Human malaria has been known to be caused by 4 Plasmodium species, with Plasmodium falciparum causing the most-severe disease. Recently, numerous reports have described human malaria caused by a fifth Plasmodium species, Plasmodium knowlesi, which usually infects macaque monkeys. Hundreds of human cases have been reported from Malaysia, several cases have been reported in other Southeast Asian countries, and a few cases have been reported in travelers visiting these areas. Similarly to P. falciparum, P. knowlesi can cause severe and even fatal cases of disease that are more severe than those caused by the other Plasmodium species. Polymerase chain reaction is of value for diagnosis because P. knowlesi infection is easily misdiagnosed as less dangerous Plasmodium malariae infection with conventional microscopy. P. knowlesi infection should be suspected in patients who are infected with malaria in Southeast Asia. If human–mosquito–human transmission were to occur, the disease could spread to new areas where the mosquito vectors live, such as the popular tourist areas in western India.

More than half of the world population lives in malaria risk areas in Africa, Central and South America, and South and Southeast Asia, and the annual malaria-associated mortality approaches 1 million, with 2 children dying from the disease every minute worldwide (World Malaria report 2009, WHO). For the past 80 years, human malaria has been known to be caused by 4 Plasmodium species—Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, and Plasmodium malariae—with P. falciparum being responsible for the most-severe cases. Recently, a fifth Plasmodium species has been recognized as a cause of malaria in humans. The newcomer is Plasmodium knowlesi, which was formerly known to cause malaria only in macaques.

HISTORY

First described in 1931 in a longtail macaque (Macaca fascicularis), P. knowlesi was later found to cause naturally acquired malaria in pigtail macaques (Macaca nemestrina) and the mitred leaf monkey (Presbytis melalophos), as well. Experimentally, P. knowlesi infects several other primates, and in 1932, P. knowlesi was proved to infect humans. Iatrogenic P. knowlesi infection was initially considered to be nonhazardous, and therefore, the parasite replaced P. vivax as a pyretic agent in the treatment of neurosyphilis in the early 1930s. This practice was discontinued, however, as a result of patient deaths [1].

It was not until 1965 that the first natural infection of P. knowlesi in humans was reported in an American traveller returning from Peninsular Malaysia [2]. In 1971, another natural infection was suspected in Peninsular Malaysia [3]. Despite extensive studies in Malaysia in the 1960s, no additional reports appeared until 2004, when Singh et al [4] described 120 cases of naturally acquired P. knowlesi infection in humans in Malaysian Borneo. Since then, numerous reports have been published. By analyzing old blood films, P. knowlesi...
has been shown to have caused malaria in a great number of patients since 1996 [5].

**MONKEY MALARIA**

At least 26 *Plasmodium* species are known to infect primates, yet natural transmission of a nonhuman *Plasmodium* species to humans is rare. The host specificity has been shown to be surprisingly strict: eg, *Plasmodium reichenowi* causing malaria in chimpanzees fails to infect humans [6]. Similarly, *P. falciparum* causes only mild parasitemia in chimpanzees. These kinds of differences appear to be caused by species-specific erythrocyte recognition profiles [6] or different binding of sporozoites to liver cells [7]. *P. knowlesi* is clearly not strictly species-specific, because both experimental monkey-to-human and human-to-human transmission have proved to be possible [8]. Other mechanisms of species specificity of plasmodia are attributable to vector restriction, vector feeding preferences, and vector species specificity. *P. knowlesi* transmission is vector restricted in that the parasite can be transmitted only by certain Anopheles mosquitoes [1, 9]. At least 2 main vectors in the *Anopheles leucosphyrus* group, *Anopheles latens* and *Anopheles cracens*, are known to be forest feeders, biting both humans and macaques at evening or during the night [9, 10]. Taken together, numerous mechanisms restrict the nonhuman *Plasmodium* species from infecting humans, yet *P. knowlesi* has proved to make a significant exception to this rule.

In the natural hosts, *P. knowlesi* causes an asymptomatic low-grade parasitism or mild disease [4, 10]. It is not known whether human *P. knowlesi* infections are obtained only from mosquitoes fed on macaques or whether natural human-mosquito-human transmission occurs. The zoonotic nature of the infection is suggested by the lack of case clustering in Sarawakian human settlements, where most cases have been described, and by the fact that most patients have a recent history of working or dwelling in a forest or forest fringe, which is the environment characteristic of the vector mosquitoes [10, 11]. During human infection, gametocytes are formed, but at low levels, which supports the suggestion that transmission is primarily zoonotic [12]. Because of the environmental preference of the natural vector mosquitoes, urban transmission is not likely to occur. The situation might change if human-mosquito-human transmission occurred, because at least one widely distributed urban mosquito species (*Anopheles stephensi*) has experimentally been shown to be a possible vector for *P. knowlesi* (reviewed by Coatney et al [1]).

**CASES IN MALAYSIA**

In the first study to recognize *P. knowlesi* as a causative organism of human malaria in Sarawak, Malaysia, the authors investigated cases that were identified by microscopic examination as being due to *P. malariae* but that had negative polymerase chain reaction (PCR) results [4]. When new primers were applied, 58% of the cases that originally had PCR results negative for *P. malariae* were found to have positive results for *P. knowlesi*, resulting in the identification of 120 cases of *P. knowlesi* infection in humans.

Thereafter, *P. knowlesi* was identified in 266 of the samples from 960 hospitalized Sarawakian patients with malaria [13], in 41 of 49 archived blood-films collected in Sabah [13], and 35 of 47 in Sarawak [5], in Malaysian Borneo, and in a total of 89 cases in Peninsular Malaysia [9, 13, 14]. In 2009, a prospective report on *P. knowlesi* infections in Sarawak was published that contained the clinical picture and the laboratory findings for 107 patients with *P. knowlesi* infection during 2006–2008 [15]. Since 2004, 5 fatal cases have been reported [13, 16].

**CASES IN OTHER SOUTHEAST ASIAN COUNTRIES**

After identifying cases in Malaysia, several patients with *P. knowlesi* infection have been documented in other Southeast Asian countries (Figure 1; Table 2). Diagnosis in all of these cases was based on PCR analysis, but malaria was detected in practically all of the cases using microscopy. In 2004, *P. knowlesi* malaria was described in a patient who lived in a suburb of Bangkok, Thailand, and who had visited southern Thailand near the Myanmar border [27]. Subsequently, *P. knowlesi* has been identified in 10 patients from southern and southwestern Thailand, in 22 patients from Peninsular Malaysia (reviewed by Coatney et al [1]), and in 7 patients from Indonesia. In Indonesia, *P. knowlesi* cases were found in northwestern Sumatra, and in Sulawesi, in the Selayar district [28]. In Vietnam, *P. knowlesi* was identified in 2 patients from the An Giang area [29]. In Cambodia, *P. knowlesi* infection was detected in 4 patients [30], and in Laos, it was found in 1 patient [31]. This distribution suggests that the infection of *P. knowlesi* in humans is very recent, and that it has spread very rapidly throughout Southeast Asia.
thrombocytopenia at hospital admission or on the following day, yet none of the patients had clinical coagulopathy. At hospital admission, only a few of the patients had anemia, whereas mild hepatic dysfunction was relatively common. The great majority of patients (94%) experienced no complications, and the infection responded well to chloroquine and primaquine treatment [15].

Applying the World Health Organization criteria for P. falciparum infection, P. knowlesi infection was evaluated as severe in 7% of the cases, with the most frequent complication being respiratory distress with pulmonary rather than metabolic etiology. A strong correlation was found between parasite density and the development of respiratory distress. Parasite density was also strongly and independently associated with renal dysfunction. Two of the patients died, representing a case fatality rate of 1.8% [15]. Phylogenically, P. knowlesi is relatively closely related with P. vivax; therefore, it is not surprising that infections with these 2 species share some common features, such as occasional severity and marked thrombocytopenia [35]. However, disease caused by P. knowlesi is somewhat more severe than disease caused by P. vivax, although also vivax malaria can be severe in a significant number of cases, at least in certain regions (severe malaria in ~3%, compared with 7% in individuals with P. knowlesi malaria) [15, 35].

In P. knowlesi infections, neurological symptoms are rare, and no cases of cerebral malaria have been reported [15]. A post-mortem study of a lethal P. knowlesi case suggests, however, that P. knowlesi–infected red cells can sequester in capillaries of the brain, heart, and kidneys [16].

Similarly to P. falciparum, P. knowlesi can cause severe or even fatal disease. It has a short lifecycle of 24 h [1], enabling a rapid progression of the disease (Table 1). The erythrocyte invasion by P. knowlesi is not restricted to young or old cells, which allows high parasitemia, and development of the parasite in erythrocytes is asynchronous. The threshold for hyperparasitemia in P. knowlesi infections is lower than in P. falciparum malaria and, at least theoretically, hyperparasitemia, together with the shorter asexual cycling time, may even render P. knowlesi more virulent in its severe form. In 3 of the 5 reported lethal cases, a high parasitemia was seen (15%, >10%, and >10 parasites per high-power microscope field) [13, 16]. Clinicians treating these patients should be aware of the potential lethal outcome of the infection.

**TREATMENT**

As malaria due to P. knowlesi might progress rapidly into severe disease, it should be treated like P. falciparum malaria if the species identification is based on microscopic examination alone or if co-infection with P. falciparum cannot be excluded with certainty using PCR. P. knowlesi itself appears to be susceptible to numerous alternative treatments (Table 1). The majority of
Malaysian patients were primarily treated with chloroquine [4, 15, 36], the Finnish patient was treated with quinine and doxycycline [23], the Swedish patient was treated with mefloquine [18], and the American patient was treated with the combination of atovaquone and proguanil [33]. This suggests that *P. knowlesi* malaria can be treated with all of these agents (Table 1). In general, antimalarial medication should be commenced at once after patients are found to have positive blood smear results, and the species can be identified or confirmed later. In *P. knowlesi* infection, commencing medication immediately is as important as it is in *P. falciparum* infection because of the potentially severe nature of both infections and because of the difficulty in distinguishing between these 2 species or determining whether there is a mixed infection on the basis of microscopic examination alone.

Two *plasmodium* species, *P. vivax* and *P. ovale*, may become dormant as liver hypnozoites and can cause one or more relapses of disease even years after the original infection if primaquine is not given to eradicate this form of the parasite (Table 1). After the primary treatment, Malaysian patients have been treated with primaquine to eliminate possible hypnozoites [15, 36]; however, there is no evidence from either macaque or human infections on relapses or indicating that hypnozoites exist in *P. knowlesi* infection. Therefore, it appears to be unlikely that primaquine administration is beneficial or necessary in *P. knowlesi* infection.

### Table 1. The 5 *Plasmodium*-Species Causing Malaria in Humans

<table>
<thead>
<tr>
<th>Variable</th>
<th><em>Plasmodium falciparum</em></th>
<th><em>Plasmodium knowlesi</em></th>
<th><em>Plasmodium vivax</em></th>
<th><em>Plasmodium ovale</em></th>
<th><em>Plasmodium malariae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Life threatening</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Red cells and fever cycle, days</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Infected red cells</td>
<td>All</td>
<td>All</td>
<td>Young cells</td>
<td>Young cells</td>
<td>Old cells</td>
</tr>
<tr>
<td>Parasitemia</td>
<td>Can be high</td>
<td>Can be high</td>
<td>&lt;2%</td>
<td>&lt;2%</td>
<td>&lt;2%</td>
</tr>
<tr>
<td>Liver hypnozoites</td>
<td>No</td>
<td>Probably no</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Treatment*</td>
<td>Combinations with artemisinine derivatives</td>
<td>Probably all the medications listed for the other <em>plasmodia</em></td>
<td>Chloroquine followed by primaquine</td>
<td>Chloroquine followed by primaquine</td>
<td>Chloroquine</td>
</tr>
<tr>
<td>Medical prevention</td>
<td>Atovaquone plus proguanil</td>
<td>Mefloquine</td>
<td>Quinine ± doxycycline</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* If a patient with a *P. falciparum* infection has high parasitemia, poor condition, and/or complications, intravenous medication (artesunate or quinine with or without doxycycline) is recommended. This probably applies also to *P. knowlesi* infections requiring intravenous medication. Possible use of chloroquine has been suggested as well [36], but this needs to be further explored before a recommendation can be made.
real-time PCR [38], or PCR combined with sequencing [23]. The widely used nested PCR assay (using the primers Pmk8 and Pmk9), however, occasionally gives false-positive results in either uninfected or P. vivax–infected individuals [21, 31, 39], indicating that a molecular method to test all 5 human malaria species or verification of identification by sequencing of the PCR product is needed [21, 28, 31]. A more specific set of primers has also been suggested [38, 39]. Although PCR analyses can be done by scraping material from microscopy slides, ethylenediaminetetraacetic acid-anticoagulated blood samples should be used to minimize contamination.

In several countries, traditional microscopy-based malaria diagnostics have recently been combined with, or at least partially replaced by, the use of malaria rapid diagnostic tests (RDTs). Some of the kits available are based on detection of plasmodium-specific lactate dehydrogenase, whereas others detect histidine-rich protein II (HRP-2) of P. falciparum either alone or in combination with a pan-malarial antigen, aldolase. Currently, no commercially available RDTs are designed to specifically detect P. knowlesi. Performance of these mainly P. falciparum– and P. vivax–targeted RDTs in P. knowlesi infection has been reported in a few cases [18, 25], which suggests that anti-HRP-2 antibodies do not always recognize P. knowlesi [18], whereas anti-aldolase and anti-LDH antibodies are more reliable [19, 40]. In conclusion, these tests are not recommended because of the unreliable results and low sensitivity in detecting P. knowlesi. Because knowlesi malaria can be symptomatic in patients with low parasitaemia, RDTs should detect it with sensitivity of 1 or even <1 parasite/μL of blood.

**PREVENTION OF P. KNOWLESI-INFECTION**

As with the other types of human malaria, prevention of P. knowlesi infection is based on avoiding mosquito bites and taking preventive medication when indicated. The efficacy of these measures against P. knowlesi has not been shown, but it can be assumed that its vector behaves like the vectors of other Anopheles species, and general precautions for avoiding the bites of Anopheles mosquitoes probably apply. Current indoor control measures for malaria do not, however, prevent zoonotic transmission of malaria by vectors that mainly feed in the forest [9, 10]. Zoonotic P. knowlesi infection can, accordingly, continue to be a problem for malaria control, and also poses a significant threat to the renewed efforts aimed at fully eradicating malaria.

None of the tourists with reported P. knowlesi infection used preventive medication [18, 19, 22, 23, 33]. Because chloroquine [4, 15], doxycycline [23], mefloquine [18], or atovaquone-proguanil in combination [22] have been successfully used in treatment of the disease, all of these agents could probably be used as preventive medicines against P. knowlesi, as well.
SIGNIFICANCE AND FUTURE PROSPECTS

As the number of human *P. knowlesi* cases increases, clinicians and laboratory personnel should be alerted to this emerging—and potentially lethal—cause of malaria [13, 16]. Laboratory personnel have thus far only been trained to identify the 4 traditional *Plasmodium* species. Numerous cases in several countries may have been misidentified as being due to *P. malariae*. Moreover, some malaria RDTs have failed to detect *P. knowlesi*, and all RDTs fail to identify the species [18,19]. Therefore, if rapid and reliable molecular diagnostic methods are not available to establish the diagnosis of *P. knowlesi* infection, either ordinary malaria microscopic examination or at least superficial microscopic examination together with RDTs appears to be necessary to diagnose malaria in general in patients infected with *P. knowlesi*.

So far, human *P. knowlesi* infections have been described in high numbers only in Malaysia. The major majority are from Malaysian Borneo [3, 4, 13, 15], yet numerous cases have also been described in Peninsular Malaysia [9, 13, 14, 23]. Due to the reports of *P. knowlesi* malaria obtained in the neighboring countries, Thailand [27, 28], Singapore [24–26], Brunei [20], Indonesia [21, 22], Myanmar [29, 30], Vietnam [31], and the Philippines [32, 33], it appears that *P. knowlesi* is a natural parasite of macaques throughout the Southeast Asian region. The wide distribution of human cases shows that *P. knowlesi* is generally able to infect humans, which is also suggested by the genetic polymorphism of the *P. knowlesi* identified from human samples [28]. The fact that *P. knowlesi* is being identified in increasing numbers now may be attributable in some areas to the reduction of cases of *P. falciparum* and *P. vivax* infection, whereas the number of cases of *P. knowlesi* infection remains constant. The availability and use of specific PCR methods to detect *P. knowlesi* may generate more reports of *P. knowlesi* infection, and therefore, the increase in the number of reports is not necessarily linked to the actual spread of the parasite but to the use of more-accurate diagnostic methods.

The high number of human cases (Table 2) suggests that *P. knowlesi* may be more capable of infecting humans than are the other species that cause nonhuman primate malaria. To date, only experimental, and not natural, human-mosquito-human transmission has been reported [8]. If natural human-mosquito-human transmission occurred, *P. knowlesi* could spread more widely in Asia. This is made possible by the wide distribution of at least one vector species, *Anopheles latens*, in Southeast Asia and in the southern parts of the Indian subcontinent, including the popular tourist areas in western India [10] (Figure 1). It remains to be seen whether this kind of spreading has occurred in the past or will occur in the future, but to date, neither human-mosquito-human transmission nor spreading of *P. knowlesi* has been documented.

Acknowledgments

Potential conflicts of interest. All authors: no conflicts.

References


Table 2. Reported Human *Plasmodium knowlesi* Infections

<table>
<thead>
<tr>
<th>Country/area</th>
<th>Local cases</th>
<th>Cases in travelers</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaysia/Borneo</td>
<td>570</td>
<td>3</td>
<td>[3, 4, 5, 13, 15]; [2, 18, 19]</td>
</tr>
<tr>
<td>Brunei/Borneo</td>
<td>1</td>
<td></td>
<td>[20]</td>
</tr>
<tr>
<td>Indonesia/Borneo</td>
<td>1</td>
<td>1</td>
<td>[21, 22]</td>
</tr>
<tr>
<td>Malaysia/Peninsular</td>
<td>89</td>
<td>1</td>
<td>[9, 13, 14, 23]</td>
</tr>
<tr>
<td>Singapore</td>
<td>6</td>
<td></td>
<td>[24–26]</td>
</tr>
<tr>
<td>Thailand</td>
<td>11</td>
<td></td>
<td>[27, 28]</td>
</tr>
<tr>
<td>Myanmar</td>
<td>33</td>
<td></td>
<td>[29, 30]</td>
</tr>
<tr>
<td>Vietnam</td>
<td>5</td>
<td></td>
<td>[31]</td>
</tr>
<tr>
<td>Philippines</td>
<td>5</td>
<td>1</td>
<td>[32, 33]</td>
</tr>
<tr>
<td>Total</td>
<td>720</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

* Area of infection in 1 case was unclear (Thailand, Indonesia, Peninsular Malaysia, or Vietnam) [17].


