ninh Thuan province during 2004 [31-33]. Originally diagnosed by microscopy as Pf/Pv, Pv and negative but later confirmed by PCR as Pf/Pv/Pm/Pk, Pv/Pm/Pk and Pm/Po/Pk, respectively. An additional three cases were confirmed by PCR in 2010. All were members of the Raglai ethnic group that lives and work in the forest areas of Ninh Thuan province. Four of the six cases were children and four of the six cases had more than one species of parasite. Parasite densities were low and the children were either asymptomatic or had mild symptoms. This is a pattern not seen elsewhere and may indicate that among the Raglai there is a high frequency of human exposure to *P. knowlesi* probably in part due to the fact that monkeys are often kept as pets.

The findings suggest that screening for *P. knowlesi* be done for all malaria cases diagnosed microscopically as *P. vivax*, *P. ovale*, or *P. malariae* in Ninh Thuan and other adjacent provinces. Microscopists should be retrained to suspect *P. knowlesi* but a rapid diagnostic test should also be developed. In addition, groups that stay overnight in forested areas should be informed about the need to use personal protection including insecticide-treated hammock nets.

### 3. CONCLUSIONS AND RECOMMENDATIONS

#### 3.1 Conclusions

*P. knowlesi* is a monkey malaria that infects humans. People at risk are those living or working in forested areas where there are populations of macaque monkeys and vector mosquitoes that bite both the monkeys and humans that live in forested areas throughout the South East Asia region.

The first recognized case of *P. knowlesi* in humans was reported in 1965 in Malaysia. After that there was another human case in 1971 also in Malaysia. Subsequently in 2004 there was a report of a large number of human cases in Sarawak, Malaysian Borneo based on newly developed PCR diagnosis. Since then *P. knowlesi* has been confirmed by PCR by several countries in this region.

Although it is a monkey malaria parasite, there have been confirmed human deaths in Malaysia and Brunei and with an increase level of awareness more and more cases are being

reported. It therefore has to be defined as a public health problem among population groups in forested areas.

In Sarawak, Malaysian Borneo, where extensive scientifically validated studies over the past ten years have shown that human cases of *P. knowlesi* are widespread, and retrospective and prospective studies using PCR show that virtually all cases previously diagnosed microscopically as *P. malariae* in Sarawak were actually *P. knowlesi* [3,4].

In a recent prospective study of 960 blood samples from 12 hospitals in Sarawak between 2001 and 2006, 266 samples were identified as *P. knowlesi* by PCR. Only four samples were confirmed as *P. malariae*. Single *P. vivax* and *P. falciparum* infections accounted for 440 and 219 cases, respectively.

*P. knowlesi* has been detected in Sabah, Malaysian Borneo, and there were confirmed deaths attributed to *P. knowlesi*, as previously reported for Sarawak.

There is so far no evidence of human-to-human transmission of *P. knowlesi*.

The major problem with human *P. knowlesi* is that cases are routinely diagnosed microscopically as either *P. malariae* or *P. falciparum* because the morphological characteristics are so similar to *P. knowlesi*. Proper diagnosis and treatment requires that both microscopists and clinicians are aware that what looks microscopically and clinically like *P. malariae* or *P. falciparum* should be reported as Pk/Pm and treated like *P. falciparum*. This is because *P. knowlesi* if not properly treated can quickly become severe and may result in death.

Existing RDTs that have been evaluated cannot reliably diagnose *P. knowlesi* so new RDTs are needed to confirm *P. knowlesi* and inform treatment down to the level of health services in areas of highest risk.

Currently PCR is the only definitive way to distinguish *P. knowlesi* from *P. malariae* or from the young ring forms of *P. falciparum*, but there is no standardization of the PCR primers and no standard operating procedures for *P. knowlesi* PCR. It is limited to research laboratories or a few national level reference laboratories but more countries should establish reference laboratories so that the confirmation of *P. knowlesi* is readily accessible.

More information is needed about the pathophysiology and management of human *P. knowlesi*. The only hospital-based studies have been in the Kapit Division of Sarawak and QEH Sabah, Malaysian Borneo and are still limited. There is very little information on what is happening elsewhere.

The Sarawak, Malaysian Borneo studies have shown that treatment with a standard three-day regimen of chloroquine given in a timely manner leads to the rapid clearance of parasites becoming microscopically negative within 24 hours and negative by PCR within three days.

There is no evidence of chloroquine resistance but some countries have already started to treat suspected cases of *P. knowlesi* with artemisinin-based combination therapy. More evidence is needed about the effectiveness of ACTs on *P. knowlesi*.

There is no evidence that primaquine has any specific effect on *P. knowlesi* and probably shouldn't be included in any treatment guidelines.

The currently available information on incidence and geographic distribution of human cases of *P. knowlesi* is limited. Additional longitudinal studies in humans, vectors and monkeys are needed to get a clearer picture of disease burden and risk factors.

Monkey studies are difficult to carry because of the problems associated with catching monkeys in the forest and drawing blood.

A stool test as described in the literature for primates would be a good solution but there is no experience in using this method for macaques.

Vector studies have so far indicated that only members of the *Anopheles leucosphyrus* group are vectors associated with human cases of *P. knowlesi* but knowledge is limited by limitations of sampling and the sampling methods used. Sampling mosquitoes in the forest is difficult especially when using monkey-baited traps. Human bait catches are also difficult to do because of ethical questions of their safety, but these are essential studies.

There are other monkey malaria parasites circulating in the forest in this region especially *P. inui* and *P. cynomolgi* but so far no naturally occurring infections of humans have been reported. This raises many questions about the transmission of those parasites that under laboratory conditions can infect humans. What is different about *P. knowlesi*?

There are no *P. knowlesi* specific control measures other than the rigorous application of standard malaria control strategies. Some countries have introduced prevention methods for high risk groups, such as soldiers training in jungle areas. This includes uses of chemoprophylaxis, insecticide-treated clothing, mosquito nets, fogging, repellents and health promotion materials. Other countries need to consider similar strategies for people that enter forested areas for recreational purposes, including international tourists.

One problem that should be addressed is the fact that the vectors so far described bite outside of houses early in the evening therefore providing a challenge to existing prevention strategies.

Basic research on human *P. knowlesi* has been limited to few groups in the region including the Malaria Research Centre (MRC) at UNIMAS. Wellcome Trust and the MRC are supporting some significant research on *P. knowlesi* but others partners need to be stimulated to collaborate and expand the research base. More funding opportunities need to be explored.

There has been some concern that the presence of human cases of *P. knowlesi* will have a negative impact on both countries that have already been declared malaria-free and for those countries moving towards elimination. *P. knowlesi* is a zoonosis so it is not included in the current WHO definition or guidelines on elimination. If in the future human-to-human transmission is demonstrated in nature this may change. Regardless, *P. knowlesi* will continue to be a public health problem among clearly defined population groups so countries need to develop strategies to deal with it.

The Informal Consultation on the Public Health Importance of *Plasmodium knowlesi* identified five areas for proposed research on *P. knowlesi*.

1. Estimating burden and severity of *P. knowlesi*, and other monkey malaria parasites, in humans.

Start by using Health Information System (HIS)/surveillance data to map areas with *P. knowlesi* transmission, identify hospital/health facilities as 'catchment clinics' and look for *P. knowlesi* admission. Track those back to their villages and conduct cross section surveys including blood surveys, entomology collections and information on the presence of macaques. All this information can then be used to plot areas of human transmission.

Carry out longitudinal community-based sampling in a high risk human cohort over a period of two years to determine incidence rates, frequency of asymptomatic infections, and re-infections.

Surveys of local macaque population can be carried out along forest transects. Samples collected by tail or ear pricks and examined microscopically looking for *P. knowlesi* and other primate malarias include *P. inui* and *P. cynomolgi*.

There are difficulties collecting and sampling monkeys so a feasibility study to use fecal samples to detect primate malaria DNA needs to be done. Validation of the method includes the use of paired blood and fecal samples to assess sensitivity.

Vector surveys have so far been a bottleneck to understanding the transmission of *P. knowlesi* and the other primate malarias because of the lack of expertise and problems associated with sampling across forest zones. Human landing catches are difficult to do and involve ethical issues and permission but are the best method to capture and incriminate vectors as well as to obtain information on biting preferences.

PCR is the best method for identifying *P. knowlesi* parasites in mosquitoes.

## 2. Diagnosis

It is not possible to distinguish *P. malariae* and *P. knowlesi* by microscopy so PCR is the only reliable method but there are a number of PCR being used. Some PCR methods have reported cross-reactivity and non-specific amplification and require genus specific primers followed by species specific primers. The current target is the 18srRNA gene but new targets need validation.

As a first step, it is suggested that a detailed SOP to be produced and disseminated using latest primer sets from Professor Singh's group [6], including DNA extraction. The next step suggested is the establishment of reference laboratory capacity at the national level, with establishment of external quality assurance (EQA) system (panel testing).

An RDT that is sensitive and specific for *P. knowlesi* is needed. Expanded testing of existing pre-qualified dual pLDH RDTs may identify one that works but future development of diagnostic platforms must include the ability to detect *P. knowlesi*.

## 3. Severity of *P. knowlesi* disease

The Kapit prospective study provided the first detailed information about the pathophysiology of *P. knowlesi* but that information was limited. More similar studies need to be done in other countries using sentinel hospitals (tertiary/referral).

## 4. Treatment

Chloroquine works for *P. knowlesi* but there is no evidence regarding the use of ACT for treating uncomplicated case. A prospective non-inferiority random control trial comparing ACT versus chloroquine for treatment of uncomplicated *P. knowlesi* is needed. A similar trial comparing ACT with quinine for severe cases is needed but there are insufficient cases for a clinical trial.

There is no evidence to support the use of primaquine for *P. knowlesi*. As with any use of primaquine, there is a risk related to G6PD deficiency but with *P. knowlesi* there is no demonstrable benefit.

## 5. Chemoprophylaxis

Brunei is using chloroquine for military personnel and other groups that are at risk but there is no evidence on effectiveness of artemisinin formulations as chemoprophylaxis.

### 3.2 Recommendations

Recognizing that *P. knowlesi* is a zoonosis infecting humans in many countries in South-East Asia and that it is a significant public health problem among specific population groups tied to forest activities, the following should be undertaken:

- (1) Estimate the burden of *P. knowlesi* in humans. The incidence of *P. knowlesi* should be estimated through well-designed studies in areas where macaques and vectors have been shown to exist and where transmission of *P. knowlesi* to humans has been demonstrated.

PCR is the only validated diagnostic method available to confirm *P. knowlesi*. Diagnosis of *P. knowlesi* based only on microscopy is not definitely possible. Therefore, the following recommendations are made in areas with known human cases of *P. knowlesi*:

- (2) In designated areas with known human cases of *P. knowlesi*, microscopy results recorded as *P. malariae* should be reported as *P. malariae/P. knowlesi* to guide case management.
- (3) Standard operating procedures (SOPs) for PCR for *P. knowlesi* and all other human malaria parasite species should be worked out together with quality assurance systems and should be adhered to. The procedures should be coordinated by WHO.
- (4) National reference laboratories for *P. knowlesi* should be established as part of a regional network that includes a regional reference laboratory.
- (5) *P. knowlesi* should be included in the development of new diagnostic methodologies, especially easy-to-use and rapid ones. In the meantime, existing dual pLDH malaria rapid diagnostic tests that have been pre-qualified by WHO-FIND should be evaluated for their ability to detect *P. knowlesi*.

The key to effective case management of *P. knowlesi* and to prevent deaths is rapid diagnosis and appropriate treatment. Chloroquine has been fully efficacious for treatment of *P. knowlesi*. In countries where human cases of *P. knowlesi* are known to exist, it is important that:

- (6) Clinicians and laboratory staff need to be aware of the existence of *P. knowlesi* in the area and of the possibility of atypical and severe clinical presentations to ensure rapid and effective *P. knowlesi* diagnosis and case management and to prevent deaths.
- (7) *P. knowlesi* symptoms, clinical course and management should be included in national malaria treatment guidelines, on reporting forms and in health staff training in all countries.
- (8) In countries where *P. falciparum* is prevalent, all suspected or doubtful *P. malariae/P. knowlesi* cases should be treated as *P. falciparum* according to national guidelines. Uncomplicated *P. knowlesi* cases should be monitored closely because they rapidly can lead to severe disease with a high case fatality rate.

- (9) Severe *P. knowlesi* cases should be treated like severe falciparum malaria according to national guidelines.
- (10) All severe malaria cases and deaths in hospitals routinely should be reviewed and documented according to a standard format, including confirmation of malaria by PCR that includes all species, including *P. knowlesi*.

In areas where macaques and vectors have been shown to exist and where transmission of *P. knowlesi* to humans has been demonstrated, the following prevention measures are recommended:

- (11) Personal protection measures (such as insecticide-treated mosquito nets, hammock nets, protective clothing and repellents) and/or chemoprophylaxis together with health promotion should be implemented for populations at-risk, such as those living, working and visiting forested areas.

Countries with known human *P. knowlesi* cases should formulate a comprehensive strategy to control *P. knowlesi*:

- (12) Countries should establish a comprehensive strategy to control *P. knowlesi* consisting of rapid diagnosis, appropriate timely treatment, personal protection, surveillance and health information targeting populations at-risk and health staff as well as operational research.
- (13) Control measures for *P. knowlesi* should be considered as an opportunity to strengthen comprehensively prevention and control of all malarias in vulnerable groups as well as the health system.

Transmission of *P. knowlesi* should not have a negative impact on progress towards malaria elimination or affect the status of countries already declared malaria-free because zoonotic malaria parasites are not included in the current WHO definition and guidelines for elimination. However:

- (14) Countries that have achieved malaria elimination or are close to elimination should be vigilant with regard to *P. knowlesi* and formulate a strategy to deal with it.
- (15) Surveillance should be continued to detect human-to-human transmission of *P. knowlesi*. If it is confirmed and *P. knowlesi* becomes the fifth human malaria parasite, it then would be inconsistent with malaria elimination.

Significant knowledge gaps currently exist regarding epidemiology, diagnosis and treatment, prevention and control of *P. knowlesi*.

- (16) Funds should be mobilized and key research be carried out in areas where macaques and vectors exist and human cases of *P. knowlesi* have been demonstrated or where human and vector contact is possible, including:
  - a) hospital-based studies on severity of *P. knowlesi*;
  - b) cross-sectional surveys on selected populations to collect epidemiological and ecological data;
  - c) longitudinal studies on the incidence of *P. knowlesi* (and other simian malaria parasites potentially affecting humans), geographic distribution, asymptomatic



infections, reinfections and immunity, changes over time in nonhuman primates and vectors (including SOPs to conduct vector surveys);

- d) studies to determine whether human-to-human transmission is occurring under natural conditions;
- e) a feasibility study to detect *P. knowlesi* DNA in macaque fecal samples;
- f) assessment of the therapeutic efficacy of artemisinin-based combination therapy (ACT) for *P. knowlesi*;
- g) studies on novel personal protection measures to prevent forest malaria; and
- h) development of novel easy-to-use diagnostic tools specific for *P. knowlesi*.

This Informal Consultation on the Public Health Importance of *P. knowlesi* has been the first WHO consultation on *P. knowlesi*. Given that a large number of countries in South-East Asia have documented human *P. knowlesi* cases which require a comprehensive public health response and further operational research, it is recommended that:

- (17) A further consultation on *P. knowlesi* should be organized in two years to review new scientific evidence and follow up on progress made in the implementation of the above recommendations.

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HEALTH IMPLICATIONS OF MALARIA IN  
HUMANS DUE TO PLASMODIUM KNOWLESI

WPR/DCC/MVP(2)/2011.1  
22 February 2011

Kuching, Sarawak, Malaysia  
22-24 February 2011

ENGLISH ONLY

## AGENDA

1. **Registration**
2. **Opening Ceremony**
3. **Technical session 1:** Review of available knowledge of *P. knowlesi* infections in humans
4. **Technical session 2:** Sharing of country data and experiences on epidemiology, diagnosis, treatment, prevention and control of *P. knowlesi* in the framework of current national malaria control and elimination strategic plans
5. **Group work**
  - Public health implications of *P. knowlesi* in SE Asia: appropriate diagnostic, case management, prevention and control strategies for *P. knowlesi* infection in humans, and possible implications for malaria control and elimination
  - Defining and prioritising a research agenda for diagnosis, case management, prevention and control of *P. knowlesi* infection in humans
7. **Plenary session:**
  - Presentation of group work; discussion.
8. **Wrap up of the workshop findings and way forward**
9. **Closing**

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