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Absence of *Plasmodium inui* and *Plasmodium cynomolgi*, but detection of *Plasmodium knowlesi* and *Plasmodium vivax* infections in asymptomatic humans in the Betong division of Sarawak, Malaysian Borneo

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Abstract

Background: *Plasmodium knowlesi,* a simian malaria parasite, has become the main cause of malaria in Sarawak, Malaysian Borneo. Epidemiological data on malaria for Sarawak has been derived solely from hospitalized patients, and more accurate epidemiological data on malaria is necessary. Therefore, a longitudinal study of communities affected by knowlesi malaria was undertaken.

Methods: A total of 3002 blood samples on filter paper were collected from 555 inhabitants of 8 longhouses with recently reported knowlesi malaria cases in the Betong Division of Sarawak, Malaysian Borneo. Each longhouse was visited bimonthly for a total of 10 times during a 21-month study period (Jan 2014–Oct 2015). DNA extracted from blood spots were examined by a nested PCR assay for *Plasmodium* and positive samples were then examined by nested PCR assays for *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, *Plasmodium knowlesi*, *Plasmodium cynomolgi* and *Plasmodium inui*. Blood films of samples positive by PCR were also examined by microscopy.

Results: Genus-specific PCR assay detected *Plasmodium* DNA in 9 out of 3002 samples. Species-specific PCR identified 7 *P. knowlesi* and one *P. vivax*. Malaria parasites were observed in 5 thick blood films of the PCR positive samples. No parasites were observed in blood films from one *knowlesi*-, one *vivax*- and the genus-positive samples. Only one of 7 *P. knowlesi*-infected individual was febrile and had sought medical treatment at Betong Hospital the day after sampling. The 6 *knowlesi*-, one *vivax*- and one *Plasmodium*-infected individuals were afebrile and did not seek any medical treatment.

Conclusions: Asymptomatic human *P. knowlesi* and *P. vivax* malaria infections, but not *P. cynomolgi* and *P. inui* infections, are occurring within communities affected with malaria.

Keywords: *Plasmodium knowlesi*, Malaria, Asymptomatic, Submicroscopic

Background

Prior to 2004, human malarias were thought to be caused by four species of *Plasmodium*; *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*. Discovery of a large number of *Plasmodium knowlesi* cases in the Kapit division of Sarawak [1] led to the inclusion of *P. knowlesi*, a simian malaria parasite [2], as the fifth cause of malaria in humans [3]. Morphological similarities between *P. knowlesi* and *P. malariae*, when examined by microscopy, had led to *P. knowlesi* being

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