INVITED REVIEW ARTICLE

Plasmodium knowlesi Malaria in Malaysia

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SUMMARY

***Plasmodium knowlesi,* a simian malaria parasite, is now recognised as the fifth cause of human malaria and can lead to fatal infections in humans. Knowlesi malaria cases are widely distributed in East and West Malaysia and account for more than 50% of admissions for malaria in certain hospitals in the state of Sarawak. This paper will begin with a description of the early studies on *P. knowlesi*, followed by a review of the epidemiology, diagnosis, clinical and laboratory features, and treatment of knowlesi malaria.**

KEY WORDS:
Plasmodium knowlesi, Malaria, Clinical and laboratory features, Epidemiology, Treatment

INTRODUCTION

Malaria in humans was thought to be caused by four species of *Plasmodium (P. falciparum, P. vivax, P. ovale and P. malariae)*[1] until a large number of human *P. knowlesi* infections were described in the Kapit Division of Sarawak in 2004[2]. Using molecular methods of detection, it was found that that 58% of 201 patients with malaria at Kapit hospital, Sarawak, were infected with the simian malaria parasite, *P. knowlesi*. The infections had been misdiagnosed by microscopy mainly as *P. malariae*. Subsequently, human knowlesi malaria cases have been reported in other parts of East[3,4] and West Malaysia[4-6], and in other countries in Southeast Asia including Thailand[7,8], Myanmar[9], Singapore[10-12], the Philippines[13], Vietnam[14] and Indonesia[15,16]. *P. knowlesi* is now considered to be the fifth species of *Plasmodium* that can cause human malaria[17]. Researchers in Malaysia have played a pivotal role in the discovery and descriptions of naturally-acquired human knowlesi malaria and this review will begin with a description of the discovery and early studies on *P. knowlesi* followed by details of the epidemiology, diagnosis, clinical and laboratory features, and treatment of *P. knowlesi* infections.

HISTORICAL ASPECTS

P. knowlesi was first isolated in 1931 from a long-tailed macaque *(Macaca fascicularis)* imported to India from Singapore[18]. The early experiments were mainly conducted by Knowles and Das Gupta, who observed that *P. knowlesi* causes asymptomatic and low level parasitaemia in its natural host, the long-tailed macaque *(M. fascicularis)*, but is lethal for Indian rhesus macaques *(M. rhesus)*. They further demonstrated that *P. knowlesi* was infectious to three humans by blood passage and that it has the shortest erythrocytic cycle amongst the primate malarias, multiplying in the blood and leading to fever spikes every 24 hours, as compared to 72 hours for *P. malariae* and 48 hours for *P. vivax*, *P. falciparum* and *P. ovale*. The short erythrocytic cycle prompted the use of *P. knowlesi*, instead of *P. vivax*, as a pyretic agent for the treatment of patients with neurosyphillis, until the mid 1950s when penicillin became the treatment of choice[1, 19].

Although it was known that humans could be infected with *P. knowlesi* by blood passage since 1931, it was not until over thirty years later that the first case of a human infection by mosquito bite under natural conditions was reported[20]. An American army surveyor, who had been working in the forest in Bukit Kertau, near Temerloh in Pahang, became ill upon returning to the United States of America in 1965. His blood sample was sent to a research facility where experiments on *P. malariae* in human volunteers were being conducted. Following inoculation of infected blood into rhesus macaques, it was confirmed that the surveyor was infected with *P. knowlesi*. At that time, studies had already been initiated by a team of American scientists based at the Institute for Medical Research (IMR) in Kuala Lumpur, to determine whether malaria was a zoonosis following accidental infection of humans in two malaria laboratories in the USA with another simian malaria parasite, *P. cynomolgi*, in 1960[1, 19]. These studies intensified after the description of the *P. knowlesi* case and involved inoculating rhesus macaques at IMR with pooled blood samples from 1,117 villagers living near the area in Pahang where the American surveyor acquired his infection[21,22]. When none of the rhesus macaques became infected, it was concluded that knowlesi malaria in humans was extremely rare and that zoonotic malaria would not be a major threat to the Malaria Eradication Programme that had been initiated by the World Health Organisation[1].

DISCOVERY OF LARGE FOCUS OF P. KNOWLESI MALARIA IN SARAWAK

P. malariae malaria is generally accepted as a benign disease, with infected persons having relatively low parasitaemia (seldom above 5,000 parasites per µl blood) and not requiring hospitalisation[1,23]. However, in the Kapit Division of Sarawak, cases identified as *P. malariae* by microscopy had features that were atypical for *P. malariae* malaria. These included the observation that almost all infected persons had clinical signs and sought treatment, approximately a fifth of *'P. malariae* malaria' patients had parasitaemias greater than 5,000 parasites per µl blood, and that the majority of cases occurred in adults[2]. Examination of DNA extracted from 8 patients with *'P. malariae* malaria' by nested PCR assays showed that they had Plasmodium but were negative for *P. malariae*. DNA sequencing and analysis of two genes indicated that these infections were due to *P. knowlesi* and not *P. malariae*[2]. A total of 201 samples from malaria patients at

This article was accepted: 13 October 2010
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Kapit Hospital, including 141 diagnosed by microscopy as *P. malariae*, were examined with nested PCR assays for malaria, including a newly developed assay for *P. knowlesi*. None of the 201 samples were *P. malariae*-positive, but 58% were found to be *P. knowlesi*-positive and the remainder were *P. falciparum*, *P. vivax* and *P. ovale*[2].

EPIDEMIOLOGY

Human cases of knowlesi malaria are not confined to the Kapit Division of Sarawak, as evidenced by reports of cases throughout the East Malaysian states of Sarawak[4], Sabah[4, 24] and in most of the states in West Malaysia[4-6]. The distribution and number of reported PCR-confirmed cases of knowlesi malaria in Malaysia are summarised in Figure 1. It should be emphasised that these are just the number of cases that have been confirmed by nested PCR assays and that the actual number of knowlesi malaria cases in Malaysia is much higher. It is also not known for how long knowlesi malaria cases have gone unrecognised. A study of archival blood films confirmed that human knowlesi malaria cases occurred throughout Sarawak as early as 1996 and were misdiagnosed as *P. malariae* by microscopy[25].

Sarawak has the largest number of PCR-confirmed cases, most probably due to the extensive studies that have been undertaken in that state since the year 2000[2, 4, 25, 26]. From 2000 until 2008, only six cases of *P. malariae* in Sarawak were confirmed by nested PCR at the Malaria Research Centre, Universiti Malaysia Sarawak, in contrast to over 770 *P. knowlesi* cases. All six were logging camp workers that had recently returned after working in malaria-endemic countries, indicating that these infections had been acquired overseas and no indigenous transmission of *P. malariae* is occurring in Sarawak. In view of these data, in September 2008 the Sarawak State Health Department instructed microscopists working in government hospitals and divisional health offices to report all microscopy-identified *P. malariae* cases as *P. knowlesi*, unless the patients had recently returned from working overseas in malaria-endemic countries. In Sabah and West Malaysia, 25 PCR-confirmed cases of *P. malariae* have been reported, compared with 150 *P. knowlesi*. However, detailed travel history of the *P. malariae* malaria patients was not reported so it is not possible to deduce whether indigenous transmission of *P. malariae* is occurring in these locations.

Data from the Sarawak Health Department for malaria infections in 2009 indicates that *P. knowlesi* is the second most prevalent species in the state after *P. vivax*, accounting for 41% of the 2,189 cases reported. In a number of hospitals in Sarawak, such as those in Kapit, Sarikei, Kanowit, Sibu, Lawas, Limbang and Betong, knowlesi malaria accounts for more than 50% of admissions for malaria. The vast majority of knowlesi malaria cases occur in adults and clustering of cases among longhouse communities in Kapit, Sarawak has not been observed[2]. This indicates that transmission of knowlesi malaria probably occurs away from the vicinity of longhouses, which is supported by entomological studies in Kapit[27, 28].

The mosquito vectors responsible for transmission of *P. knowlesi* belong to the *Anopheles leucosphyrus* group[1,19,29]. In the Kapit Division, *An. latens* has been incriminated as the vector[28]. This is a forest-dwelling mosquito, that prefers to feed outdoors after dusk, is equally attracted to humans and monkeys, with monkeys at a higher elevation attracting more *An. latens* compared to those at ground level[27]. In West Malaysia the vectors for *P. knowlesi* have been identified as *An. hackeri* in Selangor[1] and *An. cracens* in Kuala Lipis, Pahang[5]. Both of these mosquito species prefer to feed on monkeys rather than humans, which may explain the lower incidence of knowlesi malaria cases in West Malaysia compared with Sarawak.

The natural hosts for *P. knowlesi* in Malaysia are long-tailed macaques *(M. fascicularis)*, pig-tailed macaques *(M. nemestrina)* and banded leaf monkeys *(Presbytis melalophos)*[1,19]. These two species of macaques are the most common non-human primate species found in Malaysia and have been noted to harbour five species of *Plasmodium (P. knowlesi, P. cynomolgi, P. fieldi, P. coatneyi* and *P. inui)*[1, 19]. Although macaques are present in urban areas in Malaysia, the risk of acquiring *P. knowlesi* is very low due the absence of mosquito vectors of simian malaria belonging to the *An. leucosphyrus* group in these areas. Humans can acquire knowlesi malaria when they visit the forest habitat of macaques and the mosquito vectors. The majority of cases in Sarawak occur in people who either work or stay in the forest such as farmers, logging camp workers and hunters[30]. Tourists to Malaysia have not been spared and travellers from Finland and Sweden have returned to their home countries with knowlesi malaria after visiting rural areas of Perak and Sarawak respectively[31, 32].

DIAGNOSIS

Routine diagnosis of malaria involves identifying the various blood stages by examination of Giemsa-stained blood films under the microscope. However, this method of diagnosis is not suitable for *P. knowlesi* since the early trophozoite stages of *P. knowlesi* are morphologically identical to those of *P. falciparum*[2,33]. The later blood stages (late trophozoites, schizonts and gametocytes) are similar to those of *P. malariae*, including the presence of the typical band-forms that are normally associated with *P. malariae*. These morphological similarities were noted by earlier workers[18,19], including Garnham[1] who predicted the following 35 years ago: 'A *P. knowlesi* infection in a human being could easily pass unrecognised as such in routine laboratories, where it probably be diagnosed as *P. malariae*, or if rings only were present, as *P. falciparum*.' Due to the morphological similarities between *P. knowlesi* and *P. malariae*, it is necessary to use a polymerase chain reaction (PCR) based assay for the correct identification of *P. knowlesi*. The nested PCR assay developed at the Malaria Research Centre, Universiti Malaysia Sarawak was the first assay developed for detection of *P. knowlesi*[2] and now another nested PCR assay[34], a loop-mediated isothermal amplification (LAMP) assay[35] and a real-time PCR assay[36] have been described for *P. knowlesi*. However, these new assays have yet to be validated with a large number of clinical samples. There are no commercially available rapid diagnostic tests (RDTs) for *P. knowlesi*, but

Table I: Symptoms and clinical findings in patients at Kapit Hospital with knowlesi and other malarias

	Knowlesi (n=107)	Falciparum (n=24)	Vivax (n=21)
Symptom			
Fever/chills	100	92	95
Headache	94.4	87.5	52.4
Rigors	89.7	79.2	85.7
Malaise	89.7	91.7	66.7
Anorexia	83.2	70.8	52.4
Myalgia	87.9	79.2	90.2
Cough	56.1	54.7	47.6
Nausea	56.1	87.5	28.5
Abdominal pain	52.3	37.5	23.8
Vomiting	33.6	41.7	19
Diarrhoea	29	47.5	33.3
Clinical finding			
Axillary temperature (°C)	37.6 (37.0-38.5)	37.8 (37.0-38.5)	37.0 (36.8)
Respiratory rate (beats per minute)	26 (22-31)	25.5 (22.3-29.5)	27 (24.5-29.0)
Pulse rate (beats/min)	95±16	99±17	97±18
Arterial blood pressure (mm Hg)	89±11	85±9	89±9
Capillary refill time (secs)	2 (2-3)	2 (2-3)	2 (2-3)
Palpable liver	24.3	29.2	16.7
Palpable spleen	15	20.8	23.8

Data shown as %, median (interquartile range) or mean±standard deviation. Data reproduced from reference no. 26.

Table II: Summary of published cases of knowlesi malaria patients with severe malaria

Hyper-Parasitaemia*	Age	Sex	Case No.	Hypotension	Acute renal impairment	Jaundic	Hypo-glycaemia	Lactic acidosis	ARDS/ pulmonary oedema	Outcome	Ref no.
Yes	66	Female	1	Yes	Yes	Yes	No	-	No	Died	4
Yes	69	Male	2	Yes	Yes	Yes	-	-	Yes	Died	4
Yes	39	Male	3	Yes	Yes§	Yes	-	Yes	Yes	Died	4
Yes	40	Male	4	No	Yes	Yes	Yes	-	Yes	Died	4
Yes	40	Male	1	Yes	Yes§	-	-	-	Yes	Died	41
Yes	68	Female	1	Yes	Yes	Yes	Yes	Yes	Yes	Died	26
Yes	36	Female	8	No	No	Yes	No	No	Yes	Died	26
Yes	36	Male	2	No	Yes	No	No	No	No	Survived	26
No	50	Female	3	No	No	Yes	No	No	No	Survived	26
No	71	Male	4	Yes	No	No	No	No	No	Survived	26
No	66	Male	5	No	No	Yes	No	No	No	Survived	26
No	61	Female	6	No	No	No	No	No	Yes	Survived	26
No	69	Female	7	No	Yes	No	No	No	Yes	Survived	26
No	73	Female	9	No	No	No	No	No	Yes	Survived	26
No	54	Female	10	No	No	No	No	No	Yes	Survived	26
Yes	58	Male	2	No	Yes	Yes	-	-	-	Survived	6
Yes	54	Male	3	Yes	No	Yes	-	-	Yes	Survived	6
Yes	55	Male	5	Yes	Yes	No	Yes	Yes	Yes	Survived	6

Severe malaria was defined according to the World Health Organisation criteria for severe falciparum malaria[45]. *Hyperparasitaemia was defined as >100,000 parasites/μL; severe anemia = haemoglobin concentration <7.1g/dL; hypotension = systolic blood pressure ≤80 mmHg; acute renal impairment = serum creatinine >265 μmol/L despite rehydration (or §indicates a high serum urea concentration in the absence of a serum creatinine concentration); jaundice = serum bilirubin >43 μmol/L; hypoglycaemia = serum glucose <2.2 mmol/L; hyperlactaemia = lactate level >6.0 mmol/L; acute respiratory distress syndrome (ARDS) /acute pulmonary oedema = respiratory rate >30 breaths/minute plus oxygen saturation <94% on room air and/or pulmonary infiltrates on chest radiograph. Dashes indicate no data available. None of the patients had severe anaemia or cerebral malaria. Table reproduced and modified from reference no. 26.

Table III: Summary of reported treatments and outcomes in knowlesi malaria

Treatment	Outcome	Number of cases	Parasitaemia	Region	Ref no.
CQ+PQ	survived	82	80-117,600 parasites/μL	Sarawak	2
CQ+PQ	survived	102	6-90,214 parasites/μL	Sarawak	26
CQ+PQ/SUL/PYR	survived	10	480-80,320 parasites/μL	Sarawak	2
CQ/doxycycline	survived	1	0.10%	Selangor	6
CQ	survived	1	0.02%	Selangor	6
CQ	survived	1	84,000 parasites/μL	Sarawak	38
CQ	survived	1	1,155 parasites/μL	Thailand	7
CQ/SUL/PYR	died	1	764,720 parasites/μL	Sarawak	4
CQ/SUL/PYR	died	1	112,000 parasites/μL	Sarawak	4
CQ/PQ/SUL/PYR/Q	died	1	'++++'	Sarawak	4
Q	died	1	222,568 parasites/μL	Sarawak	26
Q	died	1	178,760 parasites/μL	Sarawak	26
Q	died	1	75,000 parasites/μL	Sarawak	4
Q	survived	1	43,560 parasites/μL	Sarawak	2
Q	survived	1	88,900 parasites/μL	Sarawak	2
Q	survived	1	39,988 parasites/μL	Sarawak	26
Q	survived	1	178,760 parasites/μL	Sarawak	26
Q	survived	1	23,816 parasites/μL	Sarawak	26
Q/doxycycline	survived	4	0-4.0%	Selangor	6
Q/doxycycline	survived	1	<1.0%	Perak	32
Q/Riamet®/doxycycline	survived	1	3.80%	Selangor	6
Mefloquine	survived	1	0.10%	Sarawak	31
Atovaquone/proguanil	survived	1	185 parasites/μL	Indonesia	15

"++++" indicates >10 parasites/high power microscopy field. CQ = Chloroquine, PQ = Primaquine, Q=Quinine, SUL=Sulphadoxine, PYR=Pyrimethamine

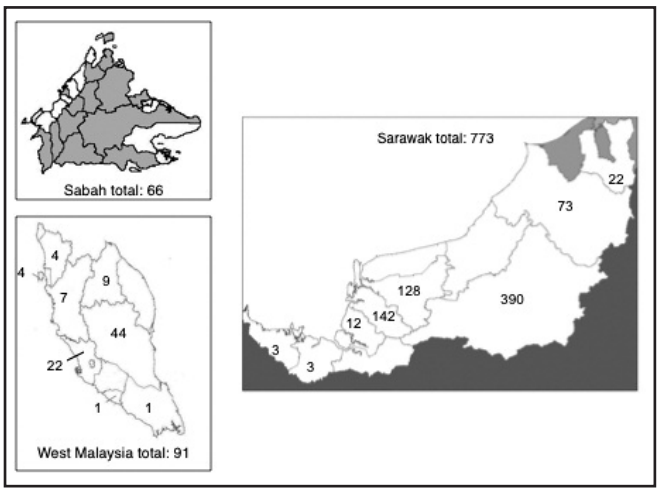

Fig. 1: Distribution and prevalence of PCR-confirmed *P. knowlesi* malaria cases in Malaysia. Districts in Sabah where knowlesi malaria cases have been detected are shaded grey, with data from reference no. 3 and 4. Number of knowlesi malaria cases detected in each state in West Malaysia are from reference no. 4, 5 and 6 and from Singh (unpublished data) for Johore and Kelantan (n=2). Number of knowlesi malaria cases detected in each administrative division in Sarawak from Singh (unpublished data) and reference no. 2, 4 and 26.

when tested with samples of *P. knowlesi*, two RDTs indicate positive results for other species of Plasmodium: the OptiMAL test for P. falciparum[12,37,38] and the Entebe Malaria Casette test for *P. vivax*[37].

For *P. knowlesi* infections, the incubation period in the liver (i.e. the time from being bitten by a mosquito to when parasites appear in the blood) is between 9 to 12 days[1]. *P. knowlesi* infections can lead to very high parasitaemias, but these are not seen in *P. malariae* infections, where the parasitaemia seldom exceeds 5,000 parasites per μl blood[1]. Therefore, if PCR assays are not available, a *P. knowlesi* infection should be strongly suspected if the patient has a *P. malariae* diagnosis by microscopy, a parasitaemia greater than 5,000 parasites per μl blood and a recent history of travel to the forest or forest fringe of Southeast Asian countries.

CLINICAL AND LABORATORY FEATURES

Our recent understanding of the clinical symptoms and laboratory features seen in naturally-acquired human infections with *P. knowlesi* is derived from single case reports[4,7,9,11,13,15,31,32, 38-41] , a small case series of 7 patients in the Klang valley, Selangor[6] a retrospective study of 94 patients in Kapit, Sarawak[2], and a prospective study, also in Kapit, describing the clinical and laboratory features in 107 patients[26]. Although the literature covers a wide geographical zone across South East Asia and includes data of patients of many ethnicities and varying background levels of malaria immunity, most of the data is weighted to Malaysia, and in particular the Kapit Division of Sarawak where the two large detailed clinical studies were conducted. In these studies from Kapit, patients were symptomatic for a median duration of 4 to 5 days at the time of presentation to a health care facility. In some cases, however, this may extend to several weeks[15,26]. The symptoms of knowlesi infection are of a non-specific infectious illness and are similar to those seen in falciparum and vivax malaria (Table I). Fevers, chills and rigors are the most dominant features, while headaches, myalgia/arthalgia, malaise and poor appetite are also commonly reported. Cough (56%), abdominal pain (52%) and diarrhoea (29%) were additional symptoms noted in a prospective study[26] and should not distract clinicians from considering malaria as a diagnosis. Gastro-intestinal symptoms were also dominant features in four fatal cases that have been previously described[4] .

The most common examination findings in 107 knowlesi patients at Kapit Hospital were tachypnoea, fever and tachycardia (Table I). A palpable liver and spleen were reported in 24% and 15% of cases respectively. Signs of a severe illness can also be present, including low oxygen saturations, tachypnoea, chest crackles (indicating acute respiratory distress or co-existing pneumonia), hypotension and jaundice[4,6,26,39]. In one fatal case with a history of poorly controlled hypertension, focal neurology was present, but it is not clear whether this was a coexistent cerebrovascular event since brain imaging was not available. A cerebral malaria-like syndrome has not been reported, but conscious levels may be impaired secondary to hypoglycaemia, and, although unusual for knowlesi malaria, should be excluded in all patients presenting with malaria[4,26].

Thrombocytopenia is very frequently seen in knowlesi infections and is also a feature of falciparum and vivax malaria, although at a lower frequency[42-44]. In the Kapit Division of Sarawak, 98% of 107 patients presented with thrombocytopenia and all were thrombocytopenic within 24 hours of admission[26]. Thrombocytopenia is a consistent feature across the published case reports of knowlesi malaria[4,6,7,11,15,31,32,38-40] and should be used as a warning flag to ensure that patients with thrombocytopenia are tested for malaria. There is a tendency among clinicians in urban areas of Southeast Asia to suspect dengue fever, rather than malaria, in thrombocytopenic patients due to the relatively high prevalence of dengue cases.

Despite the extremely high proportion of knowlesi malaria patients with thrombocytopenia, and a third of them having <50,000 platelets per µl blood, bleeding complications are rarely seen[26]. Other haematological abnormalities may be seen, including lymphopaenia (lymphocytes <800 per µl – 6.5%) and mild anaemia (haemoglobin <10g/dL – 4.6%). Severe anaemia, as seen in children with falciparum infections, does not appear to be a dominant feature of knowlesi malaria. Renal function may be significantly deranged; of 107 patients with acute infections, three had established renal failure (creatinine >265µmol/L after fluid resuscitation >24hrs)[26]. In the two survivors, careful fluid resuscitation and anti-malarial treatment were sufficient, and renal function returned to the normal limits prior to discharge. A recent case series from the Klang Valley of West Malaysia reported 2 out of 7 knowlesi malaria patients had acute renal failure[6]. It is however an ominous sign associated with fatal cases[4]. Electrolyte abnormalities including hyponatraemia (Na <136mmol/L) are frequently seen (24%), and are self-correcting with treatment of the malaria. Liver function may be abnormal, and a mild transaminitis is frequently seen.

Haematological and biochemical parameters respond rapidly following treatment, with the exception of haemoglobin, serum albumin concentration and liver enzymes, which typically return to normal limits by day 28[26].

COMPLICATED KNOWLESI MALARIA

Most cases of knowlesi malaria respond to treatment and resolve without complications. However, complicated and fatal cases have been reported[4, 6, 26, 39]. Application of the World Health Organisation criteria for severe falciparum malaria[45] indicated that 6.5% of 107 patients at Kapit Hospital had signs and laboratory features of severe disease at the time of presentation, and 2 of these died[26]. A summary of published complicated and fatal cases is provided in Table II. Patients may present with, or develop complications including acute respiratory distress syndrome (ARDS), hypotension, acute renal failure, hepatic dysfunction hypoglycaemia and metabolic acidosis. These may occur as single organ dysfunction, or as multi-organ involvement. Parasitaemia is a strong predictor of complications in knowlesi infection, with an area under the receiver operating characteristic (ROC) curve of 0.9 (95% Confidence Interval: 0.82-0.98; p<0.001). The specificity at a threshold of 100,000 parasites per µl was 100%, while the sensitivity of 30% indicates this threshold is probably too high, and highlights that severe cases can occur at relatively low parasitaemias[26].

A recent study reported for the first time, post-mortem findings of a patient in Sabah who died within 2 hours of admission, having presented in shock and found to have multi-organ failure[39]. This case showed partial sequestration of malaria parasites and haemorrhagic complications in vital organs, but a lack of chronic inflammatory infiltrate. This suggests that there are some histological similarities with falciparum malaria, but that a distinct pathophysiology may occur in severe knowlesi malaria. Studies are currently being undertaken to understand in detail the pathophysiology of knowlesi malaria.

TREATMENT

Case reports have indicated that chloroquine alone[6,7,11,38,41], atovaquone with proguanil[15], mefloquine[31], quinine and doxycycline[6,32] can be successfully used to treat knowlesi malaria patients (Table III). A regimen of chloroquine and primaquine, reviewed retrospectively, appeared to provide favourable treatment outcomes for knowlesi malaria patients at Kapit Hospital[2]. Following on from this, a recent observational study at the same hospital supports these findings of successful treatment with chloroquine[46]. In this study, 73 uncomplicated patients with PCR confirmed knowlesi malaria, who had not been exposed to anti-malarial drugs in the preceding 14 days, were treated with a total dose of 25mg/kg of chloroquine, administered as 10mg/kg, followed by 5mg/kg at 6, 24 and 48 hours. In line with the Malaysian Ministry of Health treatment guidelines for *P. malariae* infections, 2 doses of primaquine (15mg) were administered at 24 and 48 hours, mainly as a gametocidal drug, after glucose-6-pyruvate deficiency had been excluded. The findings of this study show that patients felt better within 24 hours and had a median fever clearance time of 26 hours. Parasites rapidly responded to treatment with chloroquine, and the time to clear 50% (PCT_{50}) and 90% (PCT_{90}) of the admission parasitaemia were 3.1 and 10.3 hours respectively, and significantly faster than for vivax infections. For most patients, parasite clearance occurred within 48 hours. No resistance, recrudescence or re-infection was observed during 28 days of follow-up, completed in 60 of these patients. This sensitivity to chloroquine is consistent with knowlesi infections being of zoonotic origin. Whether

primaquine is necessary seems unlikely, as gametocytes appeared to be sensitive to chloroquine and *P. knowlesi* is not known to have a latent liver (hypnozoite) stage[1].

Since there are currently no guidelines for the treatment of severe knowlesi malaria infections, such cases should be managed in accordance with the World Health Organisation guidelines for severe falciparum malaria. Before treatment studies in severe knowlesi malaria can be conducted, accurate case definitions of severe disease and the ability to rapidly confirm single knowlesi infections while excluding the presence of falciparum need to be in place. Likely candidate anti-malarial drugs would include quinine and artemisinin derivatives.

CONCLUSION

In summary, *P. knowlesi* is a significant cause of malaria in Malaysia, with cases widely distributed throughout East and West Malaysia. Knowlesi malaria is a zoonotic disease and humans acquire the infection when they enter the habitat of macaques and Anopheline mosquito vectors in the forest. Human cases had gone unrecognised, due to morphological similarities between *P. knowlesi* and *P. malariae*, until PCR-based detection assays were developed. There are no indigenous cases of *P. malariae* in Sarawak, but there may be some foci in Sabah and West Malaysia. If PCR assays are not available to confirm knowlesi malaria in West Malaysia and Sabah, a microscopy diagnosis of *P. malariae* with a parasitaemia greater than 5,000 parasites per µl blood, thrombocytopenia and a recent history of travel to the forest should strongly indicate a *P. knowlesi* infection. Unlike the benign *P. malariae* which replicates every 72 hours in the blood, *P. knowlesi* multiples every 24 hours and infections can be fatal. All patients presenting with any symptoms, signs or blood abnormalities (particularly thrombocytopenia), that may suggest an infection should be immediately tested for malaria. Knowlesi malaria results in a wide spectrum of disease and complications include acute respiratory distress syndrome, hypotension, acute renal failure, hepatic dysfunction, hypoglycaemia and metabolic acidosis. Uncomplicated knowlesi malaria responds well to oral chloroquine, and there is no evidence of chloroquine-resistance. Complicated knowlesi infections should be managed in accordance with World Health Organisation guidelines for the treatment of severe falciparum malaria. A failure to recognise knowlesi infection or a delay in treatment, especially when the parasitaemia is high, may lead to death.

ACKNOWLEDGEMENT

The authors would like to thank the Welcome Trust, The Malaysian Ministry of Science, Technology and Innovation, and Universiti Malaysia Sarawak for grants that have supported the research on knowlesi malaria in Malaysia.

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