Malaria zoonoses

J. Kevin Baird*

Eijkman-Oxford Clinical Research Unit, Jalan Diponegoro No. 69, Jakarta 10430, Indonesia and The Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, Oxford University, Oxford, United Kingdom

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Summary
The genus Plasmodium includes many species that naturally cause malaria among apes and monkeys. The 2004 discovery of people infected by Plasmodium knowlesi in Malaysian Borneo alerted to the potential for non-human species of plasmodia to cause human morbidity and mortality. Subsequent work revealed what appears to be a surprisingly high risk of infection and relatively severe disease, including among travelers to Southeast Asia. The biology and medicine of this zoonosis is reviewed here, along with an examination of the spectrum of Plasmodium species that may cause infection of humans. © 2009 Elsevier Ltd. All rights reserved.

Introduction
Endemic zoonoses represent an important segment of the range of health risks faced by travelers. This area of risk has not included parasites of the genus Plasmodium that cause malaria. No animal reservoir for these species was known and human infection by non-human species of plasmodia was limited to exceedingly rare case reports or experimental settings. Indeed, in the 1960s a comprehensive search for these zoonoses in the most likely setting (Southeast Asian jungle) produced entirely negative results. Malarialogists since then considered important malaria zoonoses improbable, despite an extraordinary confirmed case in 1965. The seminal 2004 report by Singh et al. documented routine infection of humans in Malaysian Borneo by Plasmodium knowlesi, a parasite naturally occurring among several species of macaques in Southeast Asia. This review will describe the subsequent corroborating reports affirming the problem, along with biological, geographic, clinical, and diagnostic aspects of the infection of importance to travel medicine.

The review looks beyond the specific case of P. knowlesi and describes a broader scope that includes other species of plasmodia that may also be likely to occur in humans. The incrimination of P. knowlesi as an important zoonosis ultimately rests upon a chain of unlikely events in the early 1960s characterized by Bruce-Chwatt as, “one of the most extraordinary concatenation of circumstances that reads like a detective story”. That case reported by Chin et al. and fully recounted by Coatney et al. in their 1971 monograph documented the first known case of naturally acquired simian malaria in humans. Forty years later, knowledge of that singular case brought focus to P. knowlesi in the renewed investigation applying the tools of molecular biology. The absence of confirmed naturally acquired infections among other simian species of plasmodia should not discourage consideration and investigation of them as zoonoses. This review examines underlying host–parasite biology among

* Eijkman-Oxford Clinical Research Unit, Jalan Diponegoro No. 69, Jakarta 10430, Indonesia. Tel.: +62 21 391 0414; fax: +62 21 3190 5016.
E-mail address: kbaird@eocru.org
the primate malarias in order to identify other *Plasmodium* species that would appear most likely capable of causing zoonoses. That analysis points to three species: *P. knowlesi*, *Plasmodium cynomolgi* and *Plasmodium inui*, all parasites of Southeast Asian macaques.

Illness linked to travel often prompts thorough diagnostic work up, and the opportunity for definitive diagnosis hinges upon inclusion of the real agent in the differential. This review explains the rationale for considering all four of the human malarias, along with *P. knowlesi*, *P. cynomolgi*, and *P. inui* in patients with a history of travel to Southeast Asia and a microscopic diagnosis of malaria.

**Microscopic diagnosis of the malarias**

Most physicians, laboratorians, and even scientists specializing in the study of malaria, have little appreciation of the morphological subtleties that distinguish species of plasmodia. Parasite morphology is only useful within defined limits of application, as in distinguishing the species of parasites that normally infect a particular host species. In other words, the morphological characteristics that reliably segregate *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium ovale* in humans do not constitute a basis for specific diagnosis across the genus *Plasmodium*.

Groups of species within the genus *Plasmodium* that infect primates exhibit affinities of lineage, behavior and, to a lesser extent, morphology. These groups, by virtue of our human bias, center on the species that infect us: falciparum type, vivax type, ovale type, and malariae type (Table 1). These types are grouped according to the periodicity of asexual maturation in blood, i.e., quotidian, tertian, and quartan. *P. knowlesi* stands in a class alone because it is the only primate malaria species with a quotidian (24 h) asexual blood stage development (merogonic) cycle. A group of poorly understood species, all infecting lemurs, are known only by stained blood films.

The grouping by types should not be confused with a guide to affinities strictly in the sense of appearances under the microscope. The immature trophozoites of *P. knowlesi*, for example, can look a great deal like those of *P. falciparum*, whereas older trophozoites and schizonts most closely resemble those of *P. malariae*. Any given species from any group infecting a human would appear essentially similar to one or more of the species normally found in humans. No pathognomonic forms affirm alien identity of an unmistakable plasmodia. Even an expert would have great difficulty making a specific microscopic diagnosis of a species occurring in an unnatural or incidental host regardless of species involved.

A practicing clinical microscopist will usually be called upon to make a specific diagnosis of malaria from a Giemsa-stained blood film. These practitioners would have been trained to distinguish the four species of plasmodia normally occurring in humans. Until recently, that was the universe of possibilities. In this calculus, a parasite that looks like neither *P. falciparum* nor *P. vivax* (e.g., mature trophozoites in normal-sized red blood cells) will very likely be *P. malariae*, and the distinction between *P. vivax* and *P. ovale* is quite subtle.

In the absence of a compelling suspicion of a zoonosis, any plasmodia infecting humans would very likely be diagnosed as one of the ordinary human species. This perspective largely explains a very significant and important aspect of human malaria being overlooked for

### Table 1 Affinities among the primate malarias.

<table>
<thead>
<tr>
<th>TERTIAN MALARIAS</th>
<th>Ovale type</th>
<th>Falciparum type</th>
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<tbody>
<tr>
<td><em>Plasmodium vivax</em></td>
<td><em>Plasmodium ovale</em></td>
<td><em>Plasmodium falciparum</em></td>
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<tr>
<td><em>Plasmodium cynomolgi</em></td>
<td><em>Plasmodium fieldi</em></td>
<td><em>Plasmodium coatneyi</em></td>
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<td><em>Plasmodium eylesi</em></td>
<td><em>Plasmodium simiovale</em></td>
<td><em>Plasmodium fragile</em></td>
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<td><em>Plasmodium gonderi</em></td>
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<td><em>Plasmodium reichenowi</em></td>
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<td><em>Plasmodium hylobati</em></td>
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<td><em>Plasmodium jefferyi</em></td>
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<td><em>Plasmodium pitheci</em></td>
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<td><em>Plasmodium silvaticum</em></td>
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<td><em>Plasmodium schwetzi</em></td>
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<td><em>Plasmodium simium</em></td>
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<td><em>Plasmodium youngi</em></td>
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<thead>
<tr>
<th>QUARTAN MALARIAS</th>
<th>QUOTIDIAN MALARIA</th>
<th>UNKNOWN MALARIAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malariae type</td>
<td>Knowlesi type</td>
<td>Inadequate information (all in lemurs)</td>
</tr>
<tr>
<td><em>Plasmodium malariae</em></td>
<td><em>Plasmodium knowlesi</em></td>
<td><em>Plasmodium girardi</em></td>
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<tr>
<td><em>Plasmodium inui</em></td>
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<td><em>Plasmodium lemuris</em></td>
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<td><em>Plasmodium rodhaini</em></td>
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<td><em>Plasmodium foleyi</em></td>
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<td><em>Plasmodium brasilianum</em></td>
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<td><em>Plasmodium coulangesi</em></td>
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<td><em>Plasmodium percygharnhami</em></td>
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<td><em>Plasmodium uilenbergi</em></td>
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<td></td>
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<td><em>Plasmodium bucki</em></td>
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over 40 years: simian species of plasmodia routinely infect humans in Southeast Asia, causing severe illness and at least a few deaths. The diagnosis was missed because the microscope does not provide sufficient power of discernment. Genuinely distinct species do not present sufficient morphological diversity to allow their identification under the microscope. The discovery of aberrant species infecting humans awaited PCR technology, and, to date, that technological solution has been applied only to a single simian species of malaria, P. knowlesi. Other zoonoses by the plasmodia require similar exploration.

**Historical background**

In his classic review of malaria zoonoses, Bruce-Chwatt\(^4\) attributes the discovery of plasmodia in apes and monkeys to the famous German pathologist, Robert Koch. He visited Southeast Asia in 1899 and conducted seminal surveys of human populations in Java that led to his original description of naturally acquired immunity to human falciparum malaria.\(^8\) Koch’s affinity for microscopic survey of plasmodia, an innovation at the time, apparently included examination of the blood of apes and monkeys. Although Koch documented infections by plasmodia in primates, he did no taxonomic work and species descriptions would only come later from others. Alphonse Laveran, the discoverer of the protozoan agent of malaria, nonetheless acknowledged Koch’s work in this arena by later naming a species he described from baboons *Plasmodium kochi*.\(^4\) That parasite was later reclassified into the genus *Hepatocystis*.\(^9\)

Mayer’s\(^10\) 1907 description of *P. cynomolgi* from the long-tailed macaque (*Macaca fascicularis*; synonymous with *Macaca iris*, *Macaca cynomolgus*, crab-eating macaque, Kra macaque, and other common names) from Java triggered a series of similar discoveries among many other apes and monkeys. In the 1930s, Knowles and Das Gupta\(^15\) working in India isolated and described *P. knowlesi* from a long-tailed macaque from Malaysia. They documented the extreme virulence of *P. knowlesi* in Indian rhesus macaques (*Macaca mulatta*), and its relatively benign course in natural host species, *M. fascicularis* and *Macaca nemestrina*, the pig-tailed macaque. This fact later figures prominently in understanding the geographic distribution of *P. knowlesi* and the monkeys that harbor it. In 1932, Knowles and Das Gupta\(^11\) successfully infected humans with *P. knowlesi*. Sinton and Mulligan\(^12\) described the species that same year. This parasite was then almost immediately used as a therapeutic agent against neurosyphilis, especially in Romanian sanatoriums.\(^5\) Its ability to consistently provoke relatively high fevers appealed to the therapeutic logic of the inoculation.

Work on *P. cynomolgi* and *P. knowlesi* in the coming decades firmly established the fact that these non-human parasites could infect humans, which, in turn, could infect anopheline mosquitoes, and finally infect humans or the original monkey species host.\(^13,14\) Among plasmodia, this represents an extraordinary degree of host versatility. Most plasmodia exhibit poor tolerance of an unnatural host or, as in the case of *P. knowlesi* in rhesus macaques, the host exhibits poor tolerance of the parasite. The parasite in an alien host more typically achieves only brief parasitemia and a fleeting, if any, infectiousness to mosquitoes. The demonstration of monkey—mosquito—human—mosquito—monkey transmission of these two species served as a warning regarding animal reservoirs for malaria in humans.

A rash of laboratory-acquired infections by *P. cynomolgi* occurred in the early 1960s in the United States.\(^5,15,16\) These cases alerted malariologists to the possibility of malaria perhaps causing zoonoses of public health importance within their natural ranges. This realization prompted a heroic effort to document endemic zoonoses in peninsular Malaysia. In the 1960s, a team from the U.S. National Institutes of Health working with counterparts at the Malaysian Institute of Medical Research surveyed human populations living in proximity to monkeys for evidence of infection. Blood from 1117 people was inoculated into rhesus monkeys.\(^1\) The results were entirely negative, and the issue of simian malaria as a potentially important zoonosis fell away from serious investigation for nearly 50 years. The expeditionary work nonetheless produced a body of work that greatly expanded understanding of the biology and ecology of the plasmodia that infect simians of Southeast Asia, including the discovery and descriptions of several new species.

The accidental infections of humans by *P. cynomolgi* also prompted clinical investigations in the United States and Great Britain. Humans were demonstrated to be very suitable as hosts for this parasite, with high rates of challenge success, relatively brief incubation periods, relatively high parasitemias persisting for a week or more, and readily infecting mosquitoes that in turn readily infected monkeys.\(^3\) *P. cynomolgi* thus seemed biologically positioned to cause zoonotic infections. However, it would be *P. knowlesi* that would first be demonstrated as doing so, and the history of that finding bears directly upon the discussion of risk of zoonosis by species of plasmodia infecting animals.

Coatney et al.\(^7\) fully describe the first confirmed naturally acquired infection of a human by a species normally found in monkeys.\(^2\) The patient appears to have been an intelligence person working on behalf of the U.S. government in the then-restive peninsular Malaysia of 1965. The 37-year-old man spent 5 days in the forested area of Bukit Kertau, during which time he reported being alone, working only at night and sleeping during the day. After transiting Kuala Lumpur and while awaiting transport back to the USA in Bangkok, he became acutely ill. Although the patient had chloroquine treatment in his possession, he hesitated taking the medication without clinical evaluation. Upon arrival in Texas, a U.S. Air Force physician saw him and provided treatment for an upper respiratory infection. The next day he arrived at his home in suburban Maryland outside of Washington, DC, still very ill. His private physician examined his blood and found what he thought were parasites that cause malaria. The physician referred him for treatment to the U.S. Army’s Walter Reed Hospital, but as Saturday was not an admitting day at that hospital he was asked to hold the patient until Monday. The doctor instead turned to the Clinical Center at the National Institutes of Health in Bethesda, Maryland, where the physician on duty took an interest and accepted the patient for admission. The microscopic diagnosis in the clinic laboratory was *P. malariae*, and NIH malariologists had made it known to clinical staff that they were keenly interested in obtaining
The parasite is Southeast Asian, although it is known as far north as Taiwan. It occurs as far east as Sulawesi in the Indonesian archipelago, south to Java, and as far west as Myanmar and the far eastern edge of India (Fig. 1).

Vertebrate hosts

The long-tailed macaque (M. fascicularis) is one of several natural hosts for P. knowlesi. Others include the pig-tailed macaque (M. nemestrina) and some leaf monkeys (e.g., Presbytis melalophos25). In Taiwan, Macaca cyclopis harbored P. knowlesi.5 In these natural monkey hosts, P. knowlesi typically causes a chronic low-grade parasitemia and only mild, transient disease. However, when Philippine strains of parasite infected Malaysian M. fascicularis, a more fulminant infection occurred.26 The parasite has not been found in M. mulatta (rhesus monkey) in the wild, probably because P. knowlesi in rhesus monkey produces a fulminant and almost invariably fatal infection. The western border of anopheline species that efficiently carries P. knowlesi (see later) also represents the southeastern limit to the range of rhesus monkeys. P. knowlesi may have stood as a barrier to expansion of larger and more aggressive M. mulatta into Southeast Asia.

Anopheline vectors

Wharton and Eyles27 demonstrated Anopheles hackeri to be a natural vector of P. knowlesi in coastal peninsular Malaysia. Experimental work that excluded A. hackeri, however, demonstrated Anopheles balabacensis to be the most receptive anopheline species for this parasite.5 Earlier, the incrimination of A. hackeri as a natural vector argued against P. knowlesi as a problem for humans because that mosquito occurs almost exclusively in jungle canopy. A. balabacensis, on the other hand, is an important vector species for human malaria in much of Southeast Asia. Both species are members of the Leucosphyrus group of anophelines (19 distinct species in three subgroups; Leucosphyrus being the most important and containing two species complexes: Anopheles leucosphyrus and Anopheles dirus28). These mosquito species are restricted to South or Southeast Asia and predominantly adapted to jungle habitat. Table 2 lists the organization of the species of this group. Vythilingam et al.23 recently implicated Anopheles cracens as the principal vector of P. knowlesi in Kuala Lipis, Pahang in peninsular Malaysia. That species readily feeds on humans, as does Anopheles latens in Sarawak in Malaysian Borneo,
and this mosquito has been incriminated as a \textit{P. knowlesi} vector species.\textsuperscript{29} Little doubt remains about the suitability of at least several species of anopheline mosquitoes to feed upon both monkeys and humans.

\textbf{Infection in natural hosts}

Naturally infected monkeys maintain asymptomatic, low-grade parasitemias. Prevalence in natural host populations has not been adequately surveyed, but published values range from 0\% to 8\%.\textsuperscript{23,30,31} The infection follows a quotidian cycle, the only simian malaria that does so. The parasite does not possess a latent liver stage. In natural hosts and in man, the early asexual stages resemble \textit{P. falciparum}: both appear in large numbers, as ring forms, and as applique forms. Double nuclei may also appear, often with one or more accessory chromatin dots. More mature parasites may appear as band forms typically linked to \textit{P. malariae} and the infected red blood cell may show stippling. Fully mature schizonts fill the host red blood cell but do not enlarge it. A single mass of whitish-black pigment collects in the center of the schizont typically possessing 10 (as many as 16) merozoites. The organization, size, and number of merozoites of the schizont closely resemble \textit{P. malariae}. Gametocytes completely fill and slightly enlarge the infected red blood cell.\textsuperscript{5}

\textbf{Infection of humans}

In its earliest asexual development, \textit{P. knowlesi} in humans most resembles \textit{P. falciparum}. As the infection matures, the asexual parasites present forms that resemble \textit{P. malariae}. Mature trophozoites do not enlarge red blood cells and sometimes appear as somewhat characteristic band forms. Schizonts appear and fail to enlarge the host cell. Schizonts may present a distinctive "daisy head" appearance (characteristic of \textit{P. malariae}) with 8–10 merozoites arranged more or less symmetrically around the large central clump of pigment. Cox-Singh et al.\textsuperscript{19} found 11 cases of \textit{P. knowlesi} among 216 (5\%) cases diagnosed as \textit{P. falciparum}, but among 312 infections diagnosed as \textit{P. malariae}, 216 (6\%) were \textit{P. knowlesi}. A patient infected by \textit{P. knowlesi} certainly appears most likely, but not exclusively, to be diagnosed as infected by \textit{P. malariae}.

Clinicians who have managed human cases of \textit{P. knowlesi} in Singapore (Ref. 21 and O.T. Ng, personal communication) describe recognizing the severity of illness as inconsistent with the laboratory diagnosis of usually mild malariae malaria. In the Singapore cases, relatively severe illness with a laboratory diagnosis of \textit{P. malariae} prompted the use of molecular biological diagnostic techniques.

The course of infection by \textit{P. knowlesi} in humans has been extensively studied in experimental and therapeutic settings. Important strain-specific distinctions, variable virulence with repeated passage through humans, and acquired immunity all complicate understanding those data in a context of relevance to naturally acquired infections. When Milam and Kusch\textsuperscript{32} challenged 29 Caucasians, initial fevers ran to 102°F but later fevers peaked between 104°F and 105.8°F. These appeared daily for 10 days before finally diminishing spontaneously. The highest parasitemia levels were rarely above 100/10,000 red blood cells (<1\%), although one patient showed 12\% parasitemia. Those workers found people of African descent difficult to infect: four of six challenged developed only very mild disease and the two others none at all. \textit{P. knowlesi} shares very similar Duffy-like receptors as occurs in \textit{P. vivax} (its closest relative), and thus the Duffy negative phenotype very probably protects humans against infection by \textit{P. knowlesi}. In eight sporozoite-induced infections of humans,\textsuperscript{13} the pre-patent period was 9–12 days. Peak parasitemia occurred on the 8th day of patency and declined sharply thereafter. In this series, the peak temperature was 104.8°F and 20,850/\textmu L was the peak parasitemia (median about 1000/\textmu L).

\textbf{Disease states in humans}

Coatney et al.\textsuperscript{5} in describing their experience with 20 human volunteers challenged with \textit{P. knowlesi}, characterized the infection in humans as "...moderate to severe with attacks terminating spontaneously after two weeks". Their experience with a single strain (the one isolated from the mysterious American working in Malaysia) may not reliably capture the range of experience possible in naturally acquired infections. Cox-Singh et al.\textsuperscript{19} detail four fatal outcomes in humans apparently caused by \textit{P. knowlesi}. All of those cases had hyperparasitemia (75,000 to 764,720/\textmu L) and hepatic or renal failure, metabolic acidosis, or respiratory distress. Reported cases typically present with moderate to severe disease states, with body temperature typically near or above 40°C, elevated C-reactive protein (up to 10-fold), thrombocytopenia, hyperbilirubinemia, and mild transaminitis. Parasite counts ranged from about 1000 to 8000/\mu L, and these few observations accord well with the experimental infections described by Coatney et al.\textsuperscript{5} The sum of this scanty evidence suggests that death caused by \textit{P. knowlesi} may be a very low probability outcome, but the demonstrated possibility of a pernicious and fatal course merits aggressive diagnostic and therapeutic management of travelers from Southeast Asia presenting with relatively severe disease caused by a parasite diagnosed microscopically as any species of \textit{Plasmodium}.

\begin{table}[h]
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\begin{tabular}{|l|l|}
\hline
\textbf{LEUCOSPHYRUS SUBGROUP} & \textbf{Anopheles dirus complex} \\
\textbf{Anopheles balabacensis}\textsuperscript{a} & Anopheles balmaii \\
\textbf{Anopheles introlatus}\textsuperscript{a} & Anopheles cracens\textsuperscript{a} \\
\textbf{Anopheles latens}\textsuperscript{a} & Anopheles dirus\textsuperscript{a} \\
\textbf{Anopheles leucosphyrus}\textsuperscript{a} & Anopheles elegans\textsuperscript{a} \\
& Anopheles nemophilus \\
& Anopheles scanloni \\
& Anopheles takasagoensis \\
\hline
\textbf{HACKERI SUBGROUP} & \textbf{RIPARIS SUBGROUP} \\
\textbf{Anopheles hackeri}\textsuperscript{a} & Anopheles cristatus \\
\textbf{Anopheles pujutensis} & Anopheles macarthuri \\
\textbf{Anopheles recens} & Anopheles riparis \\
\textbf{Anopheles sulawesi} & Negros form, Colless, 1956 \\
\hline
\end{tabular}
\caption{Leucosphyrus group of mosquitoes.}
\end{table}
Diagnosis in humans
The diagnostic rule of thumb applied by some clinicians in Singapore (O.T. Ng, personal communication) may suffice for a provisional diagnosis of knowlesi malaria, i.e., a judgment of relatively severe disease in a patient with recent travel to Southeast Asia and diagnosed microscopically as infected by *P. malariae* (Fig. 2). Indeed, any microscopic diagnosis of malaria acquired in Southeast Asia (Fig. 1) should alert to the possibility of infection by *P. knowlesi* and prompt investigation with molecular diagnostics.

One report describes diagnosis of *P. knowlesi* infection using a rapid antigen (plasmodia lactate dehydrogenase, pLDH) capture diagnostic kit. The kit uses distinct bands or combinations of bands to identify particular species, and *P. knowlesi* produced two bands, one shared by *P. vivax* and the other by *P. falciparum*. Unfortunately, this makes *P. knowlesi* infection diagnosed by this kit indistinguishable from mixed infections of *P. vivax* and *P. falciparum*, which routinely occur in endemic Southeast Asia.

The definitive diagnosis of human infection by *P. knowlesi* remains as relatively sophisticated molecular biological analyses. Most investigators have applied the oligonucleotides developed by Singh et al. [Ref. 3; PmK8 and Pmkr9] to amplify small subunit ribosomal ribonucleic acid (ssr RNA) gene by PCR reaction. These may be applied within a nested PCR assay format when screening large numbers of patients, but most investigators reporting single cases report sequencing of the PCR product and alignment with published ssr RNA sequences of *P. knowlesi*.

Malaria zoonoses
Considering the extraordinary circumstances leading to the discovery of *P. knowlesi* as a significant zoonosis, it may not be reasonable to presume that the species stands alone in sometimes infecting humans. Molecular biological examinations of human malarias that include other simian species would seem likely to reveal culprit species now unknown. The aim here is not to simply list species that could infect humans, but to inform the listing with assessment of biological plausibility along with real-world probability of human infection. The filters of plausibility and probability applied across the primate malarias substantially narrow the list of likely suspects for targeting with deliberate molecular investigations. Table 3 lists biological characteristics relevant to zoonoses across the primate malarias.

Only five species exhibit known characteristics compatible with the probability of causing zoonoses. One of those has been confirmed already, *P. knowlesi*, and two of them very likely represent not zoonoses threats, but anthroposphores, i.e., infection of animals with human agents of disease. *Plasmodium simium* and *Plasmodium brasilianum*, both strictly New World species, likely represent *P. vivax* and *P. malariae* acquired by New World monkeys after the human migrations from the Old World that occurred in the 16th through 18th centuries. Those parasites apparently transmit successfully within monkey populations.

The other two species showing biological characteristics compatible with high risk of causing zoonoses are *P. cynomolgi* and *P. inui*, both naturally infecting the same species of macaques of Southeast Asia as *P. knowlesi*. Both species are commonly found among relatively common monkeys and both accidental and experimental challenges have shown the parasites to do relatively well in humans. Both species also do well in anopheline species known to be important vectors of human malaria within the same geographic range. In natural host abundance, receptivity in a range of primate and anopheline species, and proximity to humans of both *P. cynomolgi* and *P. inui* seem well positioned for causing zoonoses. The level and duration of parasitemia in experimentally challenged humans was comparable to *P. knowlesi* with *P. cynomolgi*, and only slightly less so with *P. inui*. Patients traveling to Southeast Asia and presenting with patent parasitemia microscopically diagnosed as malaria should be further examined with molecular biological probes specific for the human malarias and for *P. knowlesi*, *P. cynomolgi*, and *P. inui*.

*Plasmodium fieldi* in the same geographic range and hosts may pose a risk, but too little is known of the behavior of this parasite in humans. In contrast, *Plasmodium coatneyi* also shares the same hosts and distributions of these species, but repeated attempts to infect humans with this

![Figure 2](https://example.com/figure2.png) Giemsa-stained thin blood film from a patient in Singapore showing mature trophozoites (left) and a schizont (right) proven to be *P. knowlesi* by molecular diagnostics (photo courtesy of Dr. O.T. Ng, Tan Tock Seng Hospital, Singapore).
<table>
<thead>
<tr>
<th>Table 3</th>
<th>Evaluation of primate malarias for risk of causing zoonoses.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home range</td>
<td>Natural hosts</td>
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<tr>
<td></td>
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<tr>
<td><em>Plasmodium cynomolgi</em></td>
<td>Southeast Asia</td>
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<td><em>Plasmodium hylobati</em></td>
<td>Southeast Asia</td>
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<td><em>Plasmodium schwetzi</em></td>
<td>Africa</td>
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<td><em>Plasmodium simium</em></td>
<td>South America</td>
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<td><em>Plasmodium knowlesi</em></td>
<td>Southeast Asia</td>
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</tbody>
</table>

<sup>a</sup> Likely anthroponoses.
<sup>b</sup> $M \rightarrow H =$ monkey to human; $H \rightarrow M =$ human to monkey.
species in a variety of settings produced no detectable parasitemia. This species may thus be considered a poor candidate for zoonotic infections.

*Plasmodium fragile* and *Plasmodium simiovale* occur among macaques in Sri Lanka and parts of India. Too little is known of the suitability of humans for these parasites to reliably assess their potential as zoonoses. This is also true of many of the species listed, but when the natural host species occurs in relatively very low numbers (chimpanzees, gorillas, orangutan, gibbons, lemurs, etc.) the risk to humans must be low. The obvious exceptions to these classifications would be people in close and routine contact with these exotic animals. *Plasmodium schwetzi* of chimpanzees, for example, appears to do especially well in humans, but this parasite may be another anthroponosis involving *P. vivax*. The plasmodia of orangutan, *Plasmodium pitheci* and *Plasmodium silvaticum*, readily infect *A. balabacensis*, an important human vector of malaria. These species, microscopically resembling *P. falciparum* and *P. vivax*, respectively, may thus be considered in the differential diagnosis in a patient who may have visited one of the relatively densely populated orangutan sanctuaries in Sumatra or Borneo.

**Malaria zoonoses, public health, and travel medicine**

The extent of risk to travelers or of the public health problem caused by simian plasmodia infecting humans cannot yet be reliably estimated. The issues remain inadequately explored, both with respect to adequacy of surveys of human populations at risk and the breadth of plasmodia species diversity effectively covered (i.e., with PCR reagents capable of detecting a broader range of species) in any survey. The data available in early 2009 cover only surveys of *P. knowlesi* in humans. Table 4 summarizes that work. Most samples have been from just several hundred patients scattered across sites with a microscopic diagnosis of *P. malariae*: positivity for *P. knowlesi* ranged from about 50% to 85%. Microscopic diagnosis of *P. malariae* may thus be considered a risk factor for infection by *P. knowlesi*, at least in peninsular Malaysia and Malaysian Borneo. This was also the only zone in that region reporting survey of patients admitted to hospital with a microscopic diagnosis of malaria, with 28% of those being infected by *P. knowlesi*. Should that contribution to the overall burden of hospitalized malaria be found more widely in the region, *P. knowlesi* would certainly be considered a very significant contributor to the overall burden of morbidity caused by any species of malaria. The available data, though scanty, certainly seem consistent with *P. knowlesi* being a significant risk for travelers to forested regions of Southeast Asia.

Many wide gaps in understanding the problem of malaria zoonoses hamper assessing these as problems in travel medicine and public health. The questions below highlight the key gaps.

- What is the risk of *P. knowlesi* among humans in known areas of transmission? No survey has yet measured true prevalence in human communities at risk. Surveys have been limited to special populations.
- Do other simian malarias infect humans in Southeast Asia? Molecular biological surveys of humans with malaria have so far dealt only with the four human species and *P. knowlesi*. Broader surveys are required to grasp the scale and reach of the zoonosis problem.
- What is the prevalence of *P. knowlesi*, *P. cynomolgi*, and *P. inui* among Southeast Asian macaques? The few surveys of simian populations for plasmodia do not permit forecast of corresponding risk in human communities. Risk of malaria zoonoses can only be supposed where the natural host monkey and mosquito both occur. The surveys should aim to define risk in distinct populations of monkeys, e.g., urbanized vs. forest, and correlation with risk of human malaria transmission.
- Can humans transmit simian malarias to other humans? The possibility of human-to-human transmission of *P. knowlesi* has yet to be addressed in the field. In the laboratory that transmission was achieved with little difficulty, with *P. cynomolgi* as well.5
- Can monkeys harbor human species of plasmodia and thus serve as reservoirs of human malaria? The

<table>
<thead>
<tr>
<th>Location</th>
<th>Sample</th>
<th>Sample population</th>
<th>Number examined</th>
<th>Number PCR-positive for <em>P. knowlesi</em></th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peninsular Malaysia</td>
<td>Filter paper</td>
<td>Diagnosis of <em>P. malariae</em></td>
<td>111</td>
<td>77 (69%)</td>
<td>23</td>
</tr>
<tr>
<td>Malaysian Borneo</td>
<td>Stained blood film</td>
<td>Admitted to hospital with microscopic diagnosis of malaria</td>
<td>960</td>
<td>266 (28%)</td>
<td>19</td>
</tr>
<tr>
<td>Peninsular Malaysia</td>
<td>Filter paper</td>
<td>Diagnosis of <em>P. malariae</em></td>
<td>49</td>
<td>41 (84%)</td>
<td>19</td>
</tr>
<tr>
<td>Palawan, Philippines</td>
<td>Stained blood film</td>
<td>Diagnosis of <em>P. malariae</em></td>
<td>208</td>
<td>120 (58%)</td>
<td>3</td>
</tr>
<tr>
<td>Malaysian Borneo</td>
<td>Stained blood film</td>
<td>Diagnosis of <em>P. malariae</em></td>
<td>11</td>
<td>5 (46%)</td>
<td>20</td>
</tr>
</tbody>
</table>
examples of *P. simium* and *P. brasilianum* (*P. vivax* and *P. malariae* anthropoones, respectively) in New World monkeys should alert to this possibility.

The work on *P. knowlesi* by Singh, Cox-Singh, and their colleagues in Malaysian Borneo jarred the malaria research community out of a long denial of malaria zoonoses. The problem occurs and work is underway to grasp its scale. As this picture comes into focus, travelers coming from Southeast Asia presenting with malaria diagnosed microscopically as any human species should be considered for a diagnostic work up that includes molecular probes of *P. knowlesi*, *P. cynomolgi*, and *P. inui*.

**Conflict of interest**

The author declares no conflict of interest.

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The author is supported in part by the Southeast Asian Malaria Zoonoses grant. The work on *P. knowlesi* by Singh, Cox-Singh, and their colleagues in Malaysian Borneo jarred the malaria research community out of a long denial of malaria zoonoses. The problem occurs and work is underway to grasp its scale. As this picture comes into focus, travelers coming from Southeast Asia presenting with malaria diagnosed microscopically as any human species should be considered for a diagnostic work up that includes molecular probes of *P. knowlesi*, *P. cynomolgi*, and *P. inui*.

**References**