Plasmodium knowlesi: Finally Being Recognized

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(See the article by Putaporntip et al., on pages 1143–50.)

In the 1960s, after the accidental laboratory acquisition by humans of infections with the simian malaria parasite *Plasmodium cynomolgi* via mosquito bite, there was a concerted effort to determine whether such infections are acquired under natural conditions of transmission [1, 2]. Soon thereafter, the natural transmission of a *Plasmodium knowlesi* infection was reported in a US traveler [3]. Subsequent volunteer studies conducted at the National Institutes of Health determined that humans were indeed susceptible to infection with the simian parasites *P. cynomolgi*, *Plasmodium inui*, and *P. knowlesi* via mosquito bite, but the infections were not considered life-threatening [4, 5]. At that time, definitive diagnosis of an infection with a simian parasite depended on the subpassage of blood from the infected human into a susceptible host, such as the rhesus monkey, *Macaca mulatta*, combined with detailed examination of Giemsa-stained blood films. Previously it had been shown that the human malaria parasites, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae*, would not infect Old World *M. mulatta* monkeys.

It was deemed possible that humans could be infected when they encroached on the areas where specific vectors and primates normally maintained the transmission cycle, with infected mosquitoes that normally preferred simians transmitting the monkey parasites to the human intruders. However, after the first report in 1965, no other confirmed reports of monkey malaria in humans appeared until the recent reports of hundreds of cases of *P. knowlesi* infection in humans [6, 7]. These reports, from Borneo, have been aided by the availability of molecular diagnostic tools to distinguish *P. knowlesi* from human malarial parasites—primarily *P. malariae* [6, 7]—and they have clearly demonstrated that infections of humans with monkey malaria parasites are far from rare in this area and may cause death in some cases. With the clearing of land for agricultural purposes, humans have increasingly entered the environment in which infected vectors can feed on them, transmitting parasites. *P. knowlesi* is transmitted by members of the *Anopheles leucosphyrus* group of mosquitoes. *Anopheles latens*, a major vector of human malaria in Borneo, is a member of this group. It feeds on humans and nonhuman primates and has the ability to transmit *P. knowlesi* [8]. Ecologically, the area is well suited for an interface among humans, the vector species, the malaria parasite, and the simian host: an ideal situation for the transmission of *P. knowlesi* to humans.

There have also been recent reports of human infection with *P. knowlesi* in other areas scattered across southeastern Asia, coinciding with the range of the *A. leucosphyrus* group of mosquitoes [9–12]. Although not all members of this large group have been proved to be vectors, none of the other mosquito groups of the region have been shown experimentally to be capable of transmitting *P. knowlesi* [9–12]. This issue of the Journal includes a report by Putaporntip et al. [13] on an extensive survey conducted in Thailand to compare microscopic and polymerase chain reaction diagnosis of malaria infection. Of the 10 patients found to be infected with *P. knowlesi*, 9 were also infected with *P. falciparum* or *P. vivax*. Seven of the 10 either kept monkeys as pets or had monkeys in the vicinity. Sequence analysis demonstrated variation in the small subunit ribosomal RNA gene of the *P. knowlesi* parasites, indicating that the transmission to humans in Thailand was not from a single parasite population. It appears that humans are susceptible to infection with *P. knowlesi* strains throughout the range of distribution of this parasite.

The conclusion from this and other studies is that humans can be infected anywhere within the range of distribution of the *A. leucosphyrus* complex of mosquitoes if infected monkeys are present. The distribution of *P. knowlesi* is probably defined by that of the *A. leucosphyrus* complex. Interestingly, *P. knowlesi* is lethal to *M. mulatta* monkeys, and it is strongly be-
lieved that *P. knowlesi* and the *A. leucosphyrus* vector have combined to limit the distribution of this highly aggressive and successful primate. The other monkey hosts for *P. knowlesi* in the area of this vector, such as *Macaca fascicularis*, *Macaca nemestrina*, and *Prebytes* species, are much more tolerant of or resistant to the parasite. In most instances, the vectors of this parasite are forest-dwelling, canopy-feeding mosquitoes. Workers and travelers who enter this environment are at risk of being fed on by infected mosquitoes.

Two other species of simian malaria parasites are known to infect humans: *P. cynomolgi* and *P. inui*. These are also readily transmitted by the same mosquitoes, the *A. leucosphyrus* group. Molecular studies have been conducted to look for infections with these 2 parasites. In the study by Putaporntip et al. [13], tests were conducted to identify only the 4 human species in addition to *P. knowlesi*. The question arises as to whether human-to-human transmission of *P. knowlesi* occurs. In human volunteer studies, each of these parasites has been shown experimentally to infect other mosquitoes. *P. knowlesi* is transmitted from human to human only by other members of the *A. leucosphyrus* group, such as *A. latens*, *A. leucosphyrus*, and *A. dirus*, all of which are basically forest dwellers. In contrast, *P. cynomolgi* and *P. inui* are infective to humans and can be transmitted between humans by these mosquitoes and many other vectors, such as the *Anopheles* species *A. maculatus*, *A. stephensi*, and *A. sundicus*, which occupy many different non-forest environments in which an infected person may live after leaving the forest.

There is little doubt that the incidence of this disease is lower outside Borneo, but further studies are needed to determine the real distribution of *P. knowlesi* and how frequently humans are infected. Improved rapid diagnostic techniques are needed to determine the scope of the problem. A recently described rapid diagnostic test using different anti-*Plasmodium* lactate dehydrogenase antibodies may have application in diagnosing infections with the 4 human species as well as infections with *P. knowlesi* [14]. However, further refinement will be needed, because *P. knowlesi* antibodies also cross-reacted with *P. falciparum*.

In experimental volunteer studies, the clinical responses in most patients infected with *P. knowlesi* or the other species of simian malaria were characterized by periods of high fever, but the infections usually persisted for only a few days or weeks [4]. Parasite counts were generally low, compared with those in simian hosts. Historically, serial passage of *P. knowlesi* in humans has resulted in increasingly higher parasite counts, until they reached life-threatening levels [15]. Many natural infections recently reported in humans also reached high parasite densities and were life-threatening, causing concerns about infections that go unrecognized or misidentified in populations in which the infection is endemic and in travelers. *P. knowlesi* malaria has been referred to as the fifth human malaria [16]. In fact, however, it is a simian malaria, whether or not it is occasionally (or, in some areas, more frequently) transmitted to humans. Until it is established that *P. knowlesi* is cyclically transmitted by mosquitoes from human to human, it should be considered a simian malaria and, hence, a zoonotic infection. If humans were removed from the scene, the parasite would still persist in the monkey population. However, *P. knowlesi* is a threat to human health and should be monitored closely.

**References**