Efficient Surveillance of *Plasmodium knowlesi* Genetic Subpopulations, Malaysian Borneo, 2000–2018

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Population genetic analysis revealed that *Plasmodium knowlesi* infections in Malaysian Borneo are caused by 2 divergent parasites associated with long-tailed (cluster 1) and pig-tailed (cluster 2) macaques. Because the transmission ecology is likely to differ for each macaque species, we developed a simple genotyping PCR to efficiently distinguish between and survey the 2 parasite subpopulations. This assay confirmed differences in the relative proportions in areas of Kapit division of Sarawak state, consistent with multilocus microsatellite analyses. Analyses of 1,204 human infections at Kapit Hospital showed that cluster 1 caused approximately two thirds of cases with no significant temporal changes from 2000 to 2018. We observed an apparent increase in overall numbers in the most recent 2 years studied, driven mainly by increased cluster 1 parasite infections. Continued monitoring of the frequency of different parasite subpopulations and correlation with environmental alterations are necessary to determine whether the epidemiology will change substantially.

The monkey parasite *Plasmodium knowlesi* was discovered to be a common cause of malaria in humans in 2004, initially from investigations in the Kapit division of Sarawak state, Malaysian Borneo (*1*). Humans acquire infection primarily from wild long-tailed (*Macaca fascicularis*) and pig-tailed (*M. nemestrina*) macaque reservoirs (*2*); *Anopheles* mosquitoes of the leucosphyrus group are vectors (*3*,*4*). *P. knowlesi* malaria has been described across Southeast Asia, but most clinical cases are still reported in Malaysian Borneo (*3*,*5*–*8*). In 2017 and 2018, a total of 7,745 cases were reported in Malaysia, 86.8% of which were detected in Malaysian Borneo (B. Singh, unpub. data) (*9*). *P. knowlesi* infections can be asymptomatic (*10*,*11*), and clinical cases exhibit a wide spectrum of disease ranging from mild symptoms to death (*3*).

Population genetic surveys of *P. knowlesi* infections in humans across Malaysia have revealed 2 divergent subpopulations of the parasite in Malaysian Borneo that are associated with the 2 macaque species locally, suggesting 2 independent zoonoses (*12*,*13*). The cluster 1 type has been associated with long-tailed macaques and the cluster 2 type with pig-tailed macaques (*12*). The existence of 2 sympatric subpopulations also has been confirmed by whole-genome sequencing (WGS) of *P. knowlesi* from patients in Malaysian Borneo (*13*,*14*). In peninsular Malaysia on the Asia mainland, all cases have been caused by another subpopulation, cluster 3, that has not been detected in Malaysian Borneo (*13*,*15*). Limited WGS (*14*,*15*) and microsatellite (*13*) genotyping of *P. knowlesi* isolates derived from human and only long-tailed macaque hosts from peninsular Malaysia showed allopatric divergence for this subpopulation cluster from those of Malaysian Borneo because of geographic separation by the South China Sea.

Increasing numbers of *P. knowlesi* malaria cases detected might be due to increased zoonotic exposure along with a reduction of endemic malaria parasite species (*16*). With the recent identification of different zoonotic *P. knowlesi* genetic subpopulations, determining whether these populations vary in frequency over space and time is important. Interactions with

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