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Plasmodium knowlesi from archival blood films: Further evidence that human infections are widely distributed and not newly emergent in Malaysian Borneo

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ABSTRACT

Human infections with *Plasmodium knowlesi* have been misdiagnosed by microscopy as *Plasmodium malariae* due to their morphological similarities. Although microscopy-identified *P. malariae* cases have been reported in the state of Sarawak (Malaysian Borneo) as early as 1952, recent epidemiological studies suggest the absence of indigenous *P. malariae* infections. The present study aimed to determine the past incidence and distribution of *P. knowlesi* infections in the state of Sarawak based on archival blood films from patients diagnosed by microscopy as having *P. malariae* infections. Nested PCR assays were used to identify *Plasmodium* species in DNA extracted from 47 thick blood films collected in 1996 from patients in seven different divisions throughout the state of Sarawak. *Plasmodium knowlesi* DNA was detected in 35 (97.2%) of 36 blood films that were positive for *Plasmodium* DNA, with patients originating from all seven divisions. Only one sample was positive for *P. malariae* DNA. This study provides further evidence of the widespread distribution of human infections with *P. knowlesi* in Sarawak and its past occurrence. Taken together with data from previous studies, our findings suggest that *P. knowlesi* malaria is not a newly emergent disease in humans.

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1. Introduction

Plasmodium knowlesi, a malaria parasite of Old World monkeys (Garnham, 1966), is one of the five malaria species known to cause human malaria (Cox-Singh and Singh, 2008). Following our report of a large focus of human *P. knowlesi* infections in the Kapit division of Sarawak, Malaysian Borneo (Singh et al., 2004), cases of naturally acquired human infections with *P. knowlesi* have been reported from many areas in Southeast Asia including Thailand (Jongwutiwes et al., 2004), Myanmar (Zhu et al., 2006), the Philippines (Luchavez et al., 2008), Singapore (Ng et al., 2008), Sabah State, Malaysian Borneo (Cox-Singh et al., 2008) and Peninsular Malaysia (Cox-Singh et al., 2008; Vythilingam et al., 2008).

Plasmodium knowlesi malaria in humans is routinely misdiagnosed by microscopy as *Plasmodium malariae* malaria due to the morphological similarities between the two species and the only reliable diagnostic method to correctly distinguish between the two species is two nested PCR assays (Cox-Singh and Singh, 2008). Our prospective molecular epidemiological studies conducted on 960 samples collected from malaria patients in Sarawak between 2000 and 2006 showed that by using nested PCR assays 266 were diagnosed as *P. knowlesi* and only four were *P. malariae*

cases, although 312 had been diagnosed as *P. malariae* by microscopy (Singh et al., 2004; Cox-Singh et al., 2008). All four *P. malariae* infections had been acquired by logging camp workers who had recently returned from malaria-endemic countries. The apparent lack of indigenous *P. malariae* cases in Sarawak raised the question of whether previous malaria cases identified by microscopy as *P. malariae* were *P. knowlesi* malaria. Here, we present further evidence of the past incidence and widespread distribution of human *P. knowlesi* infections in the state of Sarawak based on a retrospective study using DNA extracted from archival malaria blood films diagnosed as *P. malariae* by microscopy.

2. Materials and methods

2.1. Malaria blood films

A total of 47 thick blood films prepared in 1996 from malaria patients diagnosed by microscopy as having *P. malariae* from seven administrative divisions in the state of Sarawak were obtained from the Parasitology Diagnostic Laboratory of the Sarawak State Health Department. They had been collected at hospitals and health clinics in the following administrative divisions; Sri Aman, Sarikei, Sibul, Bintulu, Kapit, Miri and Limbang (Fig. 1). Malaria blood films collected in 1996 were the oldest samples that we were able to obtain at the time of this study. A total of 392 blood films

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