Leptospirosis as Cause of Febrile Icteric Illness, Burkina Faso

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Patients in Burkina Faso who sought medical attention for febrile jaundice were tested for leptospirosis. We confirmed leptospirosis in 27 (3.46%) of 781 patients: 23 (2.94%) tested positive using serologic assays and 4 (0.51%) using LipL32 PCR. We further presumed leptospirosis in 16 (2.82%) IgM-positive specimens.

Worldwide, approximately 1 million cases of human leptospirosis occur each year, resulting in >60,000 deaths (1). Although epidemiologic data for Africa are scarce, especially in semi-arid and arid regions, some observations suggest that Leptospira spp. may be more prevalent than previously thought (2). In our study, we tested the hypothesis that leptospirosis is a cause of febrile jaundice in Burkina Faso.

The Study
We conducted the study at Centre Muraz (Bobo Dioulasso, Burkina Faso), a central reference laboratory responsible for the national surveillance of yellow fever. We identified confirmed leptospirosis cases in accordance with World Health Organization criteria (3) by symptoms consistent with leptospirosis and the presence of IgM. Specimens testing negative for serologic and PCR were considered tentative with leptospirosis and the presence of IgM. Specimens testing negative for serologic and PCR were considered negative. We retrospectively tested samples collected during January 2014–July 2015 from adults and children with jaundice and fever ≥38.5°C for the presence of IgM against Leptospira spp. using an in-house ELISA (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/24/8/17-0436-Techapp1.pdf). We assessed serum that tested positive by ELISA for antibodies to Leptospira bacteria in the bacteriology laboratory of Montpellier University Hospital (Montpellier, France), using MAT to confirm the serologic results with a panel of 7 reference serogroups. We also tested for leptospirosis specimens for which a sufficient volume of serum was available by MAT in the French National Reference Center (Paris, France), using a larger panel of 24 serogroups, including the first 7 serogroups (Table 1). We performed real-time PCR for leptospirosis at Centre Muraz using PCR (PUMA LEPTO Kit; Omunis, Clapiers, France) targeting the lipL32 gene, which is present exclusively in pathogenic Leptospira spp. bacteria (4).

Of 781 samples, 45 (5.57%) tested positive for leptospira IgM by ELISA (Figure 1). Among those samples, 23 (2.94%) were positive by MAT (≥1:400); consequently, these cases were considered to be confirmed. We considered 6 samples tested negative by MAT and 16 samples with MAT titer ranging from 1:100 to 1:200 (combined, 2.82%) to be presumptive cases. MAT results suggested the existence of multiple serogroups (Table 2), including reacting serogroups Australis, Ballum, Canicola, Grippotyphosa, Icterohaemorrhagiae, Pomona, and Sejroe. In addition, we performed MAT in the Leptospirosis National Reference Laboratory using a larger panel of serogroups applied to 33 ELISA-positive samples. Ten samples tested positive, and we confirmed the presence of all except the Ballum serogroup (data not shown). In 1 sample, we were able to identify Mini as an additional serogroup with a 1:400 titer. In addition to the serologic test, we confirmed leptospirosis cases by lipL32 PCR in 4/781 (0.51%) samples. All were negative for IgM, but 3 had optical density just above the positive threshold; signal to mean value of the negative controls was between 2 and 3 (data not shown). Hence, screening by serologic assay plus PCR identified a total of 27 (3.46%) cases of confirmed leptospirosis.

Median age for all patients was 20 years (interquartile range [IQR] 12–30 years); 61% were male (p = 0.65 by χ² test). We observed the highest number of confirmed cases in the age group 10–19 years (data not shown), but the frequencies were not significantly different when cases were analyzed by age group (p = 0.41 by χ² test). This observation was not unexpected because the population of...
BURKINA FASO IS YOUNG; ALMOST TWO-thirds OF THE POPULATION IS <25 YEARS OF AGE. THERE WAS NO PARTICULAR GENDER DISTRIBUTION FOR PERSONS WITH CONFIRMED CASES (13 WOMEN AND 14 MEN; \( p = 0.33 \) BY \( \chi^2 \) TEST). THE REPARTITION OF CONFIRMED, PRESUMPTIVE, AND NEGATIVE CASES ACCORDING TO RAINY SEASON (MAY–MID-OCTOBER) VS. DRY SEASON FROM MID-OCTOBER–APRIL WAS UNEQUAL (\( p = 0.0035 \) BY \( \chi^2 \) TEST), WITH A TREND FOR A HIGHER PROPORTION OF CONFIRMED CASES AMONG SAMPLES RECEIVED DURING THE RAINY SEASON WHEN COMPARED WITH NEGATIVE CASES (\( p = 0.065 \) BY \( \chi^2 \) TEST; FIGURE 2).

Our data were in line with a recent publication estimating that some of the West Africa countries, including those in semiarid regions, may have among the highest rates of disability-adjusted life years due to leptospirosis; in Burkina Faso, the rate may be 60–70/100,000 population/year (5). Leptospirosis infections have been reported in various parts of West Africa in humans (6–10). Studies in Senegal and Mali have shown that cattle, pigs, and sheep are frequently infected (11,12). Detection of leptospirosis was also recently reported in rodents in Niamey.

**Figure 1.** Flowchart used in study of leptospirosis in persons who sought medical attention for febrile jaundice, Burkina Faso. MAT, microscopic agglutination.
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Niger, especially in urban agricultural settings (13). In Burkina Faso, agricultural and livestock sectors represent 30% of the gross domestic product and are the backbone of the economy with ≈80% of the working-age population involved in these activities (14). Hence, human exposure to *Leptospira* spp. bacteria is probably frequent. Studies conducted in Ghana on patients with febrile illness without an obvious cause of disease found a frequency of 3.2% of confirmed leptospirosis cases and 7.8% of probable cases among icteric patients (2). In our study, half of the probable leptospirosis cases characterized by clinical signs consistent with leptospirosis and screened positive for *Leptospira* IgM were confirmed by MAT with a titer >1:400; two thirds had a titer >1:100 that may also be leptospirosis cases. Collecting and testing a convalescent serum sample might have confirmed the presumptive cases. In addition, the MAT has been shown to be less sensitive than IgM detection using ELISA, especially in acute-phase serum samples. In addition, 2 instances of seroreactivity against Ballum serogroup observed in the first MAT performed in the Montpellier University Hospital were not confirmed by using an enlarged panel in the National Center for Leptospirosis. This finding may be related to prolonged sample storage and multiple freeze/thaw cycles before testing in the national reference laboratory.

**Conclusions**

Leptospirosis appears to be an important cause of febrile jaundice in Burkina Faso, suggesting that leptospirosis is probably endemic in this country. Further studies are required to explore animal reservoirs and occupational risk factors associated with human leptospirosis. Awareness of leptospirosis among clinicians, funding for further study, and the possibility of conducting laboratory tests in the field are needed to clarify the extent of the problem in sub-Saharan Africa.

**About the Author**

Dr. Zida works at the Muraz Center as a pharmacist. She is currently preparing a PhD at the University of Montpellier as a member of the U1058 INSERM team.

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<table>
<thead>
<tr>
<th>Serogroup</th>
<th>No. positive, N = 23</th>
<th>Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ballum</td>
<td>11 (††)</td>
<td>1:400–1:1,600</td>
</tr>
<tr>
<td>Grippotyphosa</td>
<td>4 (†‡)</td>
<td>1:400–1:1,600</td>
</tr>
<tr>
<td>Australis</td>
<td>2</td>
<td>1:400 and 1:800</td>
</tr>
<tr>
<td>Canicola</td>
<td>1 ††</td>
<td>1:400 and 1:800</td>
</tr>
<tr>
<td>Sejroe</td>
<td>1††</td>
<td>1:400</td>
</tr>
<tr>
<td>Icterohaemorrhagiae</td>
<td>1</td>
<td>1:800</td>
</tr>
<tr>
<td>Pomona</td>
<td>1</td>
<td>1:400</td>
</tr>
<tr>
<td>Mini</td>
<td>1§</td>
<td>1:400</td>
</tr>
</tbody>
</table>

*Values in parentheses represent duplicates, which are not included in the total count.

††Titers were equal for serogroups Ballum and Sejroe.

†‡Titers were equal for serogroups Grippotyphosa and Canicola.

§Titers were equal for serogroups Mini and Sejroe.

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Figure 2. Confirmed cases of leptospirosis among samples received in Centre Muraz within the national network for yellow fever surveillance in Burkina Faso, January 2014–July 2015. White bars indicate months of the dry season, gray bars months of the rainy season. Numbers above bars indicate number of confirmed leptospirosis and the number of specimens tested.
References


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Technical Appendix

IgM ELISA

We tested serum samples for the presence of IgM antibodies directed against *Leptospira* spp. by an in-house ELISA performed in Centre Muraz. The assay used an antigenic preparation of *Leptospira interrogans* serovar icterohaemorrhagiae provided by the French National Reference Centre for Leptospirosis at Pasteur Institute. Briefly, we inactivated cultures of bacteria at 56°C during 30 minutes, centrifuged them, and washed them in NaN₃ alkaline buffer. We centrifuged bacteria at 30 minutes at 4000 rpm and washed them with phosphate-buffered saline, and resuspended the pellets in NaHCO₃ alkaline buffer. We coated polystyrene microtiter plates (Falcon Dominique Dutscher, Issy-les-Moulineaux, France) overnight at 4°C with 200 µL of *Leptospira* spp. suspension. On the second day, we rinsed the plates twice with NaHCO₃ alkaline buffer and repeated the coating step once in the same conditions. After washing, we packaged the plates and stored them at −20°C until use. The antigen-covered plates were stable for 3 months. We used a phosphate buffered saline (PBS)/Tween buffer (manufactured in-house) containing milk to make 1:100-fold dilution of the serum samples. We dispensed 200 µL per well, incubated them for 1 hour at 37°C, and washed them 3 times in NaCl/Tween buffer (manufactured in-house). We added alkaline phosphatase conjugated goat anti–human IgM (Rockland TEBU BIO, Rockland Inc., Limerick, PA, USA) at a dilution of 1:1,600 in PBS/Tween and incubated for 1 hour at 37°C. After washing 3 times, we added 200 µL of substrate (4 nitrophenyl phosphate [Reference A12310.06] Alfa Aesar, A Johnson Matthey Company, Heysham, UK) for 15 min at room temperature. We measured optical density at 405 and 630 nm. We used 5 negative controls and 2 positive controls for each microtiter plate. We calculated the positive threshold based on the value of negative controls (mean value × 3). Hence, samples with ratio of signal to mean value of the negative controls >3 were considered positive.