The Journal of Infectious Diseases

MAJOR ARTICLE

Contribution of *Plasmodium knowlesi* to Multispecies Human Malaria Infections in North Sumatera, Indonesia

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Background. As Indonesia works toward the goal of malaria elimination, information is lacking on malaria epidemiology from some western provinces. As a basis for studies of antimalarial efficacy, we set out to survey parasite carriage in 3 communities in North Sumatera Province.

Methods. A combination of active and passive detection of infection was carried out among communities in Batubara, Langkat, and South Nias regencies. Finger-prick blood samples from consenting individuals of all ages provided blood films for microscopic examination and blood spots on filter paper. *Plasmodium* species were identified using nested polymerase chain reaction (PCR) of ribosomal RNA genes and a novel assay that amplifies a conserved sequence specific for the *sicavar* gene family of *Plasmodium knowlesi*.

Results. Of 3731 participants, 614 (16.5%) were positive for malaria parasites by microscopy. PCR detected parasite DNA in samples from 1169 individuals (31.3%). In total, 377 participants (11.8%) harbored *P. knowlesi*. Also present were *Plasmodium vivax* (14.3%), *Plasmodium falciparum* (10.5%) and *Plasmodium malariae* (3.4%).

Conclusions. Amplification of *sicavar* is a specific and sensitive test for the presence of *P. knowlesi* DNA in humans. Subpatent and asymptomatic multispecies parasitemia is relatively common in North Sumatera, so PCR-based surveillance is required to support control and elimination activities.

Keywords. Malaria; Indonesia; *Plasmodium knowlesi.*

Malaria remains widespread across Southeast Asia. In Indonesia, 2 million cases of malaria are reported each year, with *Plasmodium falciparum* and *Plasmodium vivax* the 2 major reported causes [1]. Among other species contributing to human infections, *Plasmodium malariae* malaria may require hospitalization in the eastern province of Papua [2] but is not frequently encountered in western Indonesia. *Plasmodium knowlesi,* a parasite of long-tailed and pig-tailed macaques, is also known to infect humans. The morphological features in the blood stage are similar to those seen in *P. falciparum* and *P. malariae*, which in routine practice has led to frequent misdiagnosis [3–5]. High *P. knowlesi* parasitemia occurs in some individuals and has been reported to cause fatal disease [6]. Despite this, a proportion of *P. knowlesi* infections are asymptomatic and submicroscopic across all age groups [7]. A small number of human cases of *P. knowlesi* malaria have been documented in the province of Kalimantan, Indonesian Borneo [8] and in Aceh province [5], but this species has not yet emerged as a major cause of human malaria and is not considered in Indonesian government guidelines.

The Ministry of Health of Indonesia has implemented malaria control, aiming for elimination by 2030. Malaria surveillance relies on passive case detection by microscopic examination and rapid diagnostic tests (RDTs) at primary health care centers [9]. These tests are sufficient to detect clinical malaria infection caused by the 2 major species in Indonesia, *P. falciparum* and *P. vivax* [10]. However, identification of less common species, particularly at low-density parasitemia, is more difficult, which can lead to underdiagnosis [11]. Modeling of data from low-endemicity areas predicts that submicroscopic parasites may contribute 70%–80% of all malaria infections [12], and in vivo studies demonstrate that these contribute to ongoing malaria transmission [13]. Hence, the use of routine microscopy and RDTs in malaria surveillance fails to detect a substantial proportion of the human reservoir of infection and so may compromise malaria elimination strategies. One solution is to deploy molecular assays for parasite detection, because these can provide excellent sensitivity and specificity [14–17].

In preparation for a study of antimalarial drug efficacy in vivo, we performed intensive malaria screening in 3 regencies of the Province of North Sumatera, western Indonesia. In

Received 2 December 2016; editorial decision 8 February 2017; accepted 11 February 2017; published online February 13, 2017.

Presented in part: American Society for Tropical Medicine & Hygiene Annual Meeting, Philadelphia, Pennsylvania November 2015; SingMalNet 2016 meeting, Singapore, February 2016.

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The Journal of Infectious Diseases® 2017;215:1148–55

DOI: 10.1093/infdis/jix091