INTRODUCTION

The rapid and accurate diagnosis of malaria has the potential to improve malaria management and control in developing countries. Although microscopy has been the mainstay of malaria diagnosis, accurate microscopic diagnosis is frequently unavailable in many rural areas with significant malaria transmission. Several rapid malaria diagnostic assays have recently been developed which have the potential to facilitate malaria treatment and control measures in rural areas.

Lao PDR is a developing country of about 4.5 million people with 80% of the population living in rural areas. The majority of residents must travel large distances at considerable expense in order to access accurate microscopic services for the diagnosis of malaria.

Determining the prevalence and spread of drug-resistant malaria is an important problem for many malaria-endemic countries. This is most frequently achieved by performing surveillance surveys in affected regions. Treatment of malaria in the rural areas is often delayed due to the large distances and considerable expense required to access reliable microscopic services. Unfortunately, it is logistically more complex to undertake surveys of drug resistance in remote field sites especially in rural and remote areas of developing nations make the microscopic diagnosis of malaria problematic. New rapid malaria-diagnostic assays are required to make diagnosis more affordable and accessible to achieve the goals of the malaria eradication and control programs.

MATERIALS AND METHODS

Study Site and Patients: The study took place during October and November in 1998 in the Vang Vieng District, Vientiane Province, where approximately 190,000 individuals are at risk for malaria. All febrile patients, or patients with a history of fever in the past 48 hours, were eligible for inclusion in the study. Subjects were excluded if pregnant, less than 1 year old, or had received anti-malarial treatment in the preceding month, or had a history of chloroquine intolerance. Informed consent was obtained in the local language from the village chief and from all patients (parents or guardians in the case of those <18 years old). The study was approved by the Ethical Review Committee of the Lao PDR Ministry of Health and by the Institutional Review Board of the Toronto General Hospital.

In order to enroll falciparum-infected patients in a 28-day chloroquine treatment trial in a rural site of Lao PDR we used the strategy of multiple rapid diagnostic assays to identify potential participants. Since no single assay has 100% specificity, we utilized two diagnostic assays (PATH and OptiMAL®). Flow Inc., Portland, Oregon, U.S.A.) that detect different antigens (HRP-2 and P. falciparum lactate dehydrogenase [pLDH]), respectively to reliably detect falciparum malaria infections.

Microscopy and Immunochromatographic Testing: The procedure for the use of the rapid tests is as follows:

Febrile Patient

Fingerstick Blood

Microscopy

sensitivity/specificity

Blood samples tested by optimal assay

P. falciparum+ve and Malaria-ve

Malaria+ve and Malaria-ve

PATH HRP-2 Assay

Microscopy and Immunochromatographic Testing: The procedure for the use of the rapid tests is as follows:

Febrile Patient

Fingerstick Blood

Case Study: P. falciparum detection in a febrile patient

Blood sample tested by optimal assay

sensitivity/specificity

Malaria+ve

Malaria-ve

PATH HRP-2 Assay

Microscopy

sensitivity/specificity

P. falciparum+ve

P. falciparum-ve

Malaria+ve

Malaria-ve

VIEW RESULTS

RESULT

The results of the sensitivity and specificity of the PATH test are presented in Table 1.

TABLE 1. Comparison of PATH Assay with Reference Microscopy for the Detection of P. falciparum

<table>
<thead>
<tr>
<th>PATH dipstick (n)</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>51</td>
<td>10</td>
<td>61.2</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>133</td>
<td>135.0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>53</td>
<td>143</td>
<td>196.0</td>
</tr>
</tbody>
</table>

A subset of 97 blood samples was also tested by OptiMAL® (all 61 positive samples and 36 negative samples). The sensitivity and specificity of the OptiMAL® assay for detection of P. falciparum was 90.6% and 81.8%, respectively accuracy measures may be affected by verification bias. Of the 8 of the 10 “false-positive” PATH assays were also false positive by OptiMAL®. No false positive OptiMAL® results were observed with the OptiMAL® assay.

Using microscopy and OptiMAL® as the “modified gold standard” (Table 2), the sensitivity and specificity of the PATH assay increased to 97.6% and 94.4%, respectively. Similarly, for the OptiMAL® assay, when the “modified gold standard” was defined as the combination of the PATH and microscopy results. The sensitivity and specificity of OptiMAL® were increased to 91.8% and 91.8%, respectively.

TABLE 2. Comparison of PATH Assay with the “Modified Gold Standard” for the Detection of P. falciparum

<table>
<thead>
<tr>
<th>PATH dipstick (n)</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>59</td>
<td>2</td>
<td>61.7</td>
</tr>
<tr>
<td>Negative</td>
<td>34</td>
<td>36</td>
<td>94.4</td>
</tr>
<tr>
<td>TOTAL</td>
<td>93</td>
<td>60</td>
<td>153.0</td>
</tr>
</tbody>
</table>

The limited supply and maintenance of microscopy, lack of expertise and reagents, delays in results, and inadequate control in developing nations make the microscopic diagnosis of malaria problematic. New rapid malaria-diagnostic assays are required to make diagnosis more affordable and accessible to achieve the goals of the malaria eradication and control programs.

The PATH and OptiMAL® rapid tests both identified eight samples as falciparum-positive, while microscopy was unable to detect parasites. It is possible that the true specificities of the PATH and OptiMAL® tests for the detection of P. falciparum are higher than that found by using standard microscopy as the gold standard. Specificity may have increased had the polymerase chain reaction (PCR) been used as the gold standard for the detection of parasitemia below the detection limit of microscopy.

Drug resistance often develops in rural areas that lack ready access to reliable microscopic services. Unfortunately, it is logistically more complex to undertake surveys of drug resistance in remote field sites especially with reference to microscopy. The rapid malaria assays were found to be straightforward to perform and rapidly interpretable in the field. They also eliminate the need for patients to devote most of their day waiting for results, and they eliminate inter-technician variation present in microscopy.

In conclusion, the PATH assay was highly accurate and appropriate for use in a field setting by local health care providers in Lao PDR. The PATH and OptiMAL® tests were successfully employed simultaneously to enroll patients for chloroquine treatment. Our data, therefore, supports the use of rapid dipstick assays to identify potential participants for WHO P. falciparum in vivo treatment trials in endemic field settings.

ACKNOWLEDGMENTS

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