Plasmodium knowlesi: an update

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There were only four species of *Plasmodium* that were thought to cause malaria in humans until a large number of human infections by *Plasmodium knowlesi*, a malaria parasite typically found in long-tailed and pig-tailed macaques, were reported in 2004 in Malaysian Borneo. Since then, cases of knowlesi malaria have been reported throughout South-east Asia and also in travellers returning from the region. This article describes the molecular, entomological and epidemiological data which indicate that *P. knowlesi* is an ancient parasite that is primarily zoonotic, and there are three highly divergent sub-populations. It also describes the detection methods for *P. knowlesi*, which is morphologicaly similar to *P. malariae*, and the clinical features and treatment of this malaria parasite that is potentially fatal.

Malaria parasites and discovery of large focus of human knowlesi malaria cases

Malaria is caused by parasites that belong to the genus *Plasmodium* and there are more than 150 species of *Plasmodium* that infect reptiles, birds and mammals[1]. These parasites, in general, tend to be host-specific. Long-tailed and pig-tailed macaques (*Macaca fascicularis* and *M. nemestrina* respectively) are hosts to five species (*P. knowlesi, P. inui, P. cynomolgi, P. fieldi* and *P. coatneyi*). Only four species of *Plasmodium*, namely *P. falciparum, P. vivax, P. malariae* and *P. ovale*, were thought to cause malaria in humans until a large number of human cases due to *P. knowlesi* were reported in Sarawak, Malaysian Borneo over 11 years ago[2]. The study in Kapit was prompted by observations that cases diagnosed by microscopy as *P. malariae* had high parasitaemias, required hospitalization and that 95% of patients were adults. This was in contrast to *P. malariae* infections which typically are asymptomatic with low parasitaemia and occur in all age groups. When blood samples from 208 malaria patients at Kapit Hospital were analysed by PCR assays, none were identified as *P. malariae*, although 141 had been diagnosed as *P. malariae* by microscopy. Fifty-eight percent (120) were either single *P. knowlesi* infections or mixed infections of *P. knowlesi* with *P. falciparum* and *P. vivax*. Misdiagnosis had occurred because the blood stages of *P. knowlesi* and *P. malariae* are morphologically indistinguishable[3].

Epidemiology and risk factors of acquiring knowlesi malaria

Human infections with *P. knowlesi* have been reported throughout Malaysia and in Thailand, Singapore, the Philippines, Vietnam, Cambodia, Indonesia, Brunei, Myanmar and in the Nicobar and Andaman Islands of India[4,5]. In Malaysia, *P. falciparum* and *P. vivax* cases have declined over the past five years and *P. knowlesi* has now become the most common cause of human malaria[6,7]. The true incidence of knowlesi malaria is not known in other parts of South-east Asia since not many large-scale studies have been undertaken with molecular detection assays.

The geographical distribution of human *P. knowlesi* infections is similar to that of the natural hosts of *P. knowlesi*, the long-tailed and pig-tailed macaques[8]. Reports from 1931 to 1970 identified macaques as hosts of *P. knowlesi* in Peninsular Malaysia, Singapore and the Philippines[9], and a banded leaf monkey (*Presbytis melalophos*) in Peninsular Malaysia[9]. Since 2007, *P. knowlesi* infections detected by molecular methods have been described in macaques in Peninsular Malaysia, Malaysian Borneo, Singapore and Thailand[4].

The transmission of *P. knowlesi* in nature has been shown to be restricted to mosquitoes belonging to the *Anopheles leucosphyrus* group[10]. The members of this forest-dwelling group of mosquitoes that have been identified as vectors include *An. latens* (in Sarawak, Malaysian Borneo)[11], *An. balabacensis balabacensis* (in Sabah, Malaysian Borneo), *An. dirus* (in Vietnam)[12] and *An. hackeri* and *An. cracens* (in Peninsular Malaysia)[1,13].

People that are at risk of acquiring knowlesi malaria are those that enter the habitat of the macaque reservoir hosts and the Anopheline vectors at dusk or later as this coincides with the peak biting time of the vectors[14,15]. These include subsistence farmers, timber camp workers, hunters, army personnel and also travelers to forests or forest-fringe areas. Visitors to South-east Asia from Australia, USA, Finland, Sweden, Germany, France, New Zealand, Taiwan and Japan have acquired knowlesi malaria following holidays or working visits

to Malaysian Borneo, Peninsular Malaysia, Brunei, Thailand, Indonesia and the Philippines[16].

Molecular and whole genome studies

In order to understand the molecular epidemiology and demographic history of knowlesi malaria, the mitochondrial (mt) genome sequences of *P. knowlesi* were initially studied[17]. Certain mt haplotypes were shared between humans and macaques and there were no haplotypes that were associated exclusively with either host; further evidence supporting *P. knowlesi* as a zoonotic parasite. Additional analyses indicated that *P. knowlesi* was as old as, if not older than, *P. falciparum* and *P. vivax*, and that it underwent a population expansion between 30,000 to 40,000 years ago. Macaques colonized Asia over 5 million years ago[18] and are probably the original hosts for *P. knowlesi*. A recent study, where 599 *P. knowlesi* samples from Peninsular Malaysia and Malaysian Borneo were analysed by a panel of ten microsatellite markers, showed there are two highly divergent sub-populations of *P. knowlesi*, and each of these subpopulations correspond with parasites from either long-tailed or pig-tailed macaques[19]. More recently, genome-wide sequence analysis of clinical *P. knowlesi* isolates from Malaysian Borneo shows sub-population structure that matches the analysis using microsatellite markers and also demonstrate there is a third sub-population of parasites, corresponding to laboratory strains isolated over 50 years ago from Peninsular Malaysia and the Philippines[20]. No signals of positive selection were observed in *P. knowlesi* around five orthologues of known *P. falciparum* drug resistance genes, indicating that the parasites in the reservoir macaque hosts have not been under antimalarial drug selection, thereby providing further evidence that knowlesi malaria is a zoonosis.

Diagnosis

In laboratories in malaria-endemic countries, malaria is diagnosed by examination of blood films by microscopy. Under the microscope, the early blood forms of *P. knowlesi* are identical to those of *P. falciparum*, while the other developmental stages, including the 'band forms', are similar to those of *P. malariae*[3]. There are minor morphological differences between these two species. The mature schizonts of *P. knowlesi* can contain up to 16 merozoites, whereas those of *P. malariae* have between 6–12[3]. However, mature schizonts are not found in all blood films examined and in diagnostic laboratories, where technologists are only trained to recognise *P. falciparum, P. vivax, P. ovale* and *P. malariae*, most *P. knowlesi* infections have been identified by microscopy as *P. malariae*[2,4,21]. Although morphologically similar, *P. knowlesi* parasites multiply every 24 h in the blood while this erythrocytic cycle is 72 h for *P. malariae*[9].

Molecular detection methods are the most sensitive and accurate techniques for identification of *P. knowlesi*. These include single and nested PCR assays, real-time PCR assays and loop-mediated isothermal assays[4]. However, these assays are relatively expensive, not rapid and are not readily available in resource-poor laboratories where the majority of *P. knowlesi* infections are detected. Rapid diagnostic tests (RDTs) for malaria are available, but the overall sensitivity of detection of a small number of RDTs that have been evaluated against knowlesi malaria cases varied between 26–74% and was even lower (0–45%) for parasitaemias below 1000 parasites/μL[22–24]. Due to the rapid multiplication rate of *P. knowlesi* in the blood of 24 h, sensitive RDTs capable of detecting knowlesi malaria at the early phase of infection are urgently required for rural laboratories.

Clinical and laboratory features of knowlesi malaria

P. knowlesi causes a wide spectrum of disease, from asymptomatic infections[9,25] to fatal ones[26–29]. The most common presenting signs and symptoms reported are fever with chills, followed by headache, myalgia, poor appetite, arthralgia, cough, abdominal pain and diarrhoea[27]. These are not significantly different to those observed in patients with vivax and falciparum malaria. The majority of cases (93.5%[27] and 84.5%[30]) at district hospitals in Sarawak had uncomplicated malaria with a fatality rate of 2%, whereas in a retrospective study in a referral hospital in Sabah, 61% of 56 cases were uncomplicated and the fatality rate was 27%[31]. However, subsequently at the same referral hospital, the use of intravenous artesunate for severe malaria cases and artemisinin combination therapy for non-severe cases, resulted in no deaths among 130 knowlesi malaria patients[29]. Typical complications of severe knowlesi malaria in adults include jaundice, acute kidney injury, hypotension, acute respiratory distress syndrome and metabolic acidosis[26,27,29,30,32]. In adults, severe anaemia has not been observed and neither has cerebral malaria, while severe disease has not been noted in the relatively small number of children with knowlesi malaria[4,33]. Thrombocytopaenia is very common, occurring in 97.3 to 100% of knowlesi malaria patients, and together with parasitaemia, correlates with severity of disease[27,30,31]. Following a case control study, it was recommended that any patient with a platelet count of <45 000/μL or parasitaemia of >35 000 parasites/μL should be regarded at risk of developing complications and should be treated for severe malaria[30].

Treatment of knowlesi malaria

Since knowlesi malaria is primarily a zoonosis, the parasites have been under no antimalarial drug pressure and should be susceptible to all antimalarials. This has been observed in hospital-based studies as well as case reports where several antimalarials have been used successfully to treat knowlesi malaria patients[4]. *P. knowlesi* parasites are highly sensitive to chloroquine[34] but following an informal consultation on the public health importance of knowlesi malaria organised by the WHO in 2011, it was recommended that in areas where knowlesi malaria has been detected, all infections diagnosed as *P. malariae* by microscopy should be treated and managed as for falciparum malaria[35]. Therefore, for uncomplicated knowlesi malaria cases in South-east Asia, artemisinin combination therapy is recommended. For severe knowlesi malaria, intravenous antimalarials should be administered and the use of artesunate in a tertiary referral hospital in Sabah was associated with zero mortality[29].

Future directions

The available molecular, entomological and epidemiological data strongly indicate that knowlesi malaria is primarily a zoonosis. However, human-to-human transmission has been demonstrated under experimental conditions[9] and it is not known whether it is currently occurring. The reasons for the increase in the number of knowlesi malaria cases, particularly in Malaysian Borneo, are also unknown. Whether the increase is due to increased awareness, changes in the feeding habits of the vectors, the destruction of the natural habitats of the macaque reservoir, human migration to areas close to macaque habitats, a recent adaptation of knowlesi malaria parasites to humans, or to some other factors needs to be investigated. In addition, currently available methods of control of human malaria involving the use of insecticide treated bednets and residual spraying of houses are ineffective against knowlesi malaria, where transmission primarily occurs outdoors. Therefore, effective methods of prevention and control need to be found and implemented, in order to prevent *P. knowlesi* from establishing itself in the human population.

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Biography

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