

## Case Report: Pediatric Visceral Leishmaniasis Caused by *Leishmania infantum* in Northern Cyprus

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**Abstract.** Visceral leishmaniasis (VL) is a vector-borne disease widespread in the Mediterranean basin, including Cyprus. During the last decades no cases were notified from northern Cyprus, but herein three cases of VL (female: 2, male: 1, median age: 24.6 months) diagnosed during their hospital admission between January 2011 and December 2012 are reported. Diagnosis was based on clinical findings;  $1 \geq 1/64$  titer positivity of immunofluorescence antibodies, *Leishmania* amastigotes in Giemsa-stained slides of bone marrow, as well as molecular identification confirmed that in all three the infecting pathogen was *Leishmania infantum*. Fever, splenomegaly, and hepatomegaly were the typical clinical findings. First-line treatment with liposomal amphotericin B (AmBisome<sup>®</sup>; intravenous, 3 mg/kg) on days 1–5, followed by the same on days 10 and 21 yielded a successful outcome with no relapse in all cases. These confirmed VL cases found within 2 years demonstrate the presence of VL on the island.

Leishmaniasis is a vector-borne disease widespread in the Mediterranean basin, including countries such as Turkey, Greece, and Cyprus.<sup>1–4</sup> *Leishmania infantum*, *Leishmania donovani*, *Leishmania major*, and *Leishmania tropica* are the main species observed in this region, which are the etiologic agents of cutaneous leishmaniasis (CL), visceral leishmaniasis (VL), and canine leishmaniasis (CanL).<sup>1–3</sup>

VL is a life-threatening systemic infection manifesting as a chronic febrile illness, marked by hepatosplenomegaly, pancytopenia, and hyperglobulinemia, and children are regarded to be at greater risk of infection.<sup>5–7</sup>

Cyprus is a crossroad between the two continents, Europe and Asia, and the different ecological and climatic conditions of the island provide a favorable environment for *Phlebotomus* spp., the vectors that are responsible for the transmission of leishmaniasis.<sup>2,3,8,9</sup> In particular, *L. donovani* causes anthroponotic CL and VL in Cyprus.<sup>3</sup> CL and CanL cases are reported sporadically in Cyprus and no VL cases were reported in last decades.<sup>2,10</sup> However, herein a total of three pediatric cases diagnosed with VL within the last 2 years is reported, which signifies the current status regarding the presence of leishmaniasis in Northern Cyprus.

A total of three cases (female: 2, male: 1, median age: 24.6 months) diagnosed with visceral leishmaniasis during their hospital admission between January 2011 and December 2012 are presented here, retrospectively.

Case 1 was a 16-month-old female, from Taşkent, a mountain village of Kyrenia District on the north coast of the island with caves and stone mines (Figure 1). Cases 2 and 3 were a 24-month-old female and a 34-month-old male patient, respectively, both from Sipahi village, located on the east coast of the island on the Karpasia peninsula (Figure 1).

None of the cases was associated with underlying immune failure or human immunodeficiency virus positivity. Fever, splenomegaly, and hepatomegaly were the typical findings in physical examination while marked anemia with pancytopenia,

increased erythrocyte sedimentation rate (ESR) and high levels of C-reactive protein (CRP) were the most common laboratory findings (Table 1, Figure 1).

Diagnosis was performed based on abovementioned clinical and laboratory findings, and was further substantiated by a  $1 \geq 1/128$  titer positivity of immune fluorescence antibody test,  $\geq 1/400$  titer positivity of direct agglutination test (Biomedical Research, KIT/Royal Tropical Institute, Amsterdam, The Netherlands), positivity in rk39 dipstick assay (InBios International, Inc, Seattle, WA),<sup>11,12</sup> and the identification of *Leishmania* amastigotes in Giemsa-stained slides of bone marrow. All cases were found positive with all methods. Polymerase chain reaction and restriction fragment length polymorphism analyses of bone marrow aspiration revealed that the infecting species was *L. infantum* in all three cases.<sup>13,14</sup>

As we were dealing with pediatric patients and to avoid the side effects of frequently used meglumine antimoniate, it was decided to install liposomal amphotericin B (AmBisome<sup>®</sup>; IV, 3 mg/kg) treatment, which was administered on days 1–5 followed by the same on days 10 and 21 in all cases and revealed a successful clinical outcome. There was a regression in clinical symptoms (fever, splenomegaly), and laboratory findings found improved parameters without the development of a relapse in all cases. After 6 months, except rK39 positivity, antibodies' titer levels were decreased below the cut-off level. Molecular analysis was not repeated as this needs invasive bone marrow aspiration.

Cyprus is the third largest island in the Mediterranean basin where leishmaniasis is endemic.<sup>3,9,15,16</sup> VL and CL cases were sporadically found on the island since 1935. In addition, infantile VL cases were reported in 1990, and two CL cases were reported in 1987 from the Northern Cyprus.<sup>3,9,10</sup> The climatological characteristics of Cyprus, that is, high humidity with warm and rainy winter and hot and dry summer, are comparable to other regions where cases of VL are found.<sup>1,4,7,16,17</sup> Several studies conducted in Cyprus since the 1940s indicate the presence of several *Phlebotomus* species.<sup>2,3,8–10</sup> Furthermore, World Health Organization (WHO) data state *L. infantum* as the etiologic agent of VL and *Phlebotomus tobbi* as the potential vector in Cyprus.<sup>2,3,8</sup> Taşkent where the first case of VL presented here was found, is an area of mining and with lots of caves, which provide a

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FIGURE 1. Location of the three pediatric cases diagnosed as visceral leishmaniasis in North Cyprus, January 2011 and December 2012.

good habitat for sand flies. Oztoprak and others also found an adult leishmaniasis case who was working in the mining sector of Zonguldak, Turkey.<sup>17</sup> The other two cases found in the present study were from the Karpasia peninsula, a region with low economic development and where the most important source of income is from animal husbandry. As it is indicated by WHO, people with little resources, weak immunity, poor living conditions, and malnutrition are prone to contract leishmaniasis,<sup>18</sup> and therefore, this region is of concern.

The clinical features of patients diagnosed with VL in Northern Cyprus in the present study, that is, fever, hepatosplenomegaly, pancytopenia, and weight loss, are consistent with

the Mediterranean type, which is mostly seen in children younger than 10 years.<sup>1,6,7,19</sup> Elevated levels for ESR and CRP have been reported among the basic clinical and laboratory characteristics of disease in Mediterranean basin.<sup>7,17,19</sup> Fever was evident in all cases in the present study, splenomegaly and hepatomegaly were the typical findings in physical examination, while anemia, increased ESR, and high levels of CRP were the most common laboratory findings and thus consistent with the Mediterranean type, which is common in pediatric age group in Turkey.<sup>1,19</sup>

It has been indicated that several VL cases have been undiagnosed, misdiagnosed, or unreported due to lack of access to medical facilities or limited diagnostic capabilities.<sup>4</sup> There was no possibility for serologic and molecular diagnosis of leishmaniasis in northern Cyprus when the cases were diagnosed. These cases indicated that the awareness of the clinician was crucial.

Early diagnosis of the disease is crucial and lifesaving as almost all untreated patients with VL die.<sup>19</sup> WHO data indicate that *L. infantum* is the etiological agent of VL and *P. tobbi* the potential vector in Cyprus.<sup>2,3</sup> *Leishmania infantum* was also identified by molecular methods in the three cases in the current study. This is partly in contrast to Mazeris who suggests that there are two distinct leishmaniasis transmission cycles in Cyprus. The first cycle circulates in dogs with *L. infantum* MON-1 and the second cycle in humans with *L. donovani* MON-37.<sup>3</sup>

In response to the increasing incidence of leishmaniasis worldwide, effective VL treatment strategies have been recommended.<sup>5</sup> Pentavalent antimony compounds are the

TABLE 1

Epidemiological, clinical, and laboratory findings of the three pediatric cases diagnosed as visceral leishmaniasis in north Cyprus, January 2011 and December 2012

	Case 1	Case 2	Case 3
Sex	Female	Female	Male
Age (months)	16	24	34
Location	Taşkent/Kyrenia	Sipahi/Karpasia	Sipahi/Karpasia
Spleen (cm)	10	9	9
Liver (cm)	5	4	3
Hb (g/dL)	4.3	5.8	6
WBC (/mm <sup>3</sup> )	3,200	2,400	3,000
Platelet (/mm <sup>3</sup> )	80,000	78,000	102,000
AST (U/L)	203	330	160
ALT (U/L)	188	250	98
ESR (mm/hour)	78	108	92
CRP (mg/dL)	60	42	54

ALT = alanine aminotransferase; AST = aspartate aminotransferase; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; Hb = hemoglobin; WBC = white blood cell.

first choice of treatment,<sup>19</sup> but increasing drug resistance and relapse have been documented.<sup>6</sup> Therefore, liposomal amphotericin B has been considered useful in cases of treatment failure or severe side effects with antimonials.<sup>1,5</sup> The lipid formulation of amphotericin B has been recommended as the most effective first-line drug in leishmaniasis treatment in Europe and the United States.<sup>5,20</sup>

Accordingly, to circumvent the side effects of meglumine antimoniate, liposomal amphotericin B was administered as first-line treatment to the patients with VL in our study and was found competent to cure the infection without any relapses.

In conclusion, three cases of clinically and parasitologically confirmed VL were found in northern Cyprus from January 2011 to December 2012. Given the associated efficacy, safety, and lack of relapse, liposomal amphotericin B presents as an optimal regimen as a first-choice treatment in pediatric VL. Furthermore, public health interventions need to be put in place in northern Cyprus, including awareness of the public and health professionals, vector control and appropriate diagnosis followed by effective and safe treatment to avoid further outbreaks and spread of VL on the island.

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