

Case Report: Autochthonous Case of Human Visceral Leishmaniasis in the West Bank, Palestine

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Abstract. Human visceral leishmaniasis (HVL) is a parasitic disease infecting children in the Mediterranean region. Here, we portray a case of a 2-year-old child with an epidemiological description of the situation surrounding the case. The patient was suffering from recurrent fever, weakness and abdominal discomfort associated with loss of appetite. Routine blood investigations showed pancytopenia, whereas examination revealed hepatomegaly. A diagnosis of HVL was made by demonstrating amastigotes in a Giemsa-stained smear from a bone marrow aspirate followed by genotyping by PCR and sequencing. In conclusion, early detection of VL infection followed by appropriate treatment protocols is essential to saving the patient.

INTRODUCTION

Visceral leishmaniasis (VL) in the Mediterranean Basin is a zoonotic disease caused by the protozoan parasite *Leishmania infantum* which, if not treated, is fatal to humans and canids. Visceral leishmaniasis caused by *L. infantum* is transmitted by the bite of 20 female sandfly species, seven of which have been reported in the northern and southern Mediterranean regions including *Phlebotomus larrousius*, *Phlebotomus perniciosus*, *Phlebotomus syriacus*, *Phlebotomus ariasi*, *Phlebotomus neglectus*, *Phlebotomus tobbi*, and *Phlebotomus perfiliewi*.^{1–6} Dog is the main reservoir and host showing symptoms ranging from mildly asymptomatic to apparently severe manifestations.⁷ Around 2.5 million dogs are estimated to be infected with *L. infantum* in the Mediterranean region.⁸ A study in Palestine showed that 17% of the domestic dogs suffered from canine VL caused by *L. infantum*.⁹ Human visceral leishmaniasis (HVL) commonly causes prolonged high-grade fever (up to 40°C), paleness, and hepatosplenomegaly. Other symptoms include anorexia, irritability, rhinorrhea, vomiting, and diarrhea.¹⁰ Laboratory findings in HVL cases include pancytopenia, neutropenia, thrombocytopenia, microcytic hypochromic anemia, elevated C-reactive protein (CRP) (> 100 mg/L), and erythrocyte sedimentation rate (ESR) (> 40 mm/hour).¹⁰ Approximately 50,000–90,000 new cases of HVL are estimated to occur worldwide each year, out of which only an estimated 25–45% is reported. More than 95% of new cases occurred in 10 countries: Bangladesh, Brazil, China, Ethiopia, India, Kenya, Nepal, Somalia, South Sudan, and Sudan.¹¹ In the Mediterranean region, VL is reported in 25 countries, with a total of 875 reported cases and an estimated number of cases ranging between 1,200 and 2,000 per year. In Palestine, VL is rare and only sporadically affecting children.^{9,12,13} Three of the seven potential sandfly vectors of *L. infantum* in the Mediterranean area have been caught in Palestine.¹⁴ In this study, we report a case of VL caused by *L. infantum* in a rural area in Palestine and investigate the life cycle components, vector, and reservoir, in the patient's vicinity.

METHODS

Bone marrow aspirate was taken from the back of the pelvic bone (upper iliac crest) of the patient in Beit-Jala government hospital as part of the routine diagnostic procedure as described by Bain.¹⁵ Blood was collected by venipuncture of the median cubital vein in the antecubital fossa antecubital vein for blood chemistry and hematology profile according to protocol. One drop of bone marrow aspirate was spread on a slide as thin film, air-dried, fixed in absolute methanol, and Giemsa-stained as described by others.¹⁶ The unstained bone marrow on one of the slides was scrapped off and DNA-extracted using Qiagen kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and kept at –20°C until use. PCR was used to amplify the internal transcribed spacer 1–PCR as described earlier.^{17–19} Amplicon was sent for commercial sequencing. The DNA sequence was used to identify species of leishmania by BLAST search against leishmania species in the GenBank (www.ncbi.nlm.nih.gov) database. Blood samples were collected from dogs in the patient's village (Beit Ola in Al-Khalil village) and its vicinity and processed as described earlier.⁹ Dogs' sera were tested for the presence of anti-leishmanial antibodies by ELISA as described elsewhere.⁹ Sand flies were collected from the patient's house using aspirators. Collection was conducted early morning in the month of June during the biting season. Sandfly identification and classification were conducted using conventional keys.¹⁴ Leishmanial infection rate and identification of leishmania species in sand flies were conducted simultaneously by next-generation sequencing using Illumina MiSeq next-generation sequencer as described previously.^{20,21}

CASE PRESENTATION

A 2-year-old female child from a mountainous rural area in Palestine was referred to the local government hospital in September 2018 after 2 months of suffering from prolonged high fever (41°C) and loss of appetite. Physical examination confirmed by ultrasonography manifested hepatomegaly of 12.3 cm (normal is 9 cm). In the laboratory, complete blood count revealed pancytopenia: hemoglobin level of 7.0 (reference range: 11.5–15.5 g/dL), hematocrit of 22.3 (reference range: 33–49%), erythrocyte count of $3.27 \times 10^6/\mu\text{L}$ (reference range: $3.7\text{--}5.3 \times 10^6/\mu\text{L}$), leukocyte count of $5.0 \times 10^3/\mu\text{L}$

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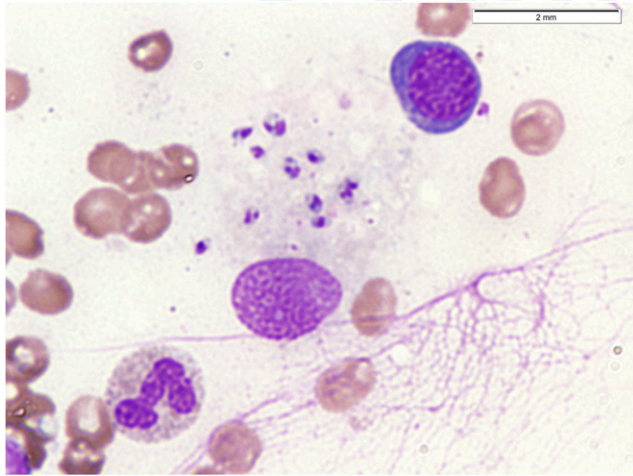


FIGURE 1. Giemsa-stained bone marrow smear showing intracellular amastigotes as pale-blue oval bodies, 2–5 μm in diameter, with a single violet-blue nucleus. The point-shaped kinetoplast is also evident. This figure appears in color at www.ajtmh.org.

(reference range: $6\text{--}17 \times 10^3/\mu\text{L}$), and thrombocyte count of $90.0 \times 10^3/\mu\text{L}$ (reference range: $140\text{--}400 \times 10^3/\mu\text{L}$), mean corpuscular volume of 68.2 (reference range: 70–86 fL), mean cell hemoglobin of 21.4 (reference range: 23–31 pg), and mean corpuscular hemoglobin concentration of 31.4 (reference range: 30–36%). C-reactive protein was high, reaching 159 (0–10 mg/L). Liver enzymes (alanine transaminase and aspartate aminotransferase) were normal. Kidney function tests (blood urea nitrogen and creatinine) were also normal. Serum electrolytes that included sodium, potassium, calcium, and magnesium were normal. Extracellular amastigotes were demonstrated in the Giemsa-stained bone marrow film, indicating a VL (Figure 1). The BLAST search of the isolated DNA sequence against leishmania sequences in the GenBank (www.ncbi.nlm.nih.gov) database revealed *L. infantum* as the causative agent. The isolated *L. infantum* sequence was deposited in GenBank under accession number MK311268. The patient was put on 2 mL/day intravenous Pentostam™ therapy (sodium stibogluconate) for 29 days, which is usually given at a rate of 0.1–0.2 mL (10–20 mg Sb^{5+})/kg for a

maximum of 8.5 mL Pentostam for 20 days. Imipenem, a potent broad-spectrum carbapenem, was given intravenously for 6 days. After 20 days of hospitalization, the hemoglobin level increased to 12.2 g/dL, erythrocytes 5.01, and leucocytes 6.1. However, platelet count remained low, whereas CRP (114 mg/L) and ESR (40 mm) were high. The erythrocyte sedimentation rate is falsely elevated because of severe anemia. Clinical chemistry profiles that included kidneys, heart, and liver function tests and serum electrolytes were performed regularly during the Pentostam therapy (Table 1).

After completion of treatment, the hemoglobin level increased to 13.5 g/dL, thrombocytes to $150,000/\mu\text{L}$, and liver size reverted to normal. Further investigation to pinpoint the source of infection in the village was conducted. The investigation targeted the components of the life cycle, namely, the sandfly vector and the reservoir/host dogs. Seventeen sandflies were collected from the indoor corners of the patient's house using a sandfly aspirator. At the same time, serum was collected from 39 dogs. All sand flies were females except two males, with 16 of them being *Phlebotomus papatasi*, the vector for *Leishmania major*. One belonged to *Sergentomyia dentata*. None of the sand flies contained *L. infantum* parasite DNA in their gut. Of the 39 dog sera, six (15.4%) were positive for antileishmanial antibodies. Three of these seropositive dogs showed at least one symptom compatible with VL, whereas the other three were asymptomatic. Seropositive dogs were quelled by the Palestinian Ministry of Health in Palestine.

DISCUSSION

This autochthonous HVL case report confirmed, by DNA sequencing, *L. infantum* as the cause of HVL in Palestine (GenBank: MK311268). Human visceral leishmaniasis has declined from 18 cases in 1996 to three cases in 2017 at an annual incidence rate ranging from 0.6 to 0.02 cases per 100,000 within the same period.¹³ The findings in this report are in agreement with previous studies on canine VL in the same area in which canine VL has been proven to be caused by *L. infantum* parasites at a seropositivity rate of 17%.^{9,12,22,23} The distribution of the cases was mainly in the rural areas of the western side of the West Bank and affecting

TABLE 1
Clinical chemistry profiles for the visceral leishmaniasis patient during Pentostam therapy

Test	Patient range	Reference value	Flag
Alanine transaminase	10–21.3	0–33 U/L	Normal
Aspartate aminotransferase	22.8–31.4	0–37 U/L	Normal
Lactate dehydrogenase	303–380	180–430 U/L	Normal
Creatine kinase, total	20–24	0–170 U/L	Normal
Creatinine	0.17–0.63	0.5–0.9 mg/dL	Normal
Blood urea nitrogen	5.3–8	5–18 mg/dL	Normal
Uric acid	3.1	1.7–5.8 mg/dL	Normal
High-sensitive C-reactive protein	114	0–5 g/L	Increased
Erythrocyte sedimentation rate	40–159	0–10 mm	Increased
Total iron binding capacity	209–228	250–425 $\mu\text{g}/\text{dL}$	Decreased
Ferritin	979	4.63–204 ng/mL	Increased
Iron, total	13.7	37–145 $\mu\text{g}/\text{dL}$	Decreased
Calcium	9.3	9–11 mg/dL	Normal
Sodium	136	136–145 mEq/L	Normal
Magnesium	2.01	1.6–2.6 mg/dL	Normal
Potassium	4.5	3.5–5.1 mEq/L	Normal
Glucose, random	123	74–110 mg/dL	Increased

children in particular. The family including the patient has no previous history with HIV. The rural area is leading a noticeable agricultural activity with domestic and stray dogs around. This case report instantiates the need to consider HVL in cases of prolonged fever of unknown origin or hepatomegaly. Early detection and timely treatment of HVL is paramount because of the high mortality rate of untreated cases. Pentostam therapy is effective in treating HVL cases in Palestine without any signs of resistance. Sandfly vectors and reservoir dogs should be considered for the control of HVL parallel to health awareness campaigns.

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