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Case Report: Molecular Detection of *Leptospira* in Two Returned Travelers: Higher Bacterial Load in Cerebrospinal Fluid versus Serum or Plasma

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Abstract. Leptospirosis is a potentially severe illness in returned travelers. Patients often present with fever, headache, and neck pain, which may lead to a workup for meningitis including the acquisition of cerebrospinal fluid (CSF). Although *Leptospira* DNA has been detected in CSF by polymerase chain reaction (PCR), little data exist regarding the utility of testing CSF in addition to serum or plasma obtained on presentation. In this report, we present two cases of leptospirosis in returned travelers presenting with fever and headache. Our first patient had neutrophilic meningitis, and *Leptospira* was detectable only in CSF obtained on admission. The second patient had a normal CSF profile, but *Leptospira* was detected in CSF at a bacterial load 5- to 10-fold higher than that in plasma. CSF is an important specimen for the diagnosis of *Leptospira* by molecular methods and may yield an actionable diagnosis in the absence of leptospiremia.

INTRODUCTION

Leptospirosis is a potentially fatal infection caused by bacteria of the genus *Leptospira*. Human infections result in a range of clinical manifestations, including fever, headache, and myalgia.[1] Neurologic manifestations of acute leptospirosis have been well documented since the first report of meningitis in 1910.[2,3] Meningitis typically occurs late in the clinical course of infection and may be quite common in severe disease.[1–3] Given this clinical presentation, returned travelers with leptospirosis often undergo lumbar puncture during their evaluation.[4] In a review of 1,994 hospitalized leptospirosis cases in the United States, lumbar puncture was performed in 20.5% patients and was the most common procedure reported.[5]

Diagnostic confirmation of leptospirosis relies on the availability of accurate laboratory tests. Microscopic agglutination testing (MAT) remains the reference standard, and assays for the detection of anti-*Leptospira* immunoglobulin M (IgM) are available.[6,7] However, MAT requires acute and convalescent serum, and IgM antibodies do not reliably develop until the second week of illness.[7] The specificity of serological testing in endemic areas is also affected by the persistence of anti-*Leptospira* antibodies for months after acute infection; this is particularly problematic if only a single specimen is obtained.[1] Molecular testing of whole blood or serum has proven more sensitive than serological methods for diagnosing acute leptospirosis,[8,9] and *Leptospira* nucleic acids have been detected in cerebrospinal fluid (CSF) during human infections.[9–13] However, the benefit of performing molecular testing on CSF in addition to serum or plasma is not clear from published accounts. In this report, we present two cases of returned travelers with leptospirosis who had molecular testing performed on CSF and paired serum or plasma. These cases demonstrate the potential utility of CSF testing for acute leptospirosis, even in the absence of meningitis or leptospiremia.

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CASE 1

In August 2014, a 23-year-old male presented with fever and headache. He had no significant medical history, but 4 days earlier he had returned from a trip to Thailand, Cambodia, and Bali. During his trip, he went rafting, hiked in the jungle, and was thrown by an elephant into a large pond. Six days before presentation, he began having fever and chills. He subsequently developed headaches, myalgia, nausea, vomiting, and loose stools. On the day of presentation, his headache worsened, and he developed a stiff neck and photophobia. In the emergency department, he had a temperature of 38.2°C, pulse of 84, blood pressure of 108/73, and room air oxygen saturation of 97%. He was alert and oriented and expressed pain when touching his chin to his chest and rotating his head. The remainder of his physical exam, including his neurological exam, was unremarkable. Lumbar puncture was performed in the emergency room. Results of laboratory studies are shown in Table 1. Empiric treatment was started with vancomycin, ceftriaxone, acyclovir, and doxycycline.

The patient's serum and CSF, both obtained on hospital day 1, were tested in a *Leptospira* polymerase chain reaction (PCR) targeting the 16S rRNA gene.[14,15] This internally controlled assay detects all *Leptospira* species and differentiates pathogenic species. His CSF tested positive for pathogenic *Leptospira*, though his serum tested negative. Results were repeated on a second run. Anti-*Leptospira* IgM was detected in serum obtained on hospital day 3 (ARUP Laboratories, Salt Lake City, UT). As the patient improved, antibiotics were narrowed to doxycycline alone on hospital day 3, and he was discharged home on day 6.

CASE 2

In December 2014, a 27-year-old, previously healthy male presented with 4 days of fever and diffuse myalgia. One week before admission, he had returned from Colombia and Costa Rica where he had spent 3 weeks surfing, rafting, and hiking. Four days before admission, he developed fever and myalgia in his neck and shoulders. Two days before admission he developed nausea, vomiting, retro-orbital headache associated

TABLE 1
Results of laboratory studies obtained at patient presentation

Laboratory test	Case 1	Case 2
Whole blood, serum, or plasma		
White blood cell count, 10^3 cells/μL	7.3	9.5
Hemoglobin, g/dL	13.7	15.4
Platelet count, 10^3 cells/μL	238	115
Creatinine, mg/dL	1.0	2.3
Glucose, mg/dL	127	112
Bilirubin, total, mg/dL	0.5	2.2
Alkaline phosphatase, units/L	88	153
Aspartate transaminase, units/L	38	61
Alanine transaminase, units/L	52	136
Blood culture	No growth	No growth
Malaria peripheral smear and BinaxNOW	Negative	Negative
Human immunodeficiency virus		
Fourth-generation screen	Not tested	Negative
Quantitative RT-PCR	Not detected	Not detected
West Nile virus IgM	Negative	Not tested
Real-time RT-PCR for dengue, yellow fever, chikungunya, Zika, West Nile, and Japanese encephalitis viruses	Not detected	Not detected
CSF		
Description	Clear	Clear
White blood cell count, cells/μL	75	1*
Neutrophils, %	90	–
Lymphocytes, %	9	–
Protein, mg/dL	50	28
Glucose, mg/dL	61	75
Gram stain	No organisms	No organisms
Culture	No growth	No growth
West Nile virus IgM	Negative	Not tested
Real-time RT-PCR for dengue, yellow fever, chikungunya, Zika, West Nile, and Japanese encephalitis viruses	Not detected	Not detected
Herpes simplex virus PCR	Negative	Not tested

CSF = cerebrospinal fluid; IgM = immunoglobulin M; RT-PCR = reverse transcriptase polymerase chain reaction.
*White blood cell differential was not performed.

with photophobia, and a faint rash over his upper chest. On presentation, the patient was febrile to 39.0°C and tachycardiac; other vital signs were normal. He was alert and oriented. He had subconjunctival hemorrhage and a faint erythematous, macular rash over his upper chest and bilateral upper extremities. The remainder of his physical exam, including the neurologic exam, was normal. A lumbar puncture was performed given concerns for possible meningitis. Results of initial clinical laboratory studies are shown in Table 1. He was initially treated with vancomycin and piperacillin–tazobactam; on hospital day 2, antibiotic coverage was narrowed to ceftriaxone for empirical treatment of leptospirosis and typhoid.

CSF and plasma, obtained on admission, tested positive for pathogenic *Leptospira* by PCR. The bacterial load in CSF, as measured by quantitative PCR, was 5- to 10-fold higher than that in plasma. Whole blood, serum, and plasma obtained on hospital day 2 (after 24 hours of antibiotic treatment) were negative for *Leptospira*. Serologic testing for anti-*Leptospira* IgM was negative. The patient improved over 3 days in the hospital and was discharged home on doxycycline.

DISCUSSION

This report describes two cases of leptospirosis in returned travelers, which highlight the utility of molecular testing for *Leptospira* in CSF. In Case 1, *Leptospira* was detected only in CSF and not in serum. Timely diagnosis allowed for de-escalation of antibiotic therapy, and without testing CSF, the diagnosis may have been missed. Although several reports have documented *Leptospira* DNA detection in the CSF, we are aware of only one report, by Wilson and others, describing such discordant results in CSF and serum or plasma obtained during one clinical encounter. However, the unusual history in this case, which included a 7-month clinical course in a 14-year-old with severe combined immunodeficiency, limits the generalizability of their findings.[16] In two reports from São Paulo, Romero and others detected DNA in 39.8% and 59.0% of CSF samples from cases of aseptic meningitis using a 16S *Leptospira* PCR.[11,12] Molecular testing was not performed on other specimen types, though, to determine whether there was added benefit to evaluating the CSF for leptospirosis. A recent publication from Laos identified six cases of *Leptospira* meningitis with positive CSF PCR results.[10] The majority of these patients appear to have had negative PCR results from whole blood, but individual test results are not specifically reported.

The findings in Case 1 could have resulted from false-positive *Leptospira* detection in the CSF, though the diagnosis was eventually supported by serological results. To determine the specificity of the *Leptospira* PCR, we screened 136 CSF samples that were sent to the Stanford Clinical Virology Laboratory (Palo Alto, CA) for herpes simplex virus PCR testing. One sample was positive for pathogenic *Leptospira* (specificity, 99.3%). This sample was obtained from a 15-year-old male admitted with altered mental status 8 days after an acute febrile illness. He had been residing in a homeless shelter at the time of his illness. Unfortunately, no other specimens were available for testing. Quantitative PCR results from Case 2 provide another plausible explanation for the discordant results observed in the first case. In Case 2, *Leptospira*

was detected in the CSF at a bacterial load 5- to 10-fold higher than that in plasma. Although CSF was not necessary for the diagnosis in this case, higher bacterial loads in CSF may facilitate further evaluation of the causative *Leptospira* strain or simply improve detection rates if less sensitive assays are used.[17]

In conclusion, we report two cases of leptospirosis where the diagnosis was made or supported by molecular testing using CSF. These cases support the use of specific *Leptospira* molecular tests on CSF, which is frequently obtained during the workup of patients with leptospirosis, regardless of the CSF profile. Even with increasingly sensitive molecular diagnostics for *Leptospira*, CSF may be the only specimen that yields an actionable diagnosis.

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