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Evaluation of three rapid diagnostic tests for the detection of human infections with *Plasmodium knowlesi*

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Abstract

Background: *Plasmodium knowlesi*, a malaria parasite of Southeast Asian macaques, infects humans and can cause fatal malaria. It is difficult to diagnose by microscopy because of morphological similarity to *Plasmodium malariae*. Nested PCR assay is the most accurate method to distinguish *P. knowlesi* from other *Plasmodium* species but is not cost effective in resource-poor settings. Rapid diagnostic tests (RDTs) are recommended for settings where malaria is prevalent. In this study, the effectiveness of three RDTs in detecting *P. knowlesi* from fresh and frozen patient blood samples was evaluated.

Methods: Forty malaria patients (28 *P. knowlesi*, ten *P. vivax* and two *P. falciparum*) diagnosed by microscopy were recruited in Sarawak, Malaysian Borneo during a 16-month period. Patient blood samples were used to determine parasitaemia by microscopy, confirm the *Plasmodium* species present by PCR and evaluate three RDTs: OptiMAL-IT, BinaxNOW® Malaria and Paramax-3. The RDTs were also evaluated using frozen blood samples from 41 knowlesi malaria patients.

Results: OptiMAL-IT was the most sensitive RDT, with a sensitivity of 71% (20/28; 95% CI = 54-88%) for fresh and 73% (30/41; 95% CI = 59-87%) for frozen knowlesi samples. However, it yielded predominantly falciparum-positive results due to cross-reactivity of the *P. falciparum* test reagent with *P. knowlesi*. BinaxNOW® Malaria correctly detected non-*P. falciparum* malaria in *P. knowlesi* samples but was the least sensitive, detecting only 29% (8/28; 95% CI = 12-46%) of fresh and 24% (10/41; 95% CI = 11-37%) of frozen samples. The Paramax-3 RDT tested positive for *P. vivax* with PCR-confirmed *P. knowlesi* samples with sensitivities of 40% (10/25; 95% CI = 21-59%) with fresh and 32% (13/41; 95% CI = 17-46%) with frozen samples. All RDTs correctly identified *P. falciparum*- and *P. vivax*-positive controls with parasitaemias above 2,000 parasites/μl blood.

Conclusions: The RDTs detected *Plasmodium* in *P. knowlesi*-infected blood samples with poor sensitivity and specificity. Patients with *P. knowlesi* could be misdiagnosed as *P. falciparum* with OptiMAL-IT, *P. vivax* with Paramax-3 and more correctly as non-*P. vivax*/non-*P. falciparum* with BinaxNOW® Malaria. There is a need for a sensitive and specific RDT for malaria diagnosis in settings where *P. knowlesi* infections predominate.

Keywords: *Plasmodium knowlesi*, Malaria diagnostics, Rapid diagnostic tests

Background

Until recently only four types of *Plasmodium* (*Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae* and *Plasmodium ovale*) were known to cause malaria in humans. However, a fifth species, *Plasmodium knowlesi,* has been identified as a cause of human malaria in almost all countries in Southeast Asia (recently reviewed [1]) and extending to the Nicobar and Andaman Islands in India [2]. In Malaysian Borneo, *P. knowlesi* is the main cause of admissions for malaria in certain hospitals, including Sarikei Hospital, and can lead to fatal infections [3-9].

Plasmodium species infections are typically diagnosed by microscopic examination of stained blood films, but there are limitations in sensitivity and specificity [10]. Nested PCR assays were developed to accurately distinguish

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