## RESEARCH LETTERS

# Visceral Leishmaniasis: Clinical Observations in 4 US Army Soldiers Deployed to Afghanistan or Iraq, 2002-2004

People who traveled to Central or Southwest Asia in support of Operation Enduring Freedom and Operation Iraqi Freedom may have been exposed to various diseases endemic to this region.[1] Some of these conditions have long incubation periods and may not present for weeks or months after travelers have returned to the United States. Among such diseases is visceral leishmaniasis (VL). Visceral leishmaniasis is a chronic systemic disease caused by parasites of the *Leishmania donovani–Leishmania infantum* complex. It is classically described as a syndrome of fever, progressive spleen and/or liver enlargement, weight loss, pancytopenia, and, if untreated, possible death. Most cases are acquired via the bite of infected female sand flies, but on rare occasions, disease has been acquired by other means, most notably by blood transfusions.[2]

This case series describes 4 cases of VL and is intended to alert clinicians to the possibility of VL in patients who develop a febrile illness after returning from deployment to Central or Southwest Asia.

**Methods.** A retrospective review was performed using medical records of patients diagnosed as having VL at the Walter Reed Army Medical Center (WRAMC), a centralized military leishmaniasis treatment center in Washington, DC. Military disease surveillance systems were queried, and it was confirmed that no other cases had been reported in this time interval.

Routine clinical laboratory studies were performed at WRAMC and local military medical facilities. Histopathological samples were reviewed at the Armed Forces Institute of Pathology (AFIP), Washington, DC. Polymerase chain reaction (PCR) assays designed to amplify a portion of the ribosomal RNA gene to detect *Leishmania*,[3] rK39 dipstick assays (Kalazar Detect; InBios, Seattle, Washington),[4,5] and *Leishmania* cultures were performed at the Walter Reed Army Institute of Research (WRAIR), Silver Spring, Maryland. Anti-*Leishmania* serum indirect fluorescent antibody tests were performed at the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, with IgG titers of 1:16 or greater considered positive.

**Results.** The patients were male, aged from 23 to 39 years (**Table**). One patient was white, and 3 patients were African American. The median time to onset of symptoms after returning to the United States was 6 months (range, 3-9 months) in 3 patients; 1 patient (patient 3) became ill while still in Iraq at 11 months. Two patients acquired their infections in Afghanistan, and 2 were infected in Iraq. Of note, patient 3 denied traveling anywhere outside of "The Green Zone" in Baghdad, Iraq. Despite receiving prevention briefings prior to deployment that reviewed personal protective measures such as the proper wear of clothing and use of insect repellents, permethrin and diethyltoluamide, all patients reported limitations using these measures during their travel.

In all patients, the initial presentation included symptoms of fever, chills, and night sweats; other symptoms included cough, weight loss, anorexia, nausea, abdominal pain, and diarrhea. The median time from onset of symptoms to definitive diagnosis was 6 weeks (range, 4-8 weeks).

Physical examination findings included hepatomegaly or splenomegaly. Laboratory test results demonstrated pancytopenia and elevated liver-associated enzymes. All patients had positive serologic results when tested with the rK39 antibody dipstick assay. *Leishmania* amastigotes were visualized on histopathological examination of bone marrow samples in 2 patients and in liver biopsy samples from 2 patients whose preceding bone marrow aspirates were negative. In addition, findings from PCR analysis of tissue biopsy samples were positive in 2 patients. This testing was performed on bone marrow biopsy samples first; in 1 patient whose bone marrow sample was negative for *Leishmania*, the result of subsequent PCR testing of a liver biopsy specimen was positive.

Three patients were treated with liposomal amphotericin B (AmBisome; Gilead Sciences Inc, Deerfield, Illinois), with total dosages ranging from 21 to 30 mg/kg, resulting in a curative response in each. Patient 2 was treated with amphotericin B lipid complex (Abelcet; Enzon Pharma Inc, Indianapolis, Indiana), 5 mg/kg/d for 5 days, with an additional dose on day 10. This patient experienced an initial clinical improvement, but relapsed 4 days later with the return of symptoms including fevers, abdominal fullness, and right upper quadrant tenderness. He was then treated with a 28-day course of sodium stibogluconate (Pentostam; GlaxoSmithKline, Brentford, England) under an Investigational New Drug (IND) protocol.

**Comment.** Large numbers of Americans have traveled to Central or Southwest Asia during military deployments. Some of these individuals may have been bitten



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**Table. Characteristics of Patients Diagnosed as Having Visceral Leishmaniasis**

| Characteristic | Patient 1 | Patient 2 | Patient 3 | Patient 4 |
|---|---|---|---|---|
| Sex | Male | Male | Male | Male |
| Race | White | AA | AA | AA |
| Age, y | 32 | 39 | 23 | 35 |
| Location | Afghanistan | Afghanistan | Iraq | Iraq |
| $T_{max}$, °C | 40.0 | 40.0 | 39.5 | 40.0 |
| Chills/night sweats | Yes | Yes | Yes | Yes |
| Weight loss, kg | 5.85 | 11.25 | 9.45 | 16.65 |
| Splenomegaly | Yes | Yes | Yes | Yes |
| Hepatomegaly | No | Yes | No | Yes |
| Hct, % | 38.5 | 25 | 26 | 38 |
| WBC count, cells/μL | 2500 | 2700 | 2100 | 4300 |
| Platelet, cells/μL | 146 000 | 154 000 | 82 000 | 102 000 |
| AST, IU/L | 372 | 1308 | 81 | 97 |
| ALT, IU/L | 495 | 1012 | 95 | 125 |
| Alk Phos, IU/L | 450 | 211 | 150 | 70 |
| Alb, g/dL | 3.7 | 2.5 | 2.6 | 3.8 |
| Serology | | | | |
| rK39 Ab | Positive | Positive | Positive | Positive |
| IFA titer | 1:1024 | 1:4096 | 1:512 | 1:20 000 |
| Bone marrow biopsy | | | | |
| Histopathology | Negative | Negative | Positive | Positive |
| Culture | Negative | Negative | Positive | Not done |
| PCR | Negative | Negative | Positive | Not done |
| Liver biopsy | | | | |
| Histopathology | Positive | Positive | Not done | Not done |
| Culture | Not done | Negative | Not done | Not done |
| PCR | Negative | Positive | Not done | Not done |

Abbreviations: AA, African American; Ab, antibody; Alb, albumin; Alk Phos, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Hct, hematocrit; IFA, indirect fluorescent antibody; PCR, polymerase chain reaction; $T_{max}$, temperature maximum; WBC, white blood cell.

SI conversion factors: To convert AST, ALT, and Alk Phos to microkatals per liter, multiply by 0.0167; to convert Alb to grams per liter, multiply by 10.

by a female sand fly harboring *Leishmania* parasites capable of causing VL. The clinical course following infection may be asymptomatic infection, an acute self-limited febrile illness, or a prolonged nonspecific systemic illness that may or may not progress to overt VL.[6-9] All 4 patients in this case series presented with nonspecific symptoms of fever, chills, and night sweats, which led physicians to develop a very broad differential diagnosis that included many diseases other than VL, including malignant tumors. Patients in this series underwent numerous costly, time-consuming, invasive, and noninvasive diagnostic tests, resulting in delays of diagnosis and treatment for several weeks.

The diagnosis of VL should be suspected based on epidemiologic and clinical features including chronic fever, continued weight loss, weakness, hepatosplenomegaly, anemia, leukopenia and hypergammagloblinemia.[9] Definitive diagnosis requires tissue biopsy (bone marrow, liver, or spleen) with histological evidence of the nonflagellated amastigote, growth of the promastigote form in culture, or the identification of *Leishmania* DNA with molecular assays. The sensitivity of these diagnostic methods varies,[10] so consultation with expert laboratories may be of assistance.

A relatively new, commercially available, serodiagnostic assay is the rK39 antibody dipstick test. This assay uses the recombinant *Leishmania chagasi* antigen rk39 to identify the presence of host antibodies to the parasite's dominant, kinesin-like amastigote antigen. A positive result from this immunoassay is highly sensitive and specific for active VL in immunocompetent individuals.[4,5,11]

The only drug currently approved by the US Food and Drug Administration (FDA) for the treatment of VL is liposomal amphotericin B (AmBisome).[12] For immunocompetent patients, the FDA-approved dosage regimen is 3.0 mg/kg/d, to be given on days 1 to 5, 14, and 21. Of note, amphotericin B lipid complex (Abelcet) is not FDA-approved for this indication. When used in 1 patient in this series, as noted previously, it failed as initial therapy. An acceptable alternative treatment option is the pentavalent antimonial drug, sodium stibogluconate (Pentostam),[13] which is available under an IND protocol at WRAMC for military patients and from the CDC for civilian patients.

In conclusion, although not a common disease in returning travelers, VL has been diagnosed in 4 American servicemen returning from Central or Southwest Asia. The rK39 serologic test has proven extremely helpful in the evaluation of suspected VL, but confirmation of the diagnosis with parasitological methods (ie, tissue biopsy with histopathologic analysis, culture, or PCR) is considered the standard of care. Clinicians evaluating travelers returning from endemic regions who present with symptoms suggestive of VL are encouraged to contact the CDC (telephone: 770-488-7760) or (for Department of Defense beneficiaries) the Infectious Disease Service at WRAMC (telephone: 202-782-1663) for



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assistance with the diagnosis and treatment of patients with this disease.

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## Cytokine Profile in Fatal Human Immunodeficiency Virus–Tuberculosis–Epstein-Barr Virus–Associated Hemophagocytic Syndrome

Hemophagocytic syndrome (HPS), characterized by fever, lymphadenopathy, hepatosplenomegaly, and pancytopenia, results from the abnormal function and proliferation of macrophages and their uncontrolled phagocytosis of various reticuloendothelial cell lines. Secondary (or "reactive") HPS is associated with infection, malignancy, or autoimmune diseases, whereas primary HPS has no identifiable cause and may be genetic. Reactive HPS has been described in association with infectious agents, but Epstein-Barr virus (EBV) is the most commonly associated infection, and EBV-associated HPS is almost universally fatal.[1] At present, there is no diagnostic or treatment consensus, so HPS is generally dealt with on a case-by-case basis, depending on the associated infection(s) identified.

**Report of a Case.** A 46-year-old Chinese man diagnosed as having human immunodeficiency virus (HIV) infection 8 months earlier presented with fever, lower back pain, bilateral lower limb numbness, and foot drop. He had been receiving highly active antiretroviral treatment in the 4 months before admission, during which his CD4 cell count had increased (37/μL to 204/μL) and his HIV load decreased (130 000 to 820 copies/mL). A magnetic resonance image of his lower spine revealed an epidural mass over the L5 lamina, compressing the thecal sac, and findings from a computed tomography–guided biopsy showed caseating granulomatous inflammation, although mycobacterial cultures were negative. Antimycobacterial therapy was started.

Progressive pancytopenia with high fever (38.8°C) developed over the next 4 weeks. A bone marrow examination confirmed a histological diagnosis of HPS. Again, results from mycobacterial staining and cultures were negative. A real-time quantitative EBV assay[2] showed



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