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Knowlesi malaria: newly emergent and of public health importance?

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Abstract

Several questions on public health impact have arisen from the discovery of a large focus of the simian malaria parasite, *Plasmodium knowlesi*, in the human population. *P. knowlesi* malaria is not newly emergent and was overlooked until molecular tools to distinguish between *P. knowlesi* and the morphologically similar *Plasmodium malariae* became available. Knowlesi malaria is a zoonosis that is widely distributed in Southeast Asia and can be fatal. Information on knowlesi malaria should be included in medical and public health guidelines to encourage the accurate diagnosis and treatment of patients, and monitor the incidence and distribution of cases. A complete emergence of *P. knowlesi* into the human population could be overwhelming and, although challenging, the prevention of this situation deserves serious consideration.

Large focus of human infections with *Plasmodium knowlesi*

Human beings are the natural hosts for four species of *Plasmodium* (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*), which is the causative agent of malaria. In addition to these species, there are >100 *Plasmodium* species that infect a variety of hosts, including reptiles, birds, rodents, primates and other mammals [1]. The concept of *Plasmodium* host specificity is widely accepted, and the consensus is that only four species of *Plasmodium* cause malaria in humans. This scenario was challenged recently by the discovery of a large focus of the simian malaria parasite *Plasmodium knowlesi* in the human population of the Kapit Division of Sarawak, Malaysian Borneo [2]. It was confirmed that four malaria cases that had been diagnosed as *P. malariae* at Kapit Hospital had *Plasmodium* DNA by using a nested PCR assay. However, the infecting species could not be identified using species-specific PCR primers [3], including those for *P. malariae*. DNA sequencing and analyses of the small subunit ribosomal RNA (SSU rRNA) and circum-sporozoite (*csp*) genes of eight such '*P. malariae*' isolates indicated that these were phylogenetically indistinguishable from *P. knowlesi* and were not *P. malariae*. Knowlesi-specific PCR primers were developed and included in the nested-PCR examination of 208 malaria patients that were admitted to Kapit Hospital in 2000–2002; 120 of these (58%) were either single *P. knowlesi* infections or *P. knowlesi* mixed with other non-*P. malariae* species. This focus of knowlesi infections in humans would have been missed if molecular detection methods had not been used because the early blood stages of *P. knowlesi* are morphologically identical to those of *P. falciparum* and the other stages are similar to those of *P. malariae* (Figure 1). Here, we provide a brief background of *P. knowlesi* and address several questions including: is *P. knowlesi* newly emergent in humans? How serious is knowlesi malaria for the human population? Is the current situation likely to get worse? What can or should be done about this parasite in humans?

Isolation and historical background of *P. knowlesi*

P. knowlesi was first isolated in 1931, from a long-tailed macaque (*Macaca fascicularis*) that was imported from Singapore [4]. Experimental infections resulted in low parasitaemias in the natural long-tailed macaque hosts but fulminating infections in rhesus macaques (*Macaca mulatta*). Furthermore, *P. knowlesi* had a quotidian (24-h) asexual blood cycle, the shortest among primate malarias, and produced daily fever peaks in its hosts [5,6]. Human beings could be infected with *P. knowlesi* by blood passage, and morphological similarities between *P. knowlesi* and *P. malariae* were observed. Subsequently, *P. knowlesi* was used as a pyretic agent for the treatment of patients with neurosyphilis [7-9].

The first naturally acquired case of knowlesi malaria in a human was described in 1965 [10]. An American army surveyor working in the jungle of Pahang, Peninsular Malaysia, returned to America unwell. He was diagnosed initially with *P. falciparum* malaria and later with *P. malariae* by microscopy. After blood passage into humans that were recruited for studies on *P. malariae*, the volunteers developed daily fever spikes rather than the 72-hourly spikes expected for *P. malariae*. Blood was inoculated into rhesus macaques, which subsequently died, confirming that the surveyor was infected with *P. knowlesi* [10-12]. This case showed that humans could be naturally infected with *P. knowlesi* and confirmed earlier observations that it was not possible to differentiate *P. knowlesi* from *P. malariae* by microscopy. A presumptive human case of *P. knowlesi* was reported, again in Peninsular Malaysia, in 1971 [13].

The accidental mosquito transmission of *Plasmodium cynomolgi* from long-tailed macaques, imported from Peninsular Malaysia, to malaria researchers in the USA opened a debate on the threat of zoonotic malaria to the success of the Malaria Eradication Programme of the WHO, which was initiated in 1955 [12,14]. Studies to determine whether zoonotic malaria transmission occurred in nature, which had been initiated in Peninsular Malaysia [15], were intensified after the naturally acquired human case of *P. knowlesi*. Blood samples from 1117 people were pooled and injected into rhesus macaques [16]. None became infected with either *P. cynomolgi* or *P. knowlesi*. During the same period, *Anopheles hackeri* was identified as the vector for *P. knowlesi* in Peninsular Malaysia [17]. *Anopheles hackeri* is predominantly zoophagic and feeds mainly at the canopy level [12]. These observations indicated that *P. knowlesi* did not pose a significant threat to the WHO's Malaria Eradication Programme and consolidated the widely held view that there were only four species of *Plasmodium* causing malaria in humans. Zoonotic malaria was not included in medical textbooks and was largely forgotten.

Is *P. knowlesi* a recently emergent infection?

Malaria transmission relies on a dynamic interaction between the *Plasmodium* species, the vector and the vertebrate host. At the present time, the natural hosts of *P. knowlesi* have been identified as long-tailed and pigtailed macaques, and banded leaf monkeys [18,19]. Under experimental conditions, *P. knowlesi* also readily infects a wide range of other primates, including New World marmosets (*Callithrix jacchus*), African olive baboons (*Papio anubis*), a range of Old World macaques, and humans by blood passage and, in some cases, through infected mosquito bites [11,12,20,21]. These very different primate groups diverged several million years ago and the ability of *P. knowlesi* to infect such a variety of vertebrate hosts is unlikely to be a new adaptation [22,23]. It is, therefore, improbable that human susceptibility to *P. knowlesi* is new or that the American surveyor was the first human to be naturally infected with *P. knowlesi*. Available evidence indicates that *P. knowlesi* parasites would have had the ability to infect humans since modern *Homo sapiens* arrived in Southeast Asia ~70 000 years ago [24]. The more pertinent question is: what

prevented a full-blown emergence of human-to-human transmission giving rise to five extant malaria parasites of humans?

At present, *P. knowlesi* transmission is restricted to the *Anopheles leucosphyrus* group of mosquitoes, which currently comprises 20 species [25]. The leucosphyrus group are vectors for several simian malaria parasites and are 'jungle breeders' [12]. Two members of this group (*An. hackeri*, a zoophagic canopy feeder in Peninsular Malaysia, and *Anopheles latens*, which is found in jungle habitat in Sarawak) have been implicated in natural transmission of *P. knowlesi* [17,26]. The range of the *An. leucosphyrus* group overlaps with the long-tailed and pig-tailed macaques, and naturally acquired human *P. knowlesi* infections have been acquired within this range [27,28] (Figure 2). The most conservative explanation for *knowlesi* not crossing into the human population is vector restriction to the forest-dwelling leucosphyrus group of mosquitoes coupled with a lack of opportunity. The early radiation of humans into Southeast Asia probably took a coastal route rather than one through dense jungle [24]. Forests were inhospitable, and human populations in the forests would have been sparse. During this time, when the natural host macaques of *P. knowlesi* were abundant, there would have been little pressure for *P. knowlesi* to switch host, particularly to humans when restricted by the leucosphyrus group of vectors. Furthermore, humans in Southeast Asia had their own *Plasmodium* species: *P. falciparum*, *P. malariae* and *P. vivax* (purportedly the result of an earlier host switch from Asian macaques or their ancestors [29-31]). Because the human host niche was already occupied, perhaps not only the lack of opportunity but also competition or cross-species protection from these well-established malaria parasites prevented the full-blown entry of *P. knowlesi* into the human population [32,33].

Malaria-control programmes have succeeded in reducing the number of cases reported annually in Malaysia from 243 870 in 1961 to only 5294 in 2006 [34,35]. Furthermore, human population growth has tripled in the tropical world during the past 100 years [36]. Humans now encroach on and disturb large tracts of the natural transmission sites of *P. knowlesi*, causing habitat destruction to an extent formerly unknown, probably since the radiation of people into Southeast Asia [24]. In the absence of other species of *Plasmodium*, and with human encroachment into the transmission habitat of *P. knowlesi*, it is possible that we are setting the stage for a switch of host for *P. knowlesi*, similar to the one postulated for *P. vivax*.

How serious is *knowlesi* malaria for the human population?

Recent studies have shown that *knowlesi* malaria is widely distributed across Sarawak and Sabah in Malaysian Borneo and extends to the state of Pahang in Peninsular Malaysia [37]. Furthermore, single *knowlesi* malaria cases have been acquired in Thailand, Myanmar and Singapore and five cases have been seen in Palawan Island, the Philippines [38-41] (Table 1). The true extent of the distribution and incidence of *knowlesi* malaria will be known only when extensive studies, using PCR-based assays, are undertaken in these and other Southeast Asian countries. Clearly, *knowlesi* malaria in the human host is not rare and is widely distributed, particularly in areas inhabited by the natural macaque hosts and the vectors (Figure 2).

The public health importance of *knowlesi* malaria is underscored not only by its widespread distribution in Southeast Asia but also by the observation that *knowlesi* malaria accounts for the majority of admissions for malaria in three hospitals in Sarawak. Furthermore, *knowlesi* infections can cause fatal disease and should be considered to be a serious threat to human health [37]. The presenting signs and symptoms of severe *knowlesi* malaria have been confused with renal failure, liver failure and other non-malaria related diagnoses, and it is

not possible to estimate how many fatalities occur. However, four fatal cases were detected in Sarawak during a one-year period [37], and there have been 230–377 reported cases of microscopy-confirmed '*P. malariae*' malaria in Sarawak annually since 2000 (B.S., unpublished). Therefore, although the incidence of knowlesi malaria is low compared with falciparum malaria, the proportion of severe and fatal cases observed in knowlesi infections might rival the proportion of severe cases observed in *P. falciparum* malaria [42].

Is the current knowlesi malaria situation likely to get worse?

The evidence from Sarawak, where the largest number of human knowlesi infections has been studied, indicates that *P. knowlesi* is primarily zoonotic and people become infected when they work at the forest fringe or enter the forest. This conclusion is based on our observations that there is a lack of clustering of cases in human settlements [2], that the majority of cases are adults with a recent history of being involved in activities associated with spending time in the forest or forest-fringe area (Kim S. Lee, PhD thesis, University Malaysia Sarawak, 2006), that *An. latens* (a forest feeder that is attracted to both humans and macaques) is a vector for knowlesi [26] and that captive macaques kept at human settlements, in contrast to their wild counterparts, are rarely infected with knowlesi parasites (Paul C.S. Divis, MSc thesis, University Malaysia Sarawak, 2007). While this situation remains stable, a complete host switch and human-to-human transmission might not occur easily. However, there is a possibility that deforestation and increased human and macaque populations, coupled with changes in mosquito-vector abundance and feeding behaviour, might alter the knowlesi transmission dynamic. Furthermore, strategies that aim to eradicate malaria from humans in Southeast Asia might facilitate the establishment of *P. knowlesi* in the human population by removing species of human malaria parasites that are currently in that population. We might be witnessing either a situation in which *P. knowlesi* remains a largely unrecognized zoonosis that has been in existence for epochs or the beginning of a complete adaptive switch of this parasite into the human host.

Concluding remarks

Knowlesi malaria is zoonotic, potentially fatal, not newly emergent and widely distributed in Southeast Asia. Recognition that *P. knowlesi* is one of the five species of *Plasmodium* that causes human disease is important. Recently, descriptions of knowlesi malaria have been included in two text books [43,44], and these descriptions should be included in revised editions of other medical texts and in government and WHO guidelines on malaria diagnosis and treatment. In this way, physicians treating patients that have a *P. malariae* diagnosis by microscopy and a recent history of travel into the forests in Southeast Asia would be aware that their patients might be infected with a potentially more pathogenic species of *Plasmodium*. In addition, the provision of information about knowlesi malaria and preventive measures should be made a priority for healthcare providers and communities living in the forest fringe, for those working in forests and for anyone intending to visit the forests in Southeast Asia. Current control strategies for malaria, such as the residual spraying of human dwellings with insecticides and the provision of insecticide-treated bednets, will not prevent zoonotic-malaria transmission by vectors that feed predominantly in the forest, away from human dwellings. Theoretically, one of the most effective methods of controlling human *P. knowlesi* infections would be through the reduction of the populations of non-human primate hosts and vectors, but this would be extremely difficult to implement. Zoonotic knowlesi malaria will, therefore, continue to be a problem for malaria control, and it poses a threat to the renewed efforts directed at the eradication of malaria [14]. The situation is being monitored in Sarawak, and monitoring should be extended to other areas of Southeast Asia. The importance of early detection and containment of human-

to-human knowlesi transmission in the event of a complete emergence into the human population should not be underestimated.

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Figure 1.

Thin blood films that show morphological similarities between blood stages of *P. knowlesi*, *P. malariae* and *P. falciparum*. Giemsa-stained thin blood films show that the early trophozoites of both *P. falciparum* and *P. knowlesi* appear as discrete ring forms, at times with double chromatin dots, and individual erythrocytes can be infected by more than one parasite. The late trophozoites, schizonts and gametocytes of *P. knowlesi* and *P. malariae* are similar, including the appearance of some trophozoites as band forms. The figure is modified from Ref. [2], with permission from Elsevier.

Figure 2.

The 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. All of the human cases of *P. knowlesi* shown were confirmed by sequencing and/or PCR [2,37-41] with the exception of two single cases in Peninsular Malaysia [10,13]. The map and known distribution of the *An. leucosphyrus* group were modified from Ref. [25], and the approximate distribution of long-tailed (*M. fascicularis*) and pig-tailed (*M. nemestrina*) macaques were adapted from Refs [27,28].

Locations where infections were acquired, methods of identifying *P. knowlesi*, proportion of adults, travel history and occupations of reported naturally acquired human knowlesi malaria cases^a

Location infections were acquired	Year of report	Method of identifying <i>P. knowlesi</i>	No. of cases	% adults (>15 yrs)	Recent travel history in or near forest	Occupation	Refs
Pahang State, Peninsular Malaysia	1965	Blood passage into rhesus monkeys	1	100%	Yes	Surveyor	[10]
Johore State, Peninsular Malaysia	1971	Serology	1	100%	Yes	Field assistant	[13]
Kapit Division, Sarawak State, Malaysian Borneo	2004	Nested-PCR assay, sequencing of SSU rRNA and <i>csp</i> genes	120	92.5%	85 yes 35 NA	37 farmers, 28 logging camp workers, 5 housewives, 5 teachers, 18 miscellaneous ^b , 27 NA	[2], ^c
Prachuap Khiri Khan Province, Thailand	2004	Sequencing of SSU rRNA gene	1	100%	Yes	NA	[38]
Northern Myanmar	2006	Nested-PCR assay	1	100%	Yes	Logging camp worker	[41]
Sarawak State, Malaysian Borneo	2008	Nested-PCR assay	266	98.4% (<i>n</i> = 243)	NA	NA	[37]
Sabah State, Malaysian Borneo	2008	Nested-PCR assay	41	91.4% (<i>n</i> = 35)	NA	9 farmers, 3 logging camp workers, 2 housewives, 2 students, 25 NA	[37], ^c
Pahang State, Peninsular Malaysia	2008	Nested-PCR assay	5	80%	NA	NA	[37], ^c
Palawan Island, the Philippines	2008	Nested-PCR assay	5	60%	2 yes 3 NA	2 farmers 3 NA	[39]
Singapore	2008	Nested-PCR assay, sequencing of SSU rRNA and <i>csp</i> genes	1	100%	Yes	Soldier	[40]

^aNA: data not available.

^bMiscellaneous occupations include guard, carpenter, labourer, ranger, technician, mechanic, student and field assistant.

^cB. Singh *et al.*, unpublished.