
Phylogeographic Evidence for 2 Genetically Distinct Zoonotic *Plasmodium knowlesi* Parasites, Malaysia

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Infections of humans with the zoonotic simian malaria parasite *Plasmodium knowlesi* occur throughout Southeast Asia, although most cases have occurred in Malaysia, where *P. knowlesi* is now the dominant malaria species. This apparently skewed distribution prompted an investigation of the phylogeography of this parasite in 2 geographically separated regions of Malaysia, Peninsular Malaysia and Malaysian Borneo. We investigated samples collected from humans and macaques in these regions. Haplotype network analyses of sequences from 2 *P. knowlesi* genes, type A small subunit ribosomal 18S RNA and cytochrome c oxidase subunit I, showed 2 genetically distinct divergent clusters, 1 from each of the 2 regions of Malaysia. We propose that these parasites represent 2 distinct *P. knowlesi* types that independently became zoonotic. These types would have evolved after the sea-level rise at the end of the last ice age, which separated Malaysian Borneo from Peninsular Malaysia.

The number of malaria cases in Malaysia steadily decreased from a peak of 59,208 in 1995 to 3,850 confirmed cases in 2013; of these, 80% were reported in the 2 states of Malaysian Borneo and the remainder in 6 of the 11 states of Peninsular Malaysia (Figure 1) (1). In Malaysia, the simian malarial parasite species *Plasmodium knowlesi* is now the dominant species infecting humans and is >2 times more prevalent than *P. falciparum* or *P. vivax*.

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Humans were found to be susceptible to *P. knowlesi* when this species was experimentally transmitted to man in 1932, the year in which it was first described (2,3). In 1965, the first confirmed case of a naturally acquired infection in humans was recorded (4). The next naturally acquired confirmed cases were reported in 2004, when a stable focus of *P. knowlesi* was discovered in Sarawak, 1 of 2 states that make up Malaysian Borneo (5). Thereafter, transmission of *P. knowlesi* to humans occurred in the second state, Sabah (6,7), and in neighboring countries (8,9).

The natural hosts of *P. knowlesi* are principally the long-tailed (*Macaca fascicularis*) and pig-tailed (*M. nemestrina*) macaques (10), 2 species that are widely distributed in the Southeast Asia countries in which cases of *P. knowlesi* have been recorded. To date, human-to-human transmission has not been observed. Infections in humans can cause severe disease that can be fatal (9,11), underscoring the public health concern raised by this zoonotic simian parasite.

The 2 states of Malaysian Borneo appear to be the epicenter of zoonotic *P. knowlesi* infections: 1,391 cases in Malaysian Borneo and 423 cases in Peninsular Malaysia were recorded in 2012. A total of 1,407 PCR-confirmed cases were reported during 2004–2013 in Malaysia, which contrasts with the low number of cases (n = 136) reported from neighboring countries (9): Cambodia (n = 1), China (n = 36), Indonesia (n = 1), Myanmar (n = 14), the Philippines (n = 5), Singapore (n = 2), Thailand (n = 36), and Vietnam (n = 32). The reasons for this uneven distribution remain unclear. Geographic variation in mosquito species and human social factors could be an explanation; it is also possible that the parasite populations circulating on the island of Borneo are distinct from those found in continental Malaysia. The *P. knowlesi* strains that had been studied in earlier years displayed distinct biologic characteristics, which in some cases led malariologists to propose distinct *P. knowlesi* subspecies (12). Such differences