CO-INFECTION WITH MALARIA AND LEPTOSPIROSIS

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Abstract. Malaria and leptospirosis are both common in the tropics. Simultaneous infections are possible, although not previously reported. We report two cases of malaria from an area of Thailand on the Thailand-Myanmar border with compelling serologic evidence of simultaneous acute leptospirosis. One was a case of infection with Plasmodium falciparum with acute and convalescent microscopic agglutination test (MAT) titers for Leptospira serovar icterohaemorrhagiae of 1:200 and 1:1,600, respectively. The other was a case of infection with P. vivax that seroconverted to a titer of 1:3,200 for Leptospira serovar bataviae. Additionally, there were five probable cases of leptospirosis with patent malaria parasitemia (three P. falciparum and two P. vivax) detected. Management of dual infections is complicated by their similar clinical presentations, and because the confirmatory diagnosis of malaria is readily available as opposed to that of leptospirosis. Treatment focusing on malaria mono-infections instead of dual infections could result in a delay of specific therapy for leptospirosis and possible consequences of serious complications.

INTRODUCTION

Malaria and leptospirosis are cosmopolitan infections with overlapping geographic distributions, especially in the tropics. Their co-foci have been suggested by a seroepidemiologic study in the Peruvian Amazon. Simultaneous infections are likely, although, to our knowledge, have not previously been reported. Co-infections of malaria with filariasis, non-typhoidal Salmonella bacteremia, dengue, hantavirus, human immunodeficiency virus, and Borrelia have been reported. The similar clinical presentations of acute malaria, leptospirosis, and other undifferentiated fevers make discrimination on clinical grounds difficult. We report seven cases with clinical and laboratory evidence of co-infection with malaria and leptospirosis in western Thailand.

MATERIALS AND METHODS

Between June 1999 and March 2002, 613 patients were enrolled in a study to determine the specific etiology of febrile illnesses in residents of Sangkhlaburi District in Kanchanaburi Province, on the Thailand-Myanmar border. Patients 20 years old presenting with acute fever (within 72 hours) to the Kwai River Christian Hospital in Sangkhlaburi, Thailand were eligible for the study. Evaluation included a medical history, physical examination, and routine clinical laboratory tests. Blood specimens were obtained on presentation and at convalescence 3–4 weeks later. This study was reviewed and approved by the Thai Ministry of Public Health and the Walter Reed Army Institute of Research. All subjects provided written informed consent for participation and sample donation.

Sera were screened for leptospirosis by a dot–enzyme-linked immunosorbent assay (ELISA) (INDX Multi-test Dip-S-Ticks®; PanBio-INDX, Baltimore, MD) for the detection of Leptospira-specific total immunoglobulin G (a dot intensity score of 0–1 = negative, 1+ = borderline, and 2-4 = positive) and by an ELISA (PanBio, Ltd., Brisbane, Queensland, Australia) for IgM (positive cut-off value of 11 PanBio units is recommended by the manufacturer but based on our experience using the assay in this population, a more stringent cut-off value of 18 PanBio units was applied to improve specificity). These assays are sensitive and have the advantages of their case of use. Results were not available to affect care by the clinician. Paired sera from 96 patients (including 18 with malaria), which were suspicious of leptospirosis based on these screening tests or clinical findings, were sent for a confirmatory microscopic agglutination test (MAT) at the Veterinary Command Food Analysis and Diagnostic Laboratory. A standard MAT was performed using a battery of 24 serovars from 20 serogroups common for Asia.

Clinical case description for leptospirosis is characterized by fever, headache, chills, myalgia, conjunctival suffusion, and, less frequently, meningitis, rash, jaundice, or renal insufficiency. Laboratory criteria for a confirmed diagnosis are 1) the isolation of Leptospira from a clinical specimen, or 2) a four-fold or greater increase in Leptospira microagglutination titer between acute-phase and convalescent-phase serum specimens obtained two or more weeks apart and studied at the same laboratory, or 3) demonstration of Leptospira in a clinical specimen by immunofluorescence. Case classifications are 1) probable: a clinically compatible case with supportive serologic findings (i.e., a Leptospira microagglutination titer ≥ 200 in one or more serum specimens) and 2) confirmed: a clinically compatible case that is laboratory confirmed. We have applied an agglutination titer ≥ 800 instead of ≥ 200 (as probable acute leptospirosis) to our case series. An increased cut-off value has been suggested for populations with possibly high background exposure to leptospires.

RESULTS

Forty paired specimens were considered MAT positive for leptospirosis, seven with malaria. The other 11 malaria cases were MAT negative. These 18 patients were divided into those with confirmed leptospirosis and malaria co-infection (two cases), those with probable leptospirosis and malaria co-infection (five cases), and those with malaria-only infection (11 cases) (Table 1). Both patients in the confirmed co-infection group clearly met the criterion for acute leptospirosis. The five probable cases had either acute or convalescent or both MAT titers ≥ 1:800. The malaria-only group did not meet our stricter definition of a positive MAT result.

All seven co-infection cases were men with a median age of
TABLE 1
Laboratory findings and treatment regimens for the patients with malaria-leptospirosis co-infection (confirmed and probable) and those with malaria mono-infection

<table>
<thead>
<tr>
<th>Patient group and subject number</th>
<th>Malaria smear*</th>
<th>Malaria-specific IgM (PanBio units) acute/convalescence†</th>
<th>Leptospira-specific IgM (PanBio units) acute/convalescence</th>
<th>Leptospira-specific total Ig (dipstick intensity score)§</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co-infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Pf 37,380/µL</td>
<td>200/1,600 (icterohaemorrhagiae)</td>
<td>40.2/36.2</td>
<td>3/3</td>
<td>Artesunate, doxycycline</td>
</tr>
<tr>
<td>2</td>
<td>Pv 26/µL</td>
<td>0/3,200 (bataviae)</td>
<td>30.0/29.5</td>
<td>2/4</td>
<td>Chloroquine, doxycycline¶</td>
</tr>
<tr>
<td>Probable coinfection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Pv 20,368/µL</td>
<td>800/400 (grippotyphosa)</td>
<td>17.2/17.7</td>
<td>1/2</td>
<td>Chloroquine</td>
</tr>
<tr>
<td>4</td>
<td>Pv 1,860/µL</td>
<td>800/800 (bratislava)</td>
<td>40.6/32.9</td>
<td>1+2</td>
<td>Chloroquine</td>
</tr>
<tr>
<td>5</td>
<td>Pf 146/µL</td>
<td>800/1,600 (bataviae)</td>
<td>21.6/20.3</td>
<td>3/3</td>
<td>Artesunate, melfloquine</td>
</tr>
<tr>
<td>6</td>
<td>Pf 361,180/mcL</td>
<td>800/800 (pyrogenes)</td>
<td>&lt;11/11</td>
<td>2/2</td>
<td>Artesunate, doxycycline</td>
</tr>
<tr>
<td>7</td>
<td>Pf 10,272/µL</td>
<td>800/800 (grippotyphosa)</td>
<td>21.2/15.3</td>
<td>3/4</td>
<td>Artesunate, melfloquine</td>
</tr>
<tr>
<td>Malaria only</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Pf 3,936/µL</td>
<td>Negative</td>
<td>22.0/12.2</td>
<td>1+/3</td>
<td>Artesunate, doxycycline</td>
</tr>
<tr>
<td>9</td>
<td>Pv 8,844/µL</td>
<td>100/100 (each for bratislava, autumalis, icterohaemorrhagiae)</td>
<td>&lt;11/11</td>
<td>2/2</td>
<td>Artesunate, melfloquine#</td>
</tr>
<tr>
<td>10</td>
<td>Pf 44,240/µL</td>
<td>100/100 (pyrogenes)</td>
<td>&lt;11/11</td>
<td>3/3</td>
<td>Artesunate, melfloquine</td>
</tr>
<tr>
<td>11</td>
<td>Pf 25,404/µL</td>
<td>200/200 (bataviae)</td>
<td>8.4/22.3</td>
<td>2/1.5</td>
<td>Artesunate, melfloquine</td>
</tr>
<tr>
<td>12</td>
<td>Pv 6,600/µL</td>
<td>0/100 (cynopteri)</td>
<td>6.2/13.7</td>
<td>1/4</td>
<td>Artesunate, melfloquine#</td>
</tr>
<tr>
<td>13</td>
<td>Pf 14,004/µL</td>
<td>2000 (bratislava)</td>
<td>&lt;11/11</td>
<td>1+/2</td>
<td>Azithromycin, quinine</td>
</tr>
<tr>
<td>14</td>
<td>Pf 281/µL</td>
<td>Negative</td>
<td>&lt;11/11</td>
<td>2/2</td>
<td>Chloroquine</td>
</tr>
<tr>
<td>15</td>
<td>Pf 1,276/µL</td>
<td>200/200 (bratislava)</td>
<td>&lt;11/11</td>
<td>2/3</td>
<td>Artesunate, melfloquine</td>
</tr>
<tr>
<td>16</td>
<td>Pf 36/µL</td>
<td>200/200 (bratislava)</td>
<td>&lt;11/11</td>
<td>2/2</td>
<td>Artesunate, melfloquine</td>
</tr>
<tr>
<td>17</td>
<td>Pf 36,448/µL</td>
<td>Negative</td>
<td>&lt;11/11</td>
<td>3/1+</td>
<td>Artesunate, doxycycline</td>
</tr>
<tr>
<td>18</td>
<td>Pm 1,489/µL</td>
<td>Negative</td>
<td>&lt;11/11</td>
<td>3/2</td>
<td>Amoxicillin, chloroquine</td>
</tr>
</tbody>
</table>

* Asexual malaria parasites/microliter on presentation; Pf = Plasmodium falciparum; Pv = P. vivax; Pm = P. malariae.
† Terms in parentheses are serovars of Leptospira.
‡ By PanBio enzyme-linked immunosorbent assay kit, recommended cut-off used for this study ≥11 PanBio units.
§ By INDX Multi-test Diph-Tack® (0–1-negative; 1+-borderline positive; ≥2+-positive).
¶ Doxycycline was added because leptospirosis was clinically suspected.
# Initially misdiagnosed as a mixed infection of P. falciparum and P. vivax.

33 years (range = 20–38). The median age was 34 years (range = 20–58) among the 11 with malaria mono-infection, four of whom were women. Most patients lived in poor socioeconomic conditions, and/or had occupations such as rice farming and rubber tapping, known to be high-risk conditions for zoonotic and vector-borne diseases.

Fever, chill, headache, and myalgia were the most common symptoms among all of our patients, but jaundice was not present. Hepatomegaly (5 cm below costal margin) was detected in a case of probable co-infection (patient 4). Dysuria was present in both the co-infection cases (2 of 7) and the malaria-only cases (2 of 11). No clinical finding allowed classification of the co-infection group from the malaria mono-infection group. As shown in Table 1, the presence of co-infection did not have an impact on the level of parasitemia.

Patients with the co-infection had significantly higher white blood cell counts than the malaria-only patients (median = 7,300/µL versus 4,600/µL; P = 0.0132, by Wilcoxon rank-sum test). On average, our patients had subnormal levels of platelets, with the malaria-only group showing a more depressed level (66,000/µL) than the co-infection group (120,400/µL; P = 0.0236, by t-test). Elevated levels of γ-glutamyltransferase (> 150 units/L; normal = 8–78 units/L) and/or alanine aminotransferase (> 100 units/L; normal = 13–61 units/L in men and 3–42 units/L in women) were more frequently observed in the co-infection group (3 of 7 versus 1 of 11). Both co-infection cases (patient 1 and 2) required four days of hospitalization. Their relatively low-density parasitemia suggested that leptospirosis was predominantly responsible for their clinical presentation. With the exception of two additional cases, both malaria-only cases with relatively high parasitemia (patients 10 and 11), all other patients in our series were treated on an outpatient basis. Overall, our observations suggested a more severe clinical presentation associated with co-infection than with malaria mono-infections. All hematologic and biochemical alterations returned to their normal or nearly normal levels at follow-up.

DISCUSSION
Our observation provides compelling evidence for leptospirosis-malaria co-infection in this Thailand-Myanmar border community and raises dual issues concerning its diagnosis. First, leptospirosis is difficult to diagnose on the spot and diagnostic tools are usually unavailable in remote settings. Second, it is common practice in a malaria-endemic area that if an acutely febrile patient is found to be malaria-positive, malaria is naturally assumed as the sole cause of the fever. Failure to recognize acute leptospirosis co-infection means a delay in the initiation of its proper therapy and possibly ensuing severe complications such as Weil’s syndrome (jaundice and renal failure), pulmonary hemorrhage, and uveitis.15 No severe complications were observed among our cases. Case fatality rates of as high as 1–14% have been reported in hospital series.15,16 However, most cases of leptospirosis do not receive medical attention, are undiagnosed or untreated, and mostly result in self-recovery.
The high IgM levels during the acute phase in the confirmed co-infection cases (patients 1 and 2) with the parallel negative MAT results illustrate the known limitation of the MAT in the early detection of agglutinating antibodies. New, accurate, and rapid techniques may help in improving diagnostic practice of leptospirosis in areas where they are most needed.

No isolation of leptospires was attempted in this study because the primary objective was febrile disease surveillance and leptospirosis had not been realized a priori as a prevalent zoonotic disease in this border region. Leptospirosis in Thailand is known to be largely endemic in the northeastern part of the country. Attempts to culture leptospires will be planned for the future phase of this study.

Confirmation of co-infections with leptospirosis and malaria warrants careful diagnostic evaluation and presents a therapeutic dilemma among febrile patients in Sangkhlaburi. In the case of P. falciparum, artesunate-doxycline therapy, one of the regimens of choice for this region of Thailand known for its high prevalence of multidrug-resistant malaria, will cover both diseases. For P. vivax, and in places where doxycline is not routinely used for the treatment of P. falciparum malaria, prescription of doxycline for a case with any index of suspicion should be considered.

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