

Human and monkey infections with *Plasmodium knowlesi* in Malaysian Borneo

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Abstract

In the Kapit Division of Sarawak, Malaysian Borneo, we discovered a large focus of natural human infections with *P. knowlesi* by using molecular detection methods. These infections had been mainly diagnosed as *P. malariae* by microscopy. *P. knowlesi* has been found in long and pig-tailed macaque monkeys in Peninsular Malaysia but there have been no studies on monkeys from Malaysian Borneo. The aims of the study were to determine whether human *P. knowlesi* infections occurred in other parts of Malaysian Borneo and whether local monkeys were infected. Blood spots on filter paper from 1328 malaria patients, including 337 diagnosed as *P. malariae* by microscopy, were collected at 13 locations in Sarawak from 2000-2005. Blood films were obtained from 8 cases diagnosed by microscopy as *P. malariae* at 6 locations in Sabah. In addition, blood spots were collected from 25 monkeys in Sarawak. DNA extracted from blood spots and films were examined with a nested PCR malaria detection assay. With the exception of two imported cases in Sarawak and one local case in Sabah, none of the 1313 human malaria infections were identified as *P. malariae* by PCR. However, 333 were either single *P. knowlesi* infections or co-infections of *P. knowlesi* with other *Plasmodium* species. These human *P. knowlesi* infections were widespread throughout Sarawak and Sabah. Two out of 25 monkeys were *P. knowlesi*-positive by PCR. The small subunit ribosomal RNA and CSP genes of *P. knowlesi* from these two monkeys were phylogenetically indistinguishable from 8 causing human infections. Molecular characterization and analyses of a larger number of *P. knowlesi* samples from humans and monkeys are necessary to determine whether human *P. knowlesi* infections are acquired from monkeys or whether a parasite host switch has occurred, supporting transmission from man-to-man.

Introduction

Malaria is one of the major vector-borne diseases in Malaysia, affecting mainly the rural communities in the states of Sabah and Sarawak in Malaysian Borneo (Singh & Cox-Singh, 2001). A study was recently undertaken in the Kapit Division of Sarawak to examine atypical malaria infections thought to be due to *P. malariae*. During the study, *P. malariae* infections were not detected by molecular methods and 120 of 208 hospitalised malaria cases in Kapit were found to be due to *P. knowlesi* (Singh *et al.*, 2004). These malaria parasites have been detected in long and pig-tailed macaque monkeys in nature in Peninsular Malaysia and the Philippines (Garnham, 1966) but studies have not been conducted on monkeys from Sarawak. It is important to establish whether human *P. knowlesi* infections are acquired from monkeys or whether there has been a host switch by the parasite and transmission is human-to human as this information is essential for the implementation of appropriate malaria control programmes. The aims of the study