Plasmodium knowlesi in travellers, update 2014

Müller, Mattia; Schlagenhauf, Patricia

Abstract: OBJECTIVES: Since the initial discovery of Plasmodium knowlesi in Malaysia, cases have been reported from several neighbouring countries. Tourism has also resulted in an increasing number of cases diagnosed in Europe, America, and Oceania. In this review we focus on the risk of the travel-associated acquisition of P. knowlesi malaria. METHODS: A search of the literature in PubMed was carried out to identify articles and literature on the distribution of P. knowlesi infections in Southeast Asia and details of its acquisition and importation by travellers to other continents. The cut-off date for the search was December 1, 2013. Search words used were: "Plasmodium knowlesi”, "Plasmodium knowlesi infections”, "Plasmodium knowlesi travellers”, "Plasmodium knowlesi prevalence”, "Plasmodium knowlesi host”, "Plasmodium knowlesi vector” "Plasmodium knowlesi RDT”, and "Plasmodium knowlesi Malaysia”. Traveller numbers to Malaysia were obtained from the Tourism Malaysia website. RESULTS: A total of 103 articles were found. Using a selection of these and others identified from the reference lists of the papers, we based our review on a total of 66 articles. RESULTS: P. knowlesi malaria appears to be the most common malaria species in Malaysian Borneo and is also widely distributed on the Malaysian mainland. Furthermore, locally transmitted cases of P. knowlesi malaria have been reported in Thailand, the Philippines, Vietnam, Singapore, Myanmar, Indonesian Borneo, and Cambodia. Two cases have been reported from non-endemic countries in Asia (Japan and Taiwan) in people with a history of travel to Malaysia and the Philippines. Twelve cases were imported to their home countries by travellers from other continents: two from the USA, two from the Netherlands, two from Germany, and one each from Spain, France, Sweden, Finland, Australia, and New Zealand. In most cases, the infection was associated with a trip to or near forested areas. The symptoms were fever (n=12), headache (n=6), chills (n=6), nausea (n=4), myalgia (n=3), back pain (n=3), abdominal problems (n=1), anorexia (n=2), fatigue (n=2), malaise (n=1), arthralgia (n=1), sore throat (n=1) vomiting (n=2), and jaundice (n=1). All patients were treated successfully with currently available antimalaria treatments. The identification of the pathogen by microscopy can be problematic due to the morphological similarity of P. knowlesi to Plasmodium malariae. CONCLUSION: P. knowlesi appears to be a threat not only to the local population in Malaysia, but also to the estimated 25 million annual tourists and occupational travellers to Malaysia, especially those who visit rural, forested areas of the country. The P. knowlesi risk is not limited to Malaysia, and travellers from Southeast Asia presenting with possible malaria should be considered for a diagnostic work-up that includes P. knowlesi.

Review

Plasmodium knowlesi in travellers, update 2014

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S U M M A R Y

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1. Introduction

Plasmodium knowlesi was probably discovered by Frenchini in 1927 in its natural simian host, Macaca fascicularis, and was described for the first time in morphological detail in 1932 by Knowles and Das Gupta, who also managed to show transmission from monkeys to human volunteers under laboratory conditions.1–3 Up to 1965, P. knowlesi was known only as a simian parasite. This changed when it was found in the blood of a US Army Map Service surveyor who had spent 4 weeks in Malaysia.4 With a focus on naturally acquired P. knowlesi infections in human beings, Singh et al. showed that, in Kapit Division (Sarawak, Malaysia), 58% of 208 microscopically diagnosed malaria patients were actually positive for P. knowlesi by PCR. These findings led to the recognition of P. knowlesi as the fifth human malaria parasite.5 Since then different PCR studies have shown that P. knowlesi is a common malaria Plasmodium species in Malaysian Borneo.6–9 In 2008, 27.2% of 980 malaria patients from 12 hospitals of Sarawak showed the simian
malaria infection. In Kudat District of the Sarawak neighbour-
state Sabah, some 78% of 220 malaria-positive patients were
considered to be afflicted with P. knowlesi mono-infections and 9% with
mixed infections, while in Sandakan Division, 23.6% were
reported to be positive for this Plasmodium species. Another study
in which nested PCR was used in Sabah, showed 63 P. knowlesi single
infections and two mixed infections out of 107 Plasmodium spp
positive samples.

The analysis of archival blood films suggests that, already in
1996, P. knowlesi accounted for a large part of microscopically
diagnosed Plasmodium malariae infections in Sarawak, and
to the more recent investigations, it appears to have
increased in recent decades. Also, this Plasmodium species has
been detected in different states of Peninsular Malaysia. However
the fifth malaria parasite is not geographically limited to
Malaysian Borneo and the Malaysian mainland. In Thailand, the
existence of P. knowlesi has been reported at different locations
throughout the country. In Vietnam, three cases have been
reported from Ninth Thu Lan Province and 32 PCR-positive blood
samples from Nga Hai and Da Tai (Khanh Hoa Province). Six more
cases have been reported in the forested part of Singapore, and
from southern Myanmar; six from the Philippine island of
Palawan, and two cases have been described in Cambodia near
the border with Thailand.

P. knowlesi is transmitted by mosquitoes of the Anopheles
leucophus group. The natural hosts include long-tailed
macaques (M. fascicularis), pig-tailed macaques (Macaca
nemestrina), and banded leaf monkeys (Presbytis melalophos). The
prevalence of infection in long- and pig-tailed macaques in Kapit
Division (Sarawak, Malaysia) is up to 78%. For intercontinental travellers, the significance of P. knowlesi
infections became clear when, in 2006, a Swedish traveller
presented to a Stockholm hospital with a P. knowlesi-positive
blood picture after a 2-week holiday in Sarawak, Malaysian Borneo. Since then, there have been several reports of imported P. knowlesi cases all over the world.

This review presents an overview of available knowledge on
Plasmodium knowlesi with a focus on the risk of travel-associated
acquisition of this malaria species. (Figs. 1 and 2)

2. Methods

The data were compiled from searches of PubMed using the
following search terms, alone or in combination: “Plasmodium
knowlesi”, “Plasmodium knowlesi infections”, “Plasmodium
knowlesi travellers”, “Plasmodium knowlesi prevalence”, “Plasmodium
knowlesi host”, “Plasmodium knowlesi vector”, “Plasmodium
knowlesi RDT”, and “Plasmodium knowlesi Malaysia”. References of the
selected articles were also searched to complete the documentation.
Traveller numbers to Malaysia were sourced from the website of
Tourism Malaysia. The cut-off date for the collation of
information was December 1, 2013.

3. Results and discussion

We used a selection of the 103 papers resulting from our
PubMed screening, with the addition of further papers identified
from the references lists. Furthermore we used statistics on
tourism in Malaysia obtained from the official website of Tourism
Malaysia.

3.1. P. knowlesi in international travellers (Table 1)

The first reported case of a naturally transmitted P. knowlesi
infection concerned a surveyor from the US Army in 1965. This
early report suggested that the parasite could also be a threat to
international occupational and holiday travellers. Since then,
future cases of P. knowlesi infection probably acquired during a
holiday or work-related trip have been reported from non- endemic
countries in Asia (n = 2), as well as from other continents
(n = 11). In Europe, the two first confirmed imported P. knowlesi
cases occurred in 2006/2007. The first was a Swedish
traveller who had returned from a trekking tour of the jungle area of
the Bario Highlands in Malaysian Borneo. The second was a
Finnish man who had travelled in Peninsular Malaysia during
March 2007. Since then, six more cases have been reported in
Europe, in tourists from Spain, France, the Netherlands, and
Germany, and from a Malay (Sarawak) immigrant to the
Netherlands. In the southern hemisphere, two imported cases
are known, one in Australia and one in New Zealand. One
patient had worked several times in Kalimantan, Indonesian
Borneo and the other in Sabah and Sarawak (Malaysian Borneo). A
further P. knowlesi infection, besides the early 1965 case, was
found in the USA, in a female patient who had visited
relatives on Palawan Island in the Philippines. From these 12
reported cases in intercontinental travellers, only three cases
involved female patients. The period of travel in the risk area
varied from 1 week to several months. In nearly all cases, a
history of travel to a forested area with overnight stays was
indicated. A forest infection was reported for the two cases, a female patient visiting relatives on Palawan Island in
the Philippines and a French tourist spending his holidays on a
tourist beach on the island of Ko Panag in Thailand. In both cases, however, the travellers were on the edge of a forested area, but never entered it.

3.2. Clinical and laboratory parameters

In a clinical study, Daneshvar et al. reported fever and chills to be
the common symptoms, identified in 100% of the investigated
cases. Furthermore headache, rigors, malaise, anorexia, myalgia,
arthralgia, cough, and abdominal pain were frequently observed.
Tachycardia and tachypnea were noted as common clinical signs.

In the 12 intercontinental traveller cases, the first common
symptom was fever. Other symptoms were headache (n = 6), chills
(n = 6), nausea (n = 4), myalgia (n = 3), back pain (n = 3), abdominal
problems (n = 1), anorexia (n = 2), fatigue (n = 2), malaise (n = 1),
arthralgia (n = 1), sore throat (n = 2) vomiting (n = 1), and jaundice
(n = 1). In one case, symptoms of hypoglycaemia with slight
hearing and visual loss were reported during treatment with
quinine and doxycycline. In most cases, the first clinical
symptoms were present 2–13 days after leaving the country of
probable acquisition. Only in the case of a 32-year-old Dutch
woman were the symptoms already present in the country
travelled to. Clinical examination showed an increased
body temperature (range 38.8–40.2 °C) in all 12 traveller patients. In
three cases in whom it was measured, was anaemia found to be
present. Leukocyte counts were lowered in five cases. Thrombocytopenia was present in all patients in
whom platelet levels were determined. Six publications reported an increase in the liver enzyme alanine aminotransferase
and five an increased aspartate aminotransferase. Renal failure was diagnosed with an increase in creatinine up to 3.45 mg/
dl in a German woman. According to the adapted World Health Organization (WHO) criteria for severe malaria infection by Barber et al., two of
the traveller cases could be considered to have had severe infections. In one case this was due to high parasite and bilirubin levels,
in another to rising creatinine levels during hospitalization. Concerning the prognosis, Willmann et al. postulated that patients
with P. knowlesi infections and a parasite count > 35 000/µl or > 1%, or a platelet count ≤ 45 × 109/l, should be considered as at risk of
On the other hand, Barber et al. reported a correlation only with parasitemia (≥20,000/μl) as a significant marker of severity. Therefore, according to Willmann et al., two more patients could be considered to have been at potential risk of further complications because of their low platelet levels or high parasitemia, and a further case also by the criteria of Barber et al. (Table 1 and Table 2)

3.3. Diagnosis of P. knowlesi malaria

The ‘gold standard’ for the diagnosis of most Plasmodium species is microscopic examination because it is rapid and inexpensive and accurate in expert hands. The disadvantage in its application for the diagnosis of P. knowlesi is the large number of false-negatives or misdiagnosed cases due to the morphological similarity with P. falciparum in the early trophozoite stage and with P. malariae in the late and mature trophozoites, gametocytes, and schizonts.

The first nested PCR applied to P. knowlesi diagnosis was described by Singh et al. However, in its application, the primer pair resulted in a small number of false-positive results due to cross-reactivity with Plasmodium vivax. Since then, additional nested PCR primers and real-time PCR procedures with a higher sensitivity have been developed. Although PCR is one of the

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**Figure 1.** Distribution of reported Plasmodium knowlesi infections in Southeast Asia.

**Figure 2.** Plasmodium knowlesi cases exported from Southeast Asia to other continents.
<table>
<thead>
<tr>
<th>Ref.</th>
<th>Year</th>
<th>Country of presentation</th>
<th>Presumed area of acquisition</th>
<th>Journey to potential risky areas (forest, jungle)</th>
<th>Interval between leaving the country of acquisition and presentation with symptoms</th>
<th>Symptoms</th>
<th>Treatment</th>
<th>Prophylaxis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chin et al.</td>
<td>1965</td>
<td>USA</td>
<td>Peninsular Malaysia</td>
<td>NA</td>
<td>NA</td>
<td>Sore throat, chills, fever, sweating, anorexia, fatigue, nausea</td>
<td>Chloroquine, primaquine</td>
<td>NA</td>
</tr>
<tr>
<td>Bronner et al.</td>
<td>2006</td>
<td>Sweden</td>
<td>Sarawak, Malaysia, Borneo</td>
<td>Jungle of the Bario Highlands</td>
<td>11 days</td>
<td>Fever (40 °C), sweating, headache, fatigue</td>
<td>Mefloquine</td>
<td>No</td>
</tr>
<tr>
<td>Kantele et al.</td>
<td>2007</td>
<td>Finland</td>
<td>Kuala Lumpur, Malaysia; Ipo, Malaysia; Langkawi, Malaysia</td>
<td>Surrounding areas, jungle</td>
<td>3 days</td>
<td>Fever (38.8 °C), abdominal problems, attack of hypoglycaemia with transient mild hearing and visual loss</td>
<td>Quinine dihydrochloride, doxycycline Later: primaquine</td>
<td>No</td>
</tr>
<tr>
<td>CDC^11</td>
<td>2008</td>
<td>USA</td>
<td>Island of Palawan, Philippines</td>
<td>No (edge of forest area)</td>
<td>NA</td>
<td>Headache, fever, chills</td>
<td>Atovaquone/proguanil, primaquine</td>
<td>No</td>
</tr>
<tr>
<td>Ta et al.</td>
<td>2008/2009</td>
<td>Spain</td>
<td>Bangkok, Thailand Banda Ace + Pulau Weh, Indonesia Kuala Lumpur, Malaysia Hanoi, Vietnam</td>
<td>Rural areas</td>
<td>NA</td>
<td>Fever (40.0 °C), arthralgia, myalgia, low back pain, chills, malaise</td>
<td>Chloroquine (changed because of adverse drug reaction) Atovaquone/proguanil (took 80% of prescription)</td>
<td>No</td>
</tr>
<tr>
<td>van Hellmond et al.</td>
<td>2009</td>
<td>Netherlands</td>
<td>Kapit, Sarawak, Malaysia</td>
<td>Jungle</td>
<td>2 days</td>
<td>Fever (40.0 °C), myalgia, headache, low back pain, fever, mild headaches</td>
<td>Atovaquone/proguanil</td>
<td>-</td>
</tr>
<tr>
<td>Figtree et al.</td>
<td>2010^a</td>
<td>Australia</td>
<td>Kalimantan, Indonesia</td>
<td>Forest</td>
<td>13 days</td>
<td>Fever (39.1 °C), myalgia</td>
<td>Atovaquone/proguanil</td>
<td>-</td>
</tr>
<tr>
<td>Hoosen and Shaw</td>
<td>2010</td>
<td>New Zealand</td>
<td>Sabah, Malaysia; Sarawak, Malaysia</td>
<td>Forest</td>
<td>7–9 days</td>
<td>Fever (39.1 °C), myalgia</td>
<td>Atovaquone/proguanil</td>
<td>-</td>
</tr>
<tr>
<td>Berry et al.</td>
<td>2010</td>
<td>France</td>
<td>Ranong, Thailand; Island of Ko Payam, Thailand Borneo, Malaysia</td>
<td>No (edge of forest area)</td>
<td>9 days</td>
<td>Fever, shivering, nausea, anorexia</td>
<td>Chloroquine</td>
<td>-</td>
</tr>
<tr>
<td>Link et al.</td>
<td>2011</td>
<td>Netherlands</td>
<td>Borneo, Malaysia</td>
<td>Jungle</td>
<td>1 day before leaving country of probable acquisition</td>
<td>Fever (40.2 °C), chills, nausea, vomiting, severe, headache, backache</td>
<td>Atovaquone/proguanil</td>
<td>No</td>
</tr>
<tr>
<td>Ehrhardt et al.</td>
<td>2013^a</td>
<td>Germany</td>
<td>Ranong, Thailand</td>
<td>Forest</td>
<td>9 days</td>
<td>Fever (40.0 °C), chills, severe, headaches, fever, nausea, vomiting</td>
<td>Atovaquone/proguanil Artesunate, artemether/ lumefantrine</td>
<td>No</td>
</tr>
<tr>
<td>Orth et al.</td>
<td>2013^a</td>
<td>Germany</td>
<td>Khoa Sok, Thailand</td>
<td>National Park</td>
<td>10 days</td>
<td>Fever, nausea, vomiting</td>
<td>Atovaquone/proguanil</td>
<td>-</td>
</tr>
</tbody>
</table>

NA, information not available.

^a The year of infection was unclear, so the year of publication has been used.
most sensitive and specific known diagnostic methods, it requires time, money, and well-trained personnel, and for these reasons it is not ideal in most settings.49

Currently available rapid diagnostic tests (RDTs) based on monoclonal antibodies (MAbs) capturing pan-Plasmodium lactate dehydrogenase (pan-pLDH) and genus-specific aldolase (pan-aldolase), only have a sensitivity of 74% and 23%, respectively, and do not distinguish between the non-falciparum species.50 In 2008, the team of McCutchan analysed the reactivity of known Plasmodium LDH-specific MAbs. In this experiment, McCutchan et al. were able to distinguish P. knowlesi from P. malariae infections. However the detection of P. knowlesi infections is confounded by the difficulty in distinguishing P. knowlesi from mixed falciparum–vivax infections.51 In 2011, Piper et al. developed a model for a new test format based on three test strips, with which the four human Plasmodium species and P. knowlesi could be differentiated.52

Isenki et al. in Japan (2010) and Lau et al. in Malaysia (2011) created P. knowlesi-specific primers against β-tubulin53 and apical membrane antigen-1,46 which can be used in a loop-mediated isothermal amplification (LAMP). The advantages compared to PCR are the velocity (106 copies of a targeted gene in 1 h) and the required isothermal conditions, which allow the use of simpler and cheaper techniques such as an incubator.47 The exact niche for this diagnostic approach has yet to be established.

3.4. Treatment and chemoprophylaxis

The most frequently reported drug treatment for P. knowlesi infections in the papers reviewed was a combination of chloroquine and primaquine.4–6,8,23,34 Due to the fact that no hypnozoites have ever been described in P. knowlesi infections, primaquine is probably not required as a treatment, except in cases of mixed infection with P. vivax. In one study, the time to clear 90% of the parasitemia present at admission was defined as 10.3 h with a fever remission time of 26.5 h in adults. The dosage used was 25 mg/kg chloroquine over 2 days and additionally 15 mg primaquine.54 Children with the same drug combination showed a much slower mean parasite clearance of 2 days. A significant correlation has been shown between the parasite concentration in the blood and the time to parasite clearance.8 A recent study revealed the efficiency of artesiminin-based combination therapy (ACT) with artemether–lumefantrine and artesunate–mefloquine for non-severe P. knowlesi infections, or intravenous artesunate for severe infections.42 A faster parasite clearance was reported with artemether–lumefantrine and artesunate compared to chloroquine and quinine.42 Even if at the present time, large studies on the other established malaria treatments are missing, several single reports have underlined the efficiency of the different currently available drugs. The documented successful treatment with different combinations of chloroquine, primaquine, artesinin combinations, sulfadoxine, pyrimethamine, and quinine are shown in Table 5. Mefloquine was described three times as an effective therapy.24,29,40 In another case report involving a re-infected Malaysian businessman during an expedition in Tanjung Malim, Perak, Malaysia, the parasitemia continued to increase despite a total administration of 1.5 g of mefloquine. The man, who presented symptoms of chills, rigors, epigastric pain, nausea, and vomiting after an expedition with overnight camping, was started on initial therapy of 750 mg mefloquine with two successive doses of 500 mg after 6 h and 250 mg after 12 h. Despite treatment, the parasitemia increased from 1.0% to 2.5%. Medication was changed to quinine, doxycycline, and the combination artemether and lumefantrine. Parasitemia eventually cleared on day 5 of admission to the hospital.55 There may be potential resistance of some P. knowlesi strains against mefloquine. Experiments with infected rhesus monkeys showed a suppressive activity of mefloquine but without complete cure against P. knowlesi infections.56–58 Recent studies have indicated an innate immunity to mefloquine in vitro, as well in ex vivo experiments.59

Data regarding chemoprophylaxis for prevention are missing, but the fact that none of the infected cases reported so far had received adequate prophylaxis could indicate an effect of known preventive anti-malaria drugs against P. knowlesi as well. Only one case, a Spanish traveller, had used mefloquine and later atovaquone/proguanil chemoprophylaxis; this case developed a mild P. knowlesi infection but later recovered without further treatment.42 (Table 3, Table 4 and Table 5).

3.5. Risk and advice for travellers

In 2012, 25 032 708 tourist arrivals were registered in Malaysia. Most of them were from Asian countries, especially from Singapore, Thailand, Indonesia, Brunei, and China. In the same year, Tourism Malaysia reported an increase to 1 002 067 European travellers (from the UK, France, Germany, Netherlands, Italy, Sweden, Switzerland, Denmark, Spain, Ireland, Norway, Belgium, Austria, and Poland), 327 065 from the USA and Canada, and 573 674 from New Zealand and Australia.39 The risk of transmission therefore appears low for intercontinental travellers considering that only 14 cases have been reported beyond endemic areas.

Table 2

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Case</th>
<th>Temp, °C</th>
<th>Leukocytes*</th>
<th>Thrombocytes*</th>
<th>ALT*</th>
<th>AST*</th>
<th>Parasitemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chin et al.4</td>
<td>USA, 1965</td>
<td>40.4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>20</td>
<td>850/μl</td>
</tr>
<tr>
<td>Bronner et al.29</td>
<td>Sweden, 2006</td>
<td>40.0</td>
<td>2.2 x 10^9/l</td>
<td>34 x 10^9/l</td>
<td>1.68</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Kantele et al.10</td>
<td>Finland, 2007</td>
<td>38.8</td>
<td>2.6 x 10^9/l</td>
<td>143 x 10^9/l</td>
<td>NA</td>
<td>NA</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>CDC31</td>
<td>USA, 2008</td>
<td>NA</td>
<td>NA</td>
<td>Thrombocytopenia not specified</td>
<td>NA</td>
<td>NA</td>
<td>2.9%</td>
</tr>
<tr>
<td>Ta et al.32</td>
<td>Spain, 2008/2009</td>
<td>40.0</td>
<td>3.82 x 10^9/l</td>
<td>86 x 10^9/l</td>
<td>93 U/l</td>
<td>43 U/l</td>
<td>0.003%</td>
</tr>
<tr>
<td>van Hellmond et al.33</td>
<td>Netherlands, 2009</td>
<td>40.0</td>
<td>5.8 x 10^9/l</td>
<td>22 x 10^9/l</td>
<td>199 U/l</td>
<td>128 U/l</td>
<td>2%</td>
</tr>
<tr>
<td>Figgert et al.34</td>
<td>Australia, 2010</td>
<td>38.9</td>
<td>3.7 x 10^9/l</td>
<td>106 x 10^9/l</td>
<td>NA</td>
<td>NA</td>
<td>185/μl</td>
</tr>
<tr>
<td>Hoosen and Shaw35</td>
<td>New Zealand, 2010</td>
<td>39.1</td>
<td>4.3 x 10^9/l</td>
<td>71 x 10^9/l</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Berry et al.36</td>
<td>France, 2010</td>
<td>NA</td>
<td>Normal</td>
<td>73 x 10^9/l</td>
<td>75 U/l</td>
<td>58 U/l</td>
<td>0.8%</td>
</tr>
<tr>
<td>Link et al.37</td>
<td>Netherlands, 2011</td>
<td>40.2</td>
<td>3.5 x 10^9/l</td>
<td>72 x 10^9/l</td>
<td>80 U/l</td>
<td>76 U/l</td>
<td>0.0005%</td>
</tr>
<tr>
<td>Ehrhardt et al.38</td>
<td>Germany, 2013</td>
<td>40.0</td>
<td>4.1 x 10^9/l</td>
<td>197 x 10^9/l</td>
<td>NA</td>
<td>NA</td>
<td>0.01%</td>
</tr>
<tr>
<td>Orth et al.39</td>
<td>Germany, 2013</td>
<td>NA</td>
<td>7.36 x 10^9/l</td>
<td>27 x 10^9/l</td>
<td>277 U/l</td>
<td>237 U/l</td>
<td>0.2%</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; AST, aspartate aminotransferase; NA, not available.

* Value lower than locally used reference value; † value higher than locally used reference value.

The year of infection was unclear, so the year of publication has been used.
### Table 3
Diagnostic methods used to investigate *Plasmodium knowlesi* infections in intercontinental travellers, and the results

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Case</th>
<th>Microscopy</th>
<th>RDTs</th>
<th>Nested PCR</th>
<th>Real-time PCR</th>
<th>Other or not nearer specified PCRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chin et al.</td>
<td>USA, 1965</td>
<td>Positive for <em>P. falciparum</em> (1&lt;sup&gt;st&lt;/sup&gt; examination) Positive for <em>P. malariae</em> (2&lt;sup&gt;nd&lt;/sup&gt; examination) Later positive for <em>P. knowlesi</em>&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bronner et al.</td>
<td>Sweden, 2006</td>
<td>Suspicion for <em>P. malariae</em></td>
<td>Negative (BinaxNOW&lt;sup&gt;29&lt;/sup&gt;)</td>
<td>Positive for <em>P. knowlesi</em> (nested PCR and sequencing of the SSU rRNA)</td>
<td>-</td>
<td>Negative (PCR specific for <em>P. falciparum</em>, <em>P. vivax</em>, <em>P. ovale</em>, <em>P. malariae</em>)</td>
</tr>
<tr>
<td>Kantele et al.</td>
<td>Finland, 2007</td>
<td>Positive for <em>P. falciparum</em> (1&lt;sup&gt;st&lt;/sup&gt; examination) Positive for co-infection of <em>P. falciparum</em> and <em>P. malariae</em> (2&lt;sup&gt;nd&lt;/sup&gt; examination)</td>
<td>-</td>
<td>Negative (nested PCR with rOva1 and rPLU2 primers) Positive for <em>P. knowlesi</em> (nested PCR and sequencing with rPLU6 and rPLU2 primers)</td>
<td>-</td>
<td>Negative for: <em>P. malariae</em>, <em>P. falciparum</em>, <em>P. ovale</em>, <em>P. vivax</em> (conventional PCR targeting the SSU rRNA) Positive for <em>P. knowlesi</em> (conventional PCR with primer specific for the SSU rDNA of <em>Plasmodium</em> genus and sequencing) Positive for <em>P. knowlesi</em> (multiplex real-time PCR specific for <em>Plasmodium</em> genus, <em>P. falciparum</em>, <em>P. vivax</em> and sequencing)</td>
</tr>
<tr>
<td>CDC&lt;sup&gt;31&lt;/sup&gt;</td>
<td>USA, 2008</td>
<td>Positive for unspecific malaria</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ta et al.</td>
<td>Spain, 2008/2009</td>
<td>Positive for unspecific malaria</td>
<td>Negative for HRP2 Negative for pan-malarial aldolase (BinaxNOW&lt;sup&gt;32&lt;/sup&gt;)</td>
<td>-</td>
<td>Positive for <em>P. knowlesi</em> (real-time PCR with sequencing of the SSU rRNA)</td>
<td>-</td>
</tr>
<tr>
<td>van Hellmond et al.</td>
<td>Netherlands, 2009</td>
<td>Positive for unspecific malaria</td>
<td>Negative for HRP2 Positive for pan-malarial aldolase (BinaxNOW&lt;sup&gt;33&lt;/sup&gt;)</td>
<td>Negative for <em>P. malariae</em>, <em>P. falciparum</em>, <em>P. ovale</em>, <em>P. vivax</em> (nested PCR) Positive for <em>P. knowlesi</em> (nested PCR with rPLU1 and rPLU5 primers, species-specific primers targeting the SSU rRNA and sequencing)</td>
<td>Negative for <em>P. malariae</em>, <em>P. falciparum</em>, <em>P. ovale</em>, <em>P. vivax</em> (real-time PCR)</td>
<td>-</td>
</tr>
<tr>
<td>Figtree et al.</td>
<td>Australia, 2010&lt;sup&gt;34&lt;/sup&gt;</td>
<td>Positive for <em>P. malariae</em> or <em>P. falciparum</em></td>
<td>Negative for HRP2</td>
<td>-</td>
<td>-</td>
<td>Negative for <em>P. malariae</em>, <em>P. falciparum</em>, <em>P. ovale</em>, <em>P. vivax</em> (multiplex PCR) Positive for <em>P. knowlesi</em> (PCR and sequencing)</td>
</tr>
<tr>
<td>Hoosen and Shaw</td>
<td>New Zealand, 2010</td>
<td>Negative (1&lt;sup&gt;st&lt;/sup&gt; and 2&lt;sup&gt;nd&lt;/sup&gt; examination) Suspected <em>P. malariae</em> (3&lt;sup&gt;rd&lt;/sup&gt; examination)</td>
<td>Negative for <em>P. falciparum</em> and other <em>Plasmodium</em> antigen</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Berry et al.</td>
<td>France, 2010</td>
<td>Positive for mixed <em>P. malariae</em> and <em>P. falciparum</em> infection (1&lt;sup&gt;st&lt;/sup&gt; and 2&lt;sup&gt;nd&lt;/sup&gt; examinations)</td>
<td>Positive for <em>P. vivax</em>-specific LDH Positive for pan-<em>Plasmodium</em> LDH Negative for HRP2 (Core Malaria Pan/Pv/Pf test&lt;sup&gt;25&lt;/sup&gt;) Positive for pan-<em>Plasmodium</em> aldolase Negative for HRP2 (BinaxNOW&lt;sup&gt;26&lt;/sup&gt;) Positive for <em>P. vivax</em>-specific LDH Positive for pan-<em>Plasmodium</em> LDH Negative for HRP2 (Palutop test&lt;sup&gt;27&lt;/sup&gt;)</td>
<td>-</td>
<td>-</td>
<td>Positive for malaria Negative for <em>P. falciparum</em> (multiplex real-time PCR) Positive for <em>P. vivax</em> Negative for: <em>P. ovale</em>, <em>P. malariae</em> (real-time PCR specific for <em>P. malariae</em>, <em>P. vivax</em>, <em>P. ovale</em>) Positive for <em>P. knowlesi</em> (sequencing)</td>
</tr>
</tbody>
</table>
**Table 3 (Continued)**

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Case</th>
<th>Microscopy</th>
<th>RDTs</th>
<th>Nested PCR</th>
<th>Real-time PCR</th>
<th>Other or not nearer specified PCRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Link et al.</td>
<td>Netherlands, 2011</td>
<td>Negative (1st examination); Positive for P. malariae (2nd examination)</td>
<td>Negative (BinaxNOW&lt;sup&gt;®&lt;/sup&gt;)</td>
<td>-</td>
<td>Negative (real-time PCR specific for P. falciparum, P. vivax, P. malariae, P. ovale)</td>
<td>-</td>
</tr>
<tr>
<td>Ehrhardt et al.</td>
<td>Germany, 2013</td>
<td>Positive for mixed P. malariae and P. falciparum infection</td>
<td>-</td>
<td>-</td>
<td>Positive for P. knowlesi (PCR and sequencing)</td>
<td>-</td>
</tr>
<tr>
<td>Orth et al.</td>
<td>Germany, 2013</td>
<td>Positive for P. malariae</td>
<td>Negative for HRP2 (BinaxNOW&lt;sup&gt;®&lt;/sup&gt;); Positive for Pan-Plasmodium aldolase (BinaxNOW&lt;sup&gt;®&lt;/sup&gt;)</td>
<td>-</td>
<td>Positive for P. knowlesi (PCR and sequencing)</td>
<td>-</td>
</tr>
</tbody>
</table>

RDT, rapid diagnostic test; SSU, small subunit; LDH, lactate dehydrogenase; HRP2, P. falciparum-specific histidine-rich protein 2.

<sup>a</sup> Later positive for P. knowlesi examination of blood inoculated in rhesus monkeys and gibbons.

<sup>b</sup> BinaxNOW Malaria Test, Binax Inc., Maine, USA.

<sup>c</sup> The year of infection was unclear, so the year of publication has been used.

<sup>d</sup> Core Malaria Pan/Pv/Pf test, Core Diagnostics, Birmingham, UK.

<sup>e</sup> BinaxNOW Malaria Test, Iverness Medical, Sevres, France.

<sup>f</sup> Palatop Test, All Diag, Strasbourg, France.

Since Malaysian coastal and urban areas have been declared malaria-free zones by the WHO, travellers at potentially high risk are those undertaking trips and trekking tours in forested or rural areas in the deep hinterland. A lower risk of P. knowlesi infection is reported from Thailand, but certain forested areas are considered as malaria zones. The recent report of a French man acquiring a P. knowlesi infection probably on a tourist beach on the Island of Ko Payam, Thailand, indicates a risk in coastal areas as well, particularly if they are situated near a forest.

People travelling to known risk areas for P. knowlesi should use malaria vector protection measures, such as insect repellents, mosquito nets, mosquito coils, aerosol sprays, and protective clothes. Until more specific research contradicts this doctrine, currently available chemoprophylaxis medication is effective against P. knowlesi. A single case of possible resistance to mefloquine has been reported in a Malaysian businessman, as mentioned above. Patients presenting in non-endemic countries with malaria-typical symptoms, such as fever, headache, malaise, and...
anorexia, myalgia, cough, nausea, abdominal pain, or vomiting, and a history of travel to Malaysia, Myanmar, Thailand, Vietnam, or the state of Palawan Philippines in the last 2 weeks, should be investigated for a potential *P. knowlesi* infection.29–39 PCR investigated

The high rate of misdiagnosis of *P. knowlesi* infection as *P. falciparum* or *P. malariae* by light microscopy in non-endemic countries (Table 3) should lead to a new evaluation of diagnostic techniques,29–39 PCR shows a high specificity and sensitivity, but due to the complex process and high costs is not ideal.34,43 LAMP, as described by Iseki et al. and Lau et al., could be a possible faster and cheaper diagnostic tool, but further studies should be done to evaluate the efficiency of this procedure.49,53 A recent publication showed a low sensitivity of currently available RDTs in detecting *P. knowlesi*. The development of more sensitive tests would also bring the advantage of cheaper and faster handling under ambulatory conditions.42 The latest research on protein structures as markers for the detection of *P. knowlesi*, indicates promising progress in this field.62 Until ideal commercial diagnostic tests are developed, it is

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Drug treatment of <em>Plasmodium knowlesi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ref.</td>
<td>Drug</td>
</tr>
<tr>
<td>Singh et al.</td>
<td></td>
</tr>
<tr>
<td>Daneshvar et al.6</td>
<td>Chloroquine, primaquine</td>
</tr>
<tr>
<td>Daneshvar et al.54</td>
<td>Chloroquine, primaquine</td>
</tr>
<tr>
<td>Luchavez et al.31</td>
<td>Chloroquine, primaquine</td>
</tr>
<tr>
<td>Chin et al.4</td>
<td>Chloroquine, primaquine</td>
</tr>
<tr>
<td>Barber et al.8</td>
<td>Chloroquine, primaquine</td>
</tr>
<tr>
<td>Jongwutiwes et al.15</td>
<td>Chloroquine</td>
</tr>
<tr>
<td>Ng et al.21</td>
<td>Chloroquine</td>
</tr>
<tr>
<td>Berry et al.36</td>
<td>Chloroquine</td>
</tr>
<tr>
<td>van Hellmond et al.33</td>
<td>Chloroquine</td>
</tr>
<tr>
<td>Lee et al.66</td>
<td>Chloroquine</td>
</tr>
<tr>
<td>Singh et al.5</td>
<td>Quinine</td>
</tr>
<tr>
<td>Daneshvar et al.6</td>
<td>Quinine</td>
</tr>
<tr>
<td>Daneshvar et al.6</td>
<td>Quinine</td>
</tr>
<tr>
<td>Cox-Singh et al.7</td>
<td>Quinine</td>
</tr>
<tr>
<td>Bronner et al.24</td>
<td>Mefloquine</td>
</tr>
<tr>
<td>Tanizaki et al.41</td>
<td>Mefloquine (resistance)</td>
</tr>
<tr>
<td>Later: quinine, doxycycline</td>
<td>-</td>
</tr>
<tr>
<td>Later: quinine, artemether, lumefantrine</td>
<td>-</td>
</tr>
<tr>
<td>Lau et al.55</td>
<td>Quinine</td>
</tr>
<tr>
<td>Lee et al.66</td>
<td>Doxycycline</td>
</tr>
<tr>
<td>Later: primaquine</td>
<td>-</td>
</tr>
<tr>
<td>Lee et al.66</td>
<td>Quinine, doxycycline</td>
</tr>
<tr>
<td>Later: chloroquine</td>
<td>-</td>
</tr>
<tr>
<td>Barber et al.42</td>
<td>Artemether, lumefantrine</td>
</tr>
<tr>
<td>Barber et al.42</td>
<td>Artesunate, mefloquine</td>
</tr>
<tr>
<td>Barber et al.42</td>
<td>Artemether, lumefantrine, artesunate (intravenous)</td>
</tr>
<tr>
<td>Orth et al.39</td>
<td>Artesunate (intravenous)</td>
</tr>
<tr>
<td>Later: artemether, lumefantrine</td>
<td>-</td>
</tr>
<tr>
<td>Fitgeree et al.34</td>
<td>Atovaquone, proguanil</td>
</tr>
<tr>
<td>Link et al.37</td>
<td>Atovaquone, proguanil</td>
</tr>
<tr>
<td>Elhardt et al.31</td>
<td>Atovaquone, proguanil</td>
</tr>
<tr>
<td>Hoosen and Shaw15</td>
<td>Atovaquone, proguanil</td>
</tr>
<tr>
<td>Later: artemether, lumefantrine</td>
<td>-</td>
</tr>
<tr>
<td>CDC11</td>
<td>Atovaquone, proguanil</td>
</tr>
<tr>
<td>Later: primaquine</td>
<td>-</td>
</tr>
<tr>
<td>Singh et al.3</td>
<td>Chloroquine, primaquine, sulfadoxine, pyrimethamine</td>
</tr>
<tr>
<td>Cox-Singh et al.7</td>
<td>Chloroquine, sulfadoxine, pyrimethamine</td>
</tr>
<tr>
<td>Cox-Singh et al.7</td>
<td>Chloroquine, sulfadoxine, pyrimethamine</td>
</tr>
</tbody>
</table>
important to use the established diagnostic tools in the most efficient way. This is especially important for infections with morphological structures similar to P. malariae on light microscopy in those with a history of travel to Malaysia and a low platelet count, who should be suspected of having a possible P. knowlesi infection. 2.8.9 (Table 4)

Immediate hospitalization and initiation of treatment are imperative due to the 24-h replication cycle and a potential severity of the fifth human malaria. 53–55 Indicators for a potential severe course have been described by Willmann et al. 43 and Barber et al., 56 as mentioned above. Patients showing signs of a severe knowlesi infection should be treated according to the guidelines for severe P. falciparum infections, with parenteral medica- tion. 32,60,63

Several treatment regimens are reported to be effective against P. knowlesi, including the chloroquine/primaquine combination. 54 Due to the fact that no hypnozoites have ever been described in P. knowlesi infection, primaquine is probably not required as a treatment except in cases of mixed infection with P. vivax. Further studies are needed to evaluate the optimal prevention and treatment of P. knowlesi, especially in view of increasing resistance to the concomitantly occurring P. falciparum in Southeast Asia. Recent studies have shown the efficacy of ACT, such as artemether–lumefantrine and artesunate–mefloquine, in non-severe P. knowlesi infections, as well as intravenous artesunate in severe cases. 32,64 These treatment regimens appear promising, particularly in view of the often multi-resistant, co-endemic P. falciparum and P. vivax infections. In addition, ACTs have a significantly faster parasite clearance compared to chloroquine. 64

4. Conclusions

P. knowlesi appears to be a widespread human malaria parasite in Malaysian Borneo 53,66. This paper illustrates that the fifth human parasite is not only a threat to residents, but also to occupational and holiday travellers. Despite the increasing prevalence in Malaysia, infection rates in travellers remain low, but the extent of misdiagnosed cases remains uncertain. A special risk group are travellers to forested and rural areas. At lower, but still underestimated risk of P. knowlesi infection, are travellers to Thailand, the Philippines, Indonesia, Myanmar, and Vietnam, and probably also Cambodia. Clinicians who provide pre-travel advice or who consult ill returning travellers should be aware of the risk. Adequate preventive measures, such as mosquito nets, insect repellents, and the wearing of protective clothing, should be recommended together with appropriate antimalarials. In travellers who present with malaria-like symptoms and a recent history of travel to Malaysia or neighbouring areas in Southeast Asia, a probable P. knowlesi infection has to be taken into consideration. If blood smears are positive for P. malariae, a P. knowlesi infection has to be strongly suspected, especially if accompanied by low thrombocyte counts. Due to the fast reproduction cycle and a potentially severe progression, patients who are suspected of having a P. knowlesi infection should be hospitalized and treated immediately.

Conflict of interest: The authors declare that they have no conflict of interest with this work.

References