Natural Human Infections with *Plasmodium cynomolgi*, *P. inui*, and 4 other Simian Malaria Parasites, Malaysia

Nan Jiun Yap,[1] Hanisah Hossain,[1] Thamayanthi Nada-Raja, Romano Ngui, Azdayanti Muslim, Boon-Peng Hoh, Loke Tim Khaw, Khamisah Abdul Kadir, Paul Cliff Simon Divis, Indra Vythilingam, Balbir Singh, Yvonne Ai-Lian Lim

We detected the simian malaria parasites *Plasmodium knowlesi*, *P. cynomolgi*, *P. inui*, *P. coatneyi*, *P. inui*–like, and *P. simiovale* among forest fringe–living indigenous communities from various locations in Malaysia. Our findings underscore the importance of using molecular tools to identify newly emergent malaria parasites in humans.

Zoonotic malaria caused by *Plasmodium knowlesi*, commonly found in long-tailed macaques (*Macaca fascicularis*) and pig-tailed macaques (*M. nemestrina*), is now a major emerging disease, particularly in Malaysia (*1*,*2*). Two other simian malaria parasites, *P. cynomolgi* (*2*–*4*) and *P. inui* (*2*), have also been shown to have the potential of zoonotic transmission to humans through the bites of infected mosquitoes under natural and experimental conditions. The risk of acquiring zoonotic malaria is highest for persons living at the forest fringe and working or venturing into the forest because of their proximity with the monkey reservoir hosts and the mosquito vectors (*5*,*6*). With the aid of molecular methods, we aimed to investigate whether human infections with simian malaria parasites were present among indigenous communities in Malaysia whose villages are situated in the forest or at the forest fringe.

Author affiliations: Universiti Malaya, Kuala Lumpur, Malaysia (N.J. Yap, R. Ngui, A. Muslim, I. Vythilingam, Y.A.-L. Lim); Universiti Malaysia Sarawak, Kota Samarahan, Sarawak, Malaysia (H. Hossain, T. Nada-Raja, K.A. Kadir, P.C.S. Divis, B. Singh); Universiti Teknologi MARA (Sungai Buloh Campus), Selangor, Malaysia (A. Muslim); UCSI University, Kuala Lumpur, Malaysia (B.-P. Hoh); International Medical University, Kuala Lumpur, Malaysia (L.T. Khaw)

DOI: https://doi.org/10.3201/eid2708.204502

The Study

We examined 645 archived blood samples that we had collected during 2011–2014 among indigenous populations of various subtribes from 14 villages in 7 states in Malaysia (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/27/8/20-4502-App1.pdf). We first screened the extracted DNA samples at Universiti Malaya (UM) for the presence of *Plasmodium* with the aid of genus-specific primers (rPLU1 and rPLU5; rPLU3 and rPLU4) (Appendix). Of the 645 indigenous community samples, 102 (15.8%) were positive for *Plasmodium*. Using species-specific nested PCR assays (Appendix), we identified these infections as monoinfections with *P. knowlesi* (n = 40), *P. vivax* (n = 21), *P. cynomolgi* (n = 9), *P. falciparum* (n = 6), *P. coatneyi* (n = 3), *P. inui* (n = 3), *P. malariae* (n = 2), and *P. ovale curtisi* (n = 1) (Table 1). In 17 samples, the species could not be identified despite repeated attempts. Our species-specific primer pairs were designed on the basis of either the asexually (A) or sexually (S) transcribed forms of *Plasmodium* small subunit (SSU) rRNA genes (*7*); the genus-specific primer pairs anneal to both asexual and sexual forms of the SSU rRNA genes, and therefore the genus-specific assay is more sensitive.

We further characterized the 55 samples that tested positive for simian malaria parasites by amplifying a longer fragment of the SSU rRNA gene (914 bp–950 bp) for direct sequencing. Phylogenetic analysis using the neighbor-joining method (Figure 1) revealed the presence of *P. knowlesi* (samples PK1–40), *P. coatneyi* (UM1–3), *P. cynomolgi* (UM9, UM11, UM12, UM14, UM15, UM17, UM18), and *P. inui* (UM5–7). Meanwhile, 2 sequences derived from

[1]These authors contributed equally to this article.