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Macaca fascicularis and *Macaca nemestrina* infected with zoonotic malaria parasites are widely distributed in Sarawak, Malaysian Borneo

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Human infections with *Plasmodium knowlesi*, a malaria parasite of *Macaca fascicularis* and *Macaca nemestrina* (long-tailed and pig-tailed macaques respectively), occur throughout Southeast Asia, especially Malaysian Borneo. Other naturally-acquired human infections with malaria parasites from macaques in Southeast Asia are *P. cynomolgi*, *P. inui*-like, *P. coatneyi* and *P. simiovale*. In Sarawak, Malaysian Borneo, *M. fascicularis* and *M. nemestrina* from only the Kapit Division have been examined previously for malaria parasites. In order to determine the distribution of *P. knowlesi* and other zoonotic malaria parasites, 73 macaque blood samples derived from 7 other administrative divisions in Sarawak were studied. Of 45 blood samples from *M. fascicularis* and 28 from *M. nemestrina* tested by nested PCR assays, 23 (51.1%) *M. fascicularis* and 15 (53.6%) *M. nemestrina* samples were positive for *Plasmodium* DNA. Thirty-two of these macaques from 7 divisions sampled, harboured either single ($n=12$), double ($n=9$), triple ($n=7$) or quadruple ($n=4$) infections of *P. knowlesi*, *P. inui*, *P. cynomolgi* and *P. coatneyi*, while the infecting species of *Plasmodium* could not be identified for 6 samples. *P. knowlesi* was detected in 15.5% (7/45) *M. fascicularis* and in 7.1% (2/28) *M. nemestrina* sampled. Despite the small number of samples analysed from each administrative division, the current study indicates that macaques infected with the zoonotic malaria parasites *P. knowlesi*, *P. cynomolgi*, *P. inui* and *P. coatneyi* are widely distributed throughout Sarawak, Malaysian Borneo. Travelers to forested areas in Sarawak should be made aware of the potential risk of acquiring zoonotic malaria.

Non-human primates are reservoir hosts for malaria parasites and many other infective agents including Hepatitis B, Simian retroviruses, Macacine herpesvirus 1 and *Brugia malayi*[1,2]. There are more than 30 species of *Plasmodium* which are capable of infecting primates out of over 200 species of *Plasmodium* identified to date[3,4]. Human infections with simian malaria parasites were thought to be extremely rare until a large focus of human infections with *P. knowlesi* was reported in 2004 in the Kapit division of Sarawak, Malaysian Borneo[5]. Since then, human *P. knowlesi* infections have been reported throughout Malaysia and in all countries in Southeast Asia except Timor Leste[6–14]. Human knowlesi malaria cases are of public health concern in Malaysia, where from 2018 to 2020 they constituted all the 8,500 indigenous cases of malaria, mainly in the states of Sabah and Sarawak, Malaysian Borneo (Ministry of Health Malaysia, unpublished data)[15,16]. In Sarawak, knowlesi malaria cases have continued to increase from the 120 cases first described in the Kapit Division in 2004 to between 759 and 1,247 annual cases from 2016 to 2020, with 14 deaths (Ministry of Health Malaysia, unpublished data). There have also been case reports of tourists acquiring knowlesi malaria following visits to forested areas in Sarawak[17–19] and Southeast Asia[20].

The natural hosts for *P. knowlesi* are primarily *Macaca fascicularis* (long-tailed macaque) and *M. nemestrina* (pig-tailed macaque)[4]. *P. knowlesi* infections in these species, the most common non-human primates in Southeast Asia, have been reported from Thailand, Malaysia, Philippines and Laos[7,21–26]. There have also been reports

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