Plasmodium knowlesi Infection in Humans, Cambodia, 2007–2010

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Two cases of *Plasmodium knowlesi* infection were identified in humans in Cambodia by 3 molecular detection assays and sequencing. This finding confirms the widespread distribution of *P. knowlesi* malaria in humans in Southeast Asia. Further wide-scale studies are required to assess the public health relevance of this zoonotic malaria parasite.

In Cambodia, malaria ranks among the leading causes of illness and death. Mostly affecting the ≈3 million persons (23% of Cambodia's population) who live near forested areas, malaria remains an occupational disease in specific high-risk groups, such as forestry workers and migrant populations who have come into forested areas. However, for the past decade, the number of reported malaria cases has generally decreased but in a sawtooth pattern of periodic increases (*1*).

Four of the 5 *Plasmodium* species known to cause malaria in humans have already been described in Cambodia (*2*,*3*). *P. falciparum* remains the most frequent cause of malaria (83,777 cases in 2009, prevalence of 70%) (*1*). However, distributions of *Plasmodium* species are changing, with a particularly substantial increase of *P. vivax* malaria cases, from 4,105 (8%) cases in 2000 to 6,250 (25%) in 2009. In several areas of low transmission, the proportion of *P. vivax* infections has increased up to 50% (*2*). This trend is probably related to various effective control strategies implemented in Cambodia against *P. falciparum* malaria.

No studies in humans (*3*) and monkeys in Cambodia have identified the simian malaria parasite, *P. knowlesi*, which is causing human disease in some other countries in Southeast Asia (*4*). *P. knowlesi* parasites were not detected in blood samples collected during 2004–2007 from 138 monkeys (102 *Macaca fascicularis* monkeys; 13 *M. leonina* monkeys; 20 *Hyobates pileatus* monkeys; 2 *Presbytis cristata* monkeys; and 1 *Pongo pygmaeus* monkey) by using PCR (*cytb* and *cox1* genes) (L. Duval, unpub. data). Because these animals were confiscated by Wildlife Alliance rescue patrols from illegal traders, the locations where they were trapped in Cambodia are unknown.

We undertook a cross-sectional prospective study in 3 sites in Cambodia (Figure, panel A) (*5*). The main objective of this study was to develop evidence to guide the management of malaria parasite–negative persons with acute febrile illness and to determine whether such persons were infected with *P. knowlesi*.

The Study

We enrolled patients ≥7 years of age with acute (<8 days) febrile illness in selected outpatient clinics, where they had been tested for malaria by using rapid diagnostic test (CareStart, Access Bio Inc, Somerset, NJ, USA). A subset of nonfebrile patients were used as controls; patients in a critical clinical condition were excluded. After we obtained informed consent, each patient's history was taken, a physical examination was conducted, and blood and throat swab samples were collected. The defined test battery, including the following pathogens, was performed by using a PCR/sequencing approach: *Plasmodium* spp., *Leptospira* spp., *Rickettsia* spp. (including *Orientia tsutsugamushi*), dengue viruses, Japanese encephalitis virus, and influenza viruses. HIV infection and tuberculosis were not evaluated. DNA and RNA from the erythrocyte pellet or throat swab were extracted by using the QIAamp kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. Malaria parasites were detected by using a *Plasmodium* spp.–specific nested PCR based on the *cytb* gene followed by sequencing (*6*).

During December 2007–December 2010, a total of 1,475 patients were enrolled (621 in Soun Kouma; 650 in Ou Chra, Pailin Province; and 204 in Snoul, Kratié Province), comprising 1,193 febrile and 282 nonfebrile persons. For 564 (38.2%) patients, no pathogens were detected. A total of 754 patients (51.1%; 676 cases, 78 controls) were infected with malaria parasites, and the

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