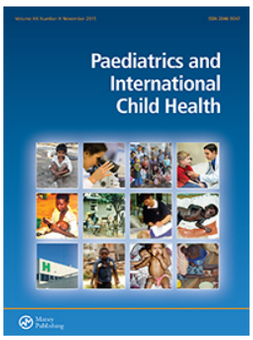

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Epidemiological and clinical features of visceral leishmaniasis in children in Alicante Province, Spain

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ABSTRACT

Background: Visceral leishmaniasis (VL) is endemic to the Mediterranean basin. In children, VL often presents with non-specific symptoms and can be life-threatening without proper treatment.

Aim: To describe the epidemiological and clinical features of pediatric VL in children in Alicante, Spain.

Methods: The study included all paediatric (<15 years) cases admitted to three hospitals in the province of Alicante from May 1992 to May 2015 with diagnosis of VL (detection was either by anti-*Leishmania* antibodies in serology or *Leishmania* in blood and/or bone marrow aspirates).

Results: There were 38 cases of pediatric VL (18 aged <24 months, 15 aged 24–59 months and 5 aged ≥5 years). The main symptoms were fever (97.4%), followed by pallor (75.0%) and loss of appetite (46.4%). Eighty-seven per cent of patients were anaemic (haemoglobin < 9 g/dL), 73.7% had neutropenia and 68.4% had thrombocytopenia. Before 2004, 92.3% of patients were treated with meglumine antimoniate (MA) and 7.7% with liposomal amphotericin B (LAmB); after 2004, 84% were treated with LAmB and just one (16%) with MA ($p < 0.001$). LAmB performed better than MA in terms of mean treatment length (7.4 days vs 25.9 days, $p < 0.001$), time to becoming afebrile (1.7 *vs* 13.7 days, $p < 0.001$), and length of hospital stay (10.9 *vs* 19.4 days, $p = 0.001$).

Conclusion: Paediatric VL in Alicante mainly affects children under five. Children aged ≤24 months present with a lower haemoglobin and white blood cell count. Treatment with LAmB reduces treatment length, time to becoming afebrile and length of hospital stay.

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Visceral leishmaniasis; paediatrics; child; epidemiology; signs and symptoms; liposomal amphotericin B; Spain

Introduction

Leishmaniasis is caused by the protozoan pathogen genus *Leishmania*. There are three major clinical forms: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis and visceral leishmaniasis (VL). Although the disease is endemic in 98 countries, more than 90% of cases occur in just six countries: India, Bangladesh, Sudan, South Sudan, Ethiopia and Brazil [1]. Georgia, Spain, Albania, Italy, Turkey, Tajikistan and Azerbaijan are the most commonly affected countries in the World Health Organization (WHO) European Region [1]. The Mediterranean region contributes only to 5–6% of the global burden of VL, which in Spain is hypo-endemic and caused by the protozoan *Leishmania infantum* [1]. The parasite is transmitted by the bite of an infected female sandfly of the phlebotomus genus (mainly *Phlebotomus perniciosus*) and is maintained in a zoonotic cycle, with dogs being the main reservoir [1–4].

Apart from the north-west, VL is reported frequently throughout Spain with the highest prevalence in the Madrid region, the Balearic Islands and along the Mediterranean coast [1–6]. Moreover, in Spain, it is one of the main causes of admission owing to parasitic infection [6].

The HIV pandemic in the 1990s modified the epidemiology of leishmaniasis [1,3–5], with *Leishmania* and HIV coinfections emerging in the Mediterranean region, particularly in Spain but also in France, Italy and Portugal [4].

Between 1997 and 2008, a third of all cases of leishmaniasis in Spain were children under 10 with the highest incidence in children under five [2,3]. The incidence rate in this age group is 1.90/100,000 person-years compared with 0.13/100,000 person-years in children aged 5–14 [2,3]. Of all new admissions owing to VL in Spain, 19.3% were in children under 5, and 2.5% were in children aged 5–14 years [2,3].

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Few studies have described the clinical profile of VL in Spanish children [8–10]. However, there are several reports of VL in children from other countries who acquired the infection whilst visiting Spain [11–13]. Given the availability of an effective therapy for this potentially life-threatening disease, some authors recommend including VL in the differential diagnosis of children presenting with persistent pancytopenia and a history of travel to an endemic area [11].

This report describes the epidemiology and clinical profile of VL in children admitted to hospital in Alicante Province on the Spanish Mediterranean coast. In addition, the epidemiology and clinical and laboratory profiles are compared between children aged ≤24 months and those aged >24 months.

Patients and methods

Study design

A retrospective study was undertaken of VL in children < 15 years treated between May 1992 and May 2015 in three public hospitals in the eastern Mediterranean province of Alicante (General University Hospital of Alicante, University Clinical Hospital of San Juan de Alicante and Hospital Marina Baixa) [14]. These hospitals serve a population of 665,965 (1.8 million inhabitants in the province), 100,366 (15.1%) of whom are under 15 years of age [15].

Source of data

The hospital admissions database which complies with the Spanish surveillance system register using the minimum basic data-set (MBDS) for hospital discharge was used. The system uses clinical codes for the Spanish version of the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM).

All hospital discharges of children < 15 years admitted during the study period with a diagnosis of VL (codes ICD-9-CM 085.0–085.9) were reviewed and data were collected from their medical records.

The case definition was a diagnosis of VL on the basis of clinical manifestations and detection either of anti-*Leishmania* antibodies in blood serology [serology > 1/80 by indirect immunofluorescence using a commercially available test (*Leishmania*-spot IF, bioMérieux)] in which promastigotes in the stationary phase are dispensed in 15-well immunofluorescence slides) or detection of *Leishmania* in blood and/or bone marrow (BM) aspirates by at least one of the following methods: (i) direct visualisation of the parasite, (ii) culture in Novy–Nicolle–McNeal's medium (NNN), (iii) and/or *Leishmania*-specific nested polymerase chain reaction (LnPCR) NNN culture: 100 µL of bone marrow aspirate dilution was cultured in NNN medium at 27 °C and examined by light microscopy every week for promastigote forms before subculturing with fresh medium. Subcultures were undertaken for 4 weeks before a negative result was returned.

The LnPCR analyses were performed in the National Centre for Microbiology, Health Institute Carlos III (Majadahonda, Madrid, Spain), as reported previously [16]. Samples shown to be positive by LnPCR were further analysed to identify the *Leishmania* species by nested amplification of the ribosomal internal transcribed spacer 1 (ITS1-PCR).

Cases described in the MBDS as cutaneous leishmaniasis and cases of VL recorded in the MBDS using ICD-9-CM codes 085.0 to 085.9 but which did not meet the inclusion criteria were excluded.

Several epidemiological, clinical, laboratory, diagnostic and treatment-related [liposomal amphotericin B (LamB) or meglumine antimonite (MA)] variables (Table 1) were analysed. VL is also known to cause secondary haemophagocytic lymphohistiocytosis (HLH), a disorder affecting the activation and proliferation of T-cells and macrophages and subsequent cytokine production, leading to uncontrolled inflammation [17]. VL patients were evaluated for HLH using the diagnostic criteria for acquired HLH syndrome published by the Hemophagocytic Lymphohistiocytosis Study Group [14] which require fulfilment of at least five of the following eight criteria: (i) fever, (ii) splenomegaly, (iii) cytopenias, (iv) hypertrigyceridaemia and or hypofibrinogenaemia (v) documented haemophagocytosis in the BM aspiration, (vi) low natural killer cell activity, (vii) ferritin ≥ 500 µg/L, and (viii) soluble CD25 ≥ 2400 U/mL. Some results on HLH for these patients have been published elsewhere [18].

Table 1. Epidemiological and clinical characteristics and outcome in 38 children with visceral leishmaniasis.

	Total
Male	18/38 (47.4)
Study period	
1992–2003	16/38 (42.1)
2004–2015	22/38 (57.9)
Symptoms	
Fever	37/38 (97.4)
Days of fever*, mean (SD)	15.9 (12.0)
Pallor	24/32 (75.0)
Loss of appetite	13/28 (46.4)
Weight loss	9/22 (40.9)
Flu-like	8/24 (33.3)
Vomiting	6/23 (26.1)
General discomfort	8/38 (21.1)
Diarrhoea	4/21 (19)
Signs	
Hepatomegaly	28/38 (73.7)
Splenomegaly	38/38 (100)
Size of splenomegaly, cm (SD)	12.3 (2.9)
Lymphadenopathy	8/21 (34.8)
Cardiac murmur	4/38 (10.5)
Outcome	
No of days in hospital, mean (SD)	13.8 (7.7)
Cure	38/38 (100)

Notes: Unless standard deviation (SD) is indicated, figures in parentheses are percentages.
*Days of fever before admission.

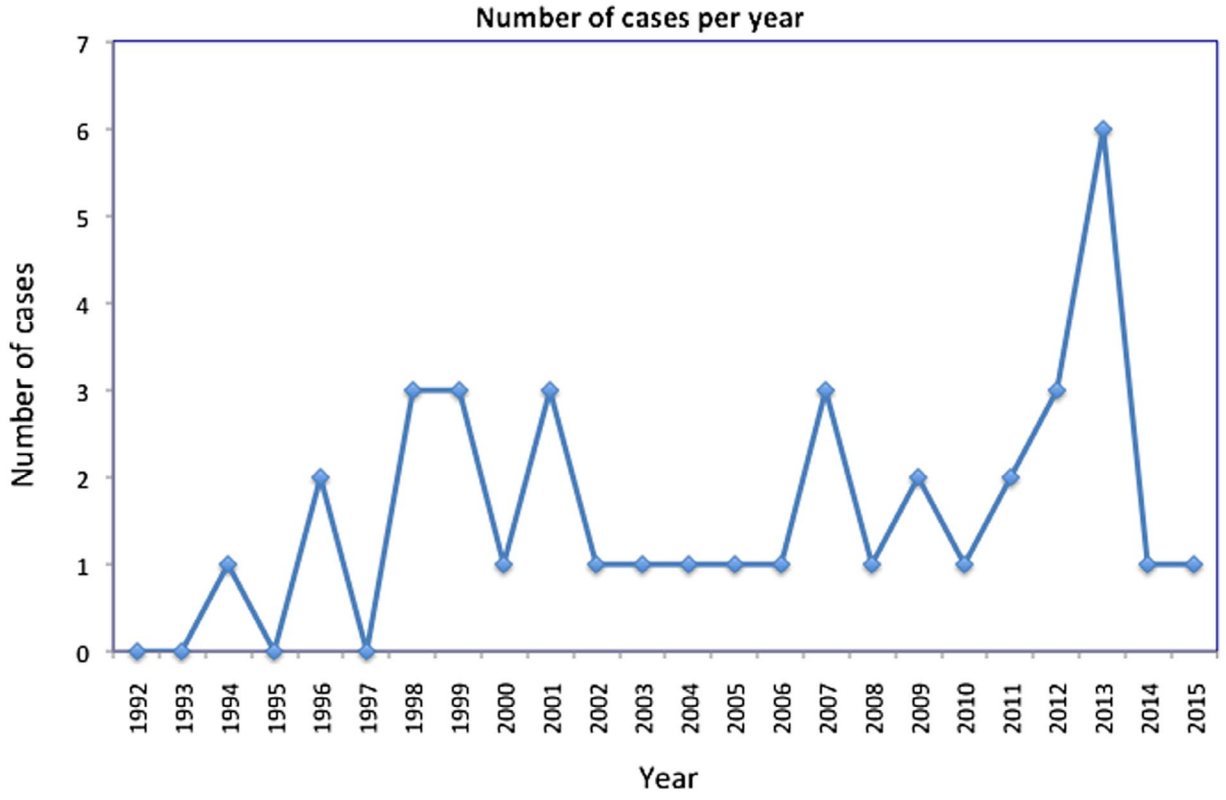

Figure 1. Annual distribution of cases of visceral leishmaniasis in children.

Statistical analysis

Statistical analysis was performed with SPSS 21.0 (IBM, Chicago, IL, USA), and *p*-values < 0.05 were considered significant. Descriptive analysis of patients was performed including demographics, diagnoses, discharge and outcome by age group. Qualitative variables were analysed using the χ^2 or Fisher's Exact test. Quantitative variables were analysed using Student's *t*-test for normally distributed data and the non-parametric Mann–Whitney U-test for skewed distribution.

Ethics

This study was reviewed and approved by the ethics committee of Hospital Marina Baixa, Alicante. All data were anonymised.

Results

There were 125 admissions of adults with VL and 38 admissions of children (2.52/10,000 paediatric admissions) to participating hospitals during the study period (May 1992–May 2015). Of the 38 children, 18 were <24 months, 15 were 24–59 months and five were 5–14 years. The prevalence was 2.8% in children under 5 years and 0.17% in those aged 5–14 years, and overall the latter figure was relatively low throughout the study (Figure 1).

Thirty-seven patients were immunocompetent children, and one patient (2.6%) had a known congenital immunodeficiency before developing VL.

The main symptoms on presentation were fever (97.4%), followed by pallor (75%) and loss of appetite (46.4%). All children presented with splenomegaly and 73.7% with hepatomegaly. Table 1 shows the main epidemiological and clinical characteristics. Eighty-seven per cent of patients were anaemic with haemoglobin < 9 g/dL, 73.7% had neutropenia with neutrophils < 1.0×10^9/L and 68.4% had thrombocytopenia with platelet counts < 100×10^9/L. Mean (SD) haemoglobin levels in children under 24 months [7.2 g/dL (1.2)] were lower than in older children [8.0 g/dL (SD 1.4), $p = 0.04$] (Table 2). Sixty per cent of children presented with raised aspartate aminotransferase, 52.6% with raised alanine aminotransferase, 54.8% with high ferritin (>5 g/L) and 44.8% with raised triglycerides levels (>3.0 mmol/L). High ferritin levels were more common in children under 24 months than in older children (71.4% *vs* 41.2%, $p = 0.09$), as were aspartate aminotransferase levels (83.3% *vs* 21.1%, $p = 0.006$).

Serology was positive (immunofluorescence > 1/80) in 26/29 (89.7%) cases tested. Table 3 outlines the method of VL diagnosis. In 14 cases (36.8%), diagnosis was made by positive serology and positive BM aspirates by microscopy, *Leishmania* culture or positive RFLP-PCR (PCR-RFLP targeting the internal transcribed spacer-1 region was positive for *Leishmania infantum*). In 12 cases (31.5%), the diagnosis was made on the basis of positive serology and symptomatology compatible with VL (negative BM aspirate or not performed). Nine diagnoses (23.6%) were based on a positive BM aspirate, with no serology testing,

Table 2. Laboratory findings in 38 children with visceral leishmaniasis.

	Total
	Mean (SD)
Haemoglobin, g/dL	7.6 (1.4)
Leucocytes, $\times 10^9$/L	3.6 (1.7)
Neutrophils, $\times 10^9$/L	0.87 (0.72)
Platelets, $\times 10^9$/L	80.3 (40.0)
Aspartate aminotransferase, U/L (<35)	153 (246)
Alanine aminotransferase, U/L (<36)	91.5 (121)
Erythrocyte sedimentation rate, mm/hr (<10) ($n = 26$)	68.7 (26.3)
C-reactive protein, mg/L (<0.1) ($n = 36$)	7.7 (6.0)
Immunoglobulin IgG, g/L (7.0–17.4) ($n = 18$)	21.1 (11.1)
Triglycerides, mmol/L (<1.8) ($n = 29$)	3.6 (2.3)
Fibrinogen, g/L (2–4) ($n = 11$)	2.7 (1.3)
Ferritin, µg/L (20–200) ($n = 31$)	1143 (1147)
Albumin, g/L (35–55) ($n = 28$)	31 (5.0)
Alkaline phosphatise, U/L (100–180) ($n = 20$)	340 (261)
Lactate dehydrogenase, U/L (80–240) ($n = 20$)	888 (519)

Notes: *n*, the number of patients in whom not all investigations were undertaken. Normal values are in parentheses.

Table 3. Diagnosis of visceral leishmaniasis in 38 children.

	Total
	n (%)
Direct microscopy of BM aspiration	
Performed	25 (67.8)
Positive	19 (76)
Negative	6 (24)
Direct microscopy of liver biopsy	
Performed	1 (2.7)
Positive	1 (100)
Negative	0
Direct microscopy of lymph nodes	
Performed	1 (2.7)
Positive	1 (100)
Negative	0
Serology	
Performed	29 (76.3)
Positive (≥1/64)	26 (89.7)
Negative (<1/64)	3 (10.3)
Leishmania culture in BM aspiration	
Performed	5 (13.1)
Positive	2 (40)
Negative	3 (60)
Leishmania PCR of BM aspiration	
Performed	7 (18.4)
Positive	5 (71.4)
Negative	2 (28.6)

Notes: BM, bone marrow; PCR, polymerase chain reaction.

and in three cases (7.8%) there was a positive BM finding and negative serology.

Of the 38 children with VL, 25 underwent a full blood test within 96 h of diagnosis. Eight of these children (32%) met the criteria for HLH syndrome but showed no haemophagocytosis on BM aspiration. No specific treatment for HLH was given. All patients had a favourable outcome.

Thirteen (34.2%) patients were treated with MA and 25 (65.8%) with LamB, although treatment strategies changed markedly in 2004. Before then, 92.3% of patients were treated with MA and 7.7% with LAmB; after 2004, 84% were treated with LAmB and just one (16%) with MA ($p < 0.001$). LAmB outperformed MA in terms of treatment length (7.4 days *vs* 25.9 days, $p < 0.001$), time to becoming afebrile ($p < 0.001$) and duration of hospital stay ($p = 0.001$) (Table 4). Treatment was successful in all cases with no reported recurrence. No children required admission to the intensive care unit.

Table 4. Treatment of visceral leishmaniasis.

	Meglumine antimoniate	Liposomal amphotericin B	*p*-value
Number (%)	13 (34.2)	25 (65.8)	
Study period, *n* (%)			<0.001
1992–2003	12 (92.3)	1 (7.7)	
2004–2015	4 (16.0)	21 (84.0)	
No. of days of treatment, mean (SD)	25.9 (5.7)	7.4 (4.9)	<0.001
Days to become afebrile, mean (SD)	13.7 (10.2)	1.7 (0.8)	<0.001
No. of days in hospital, mean (SD)	19.4 (9.2)	10.9 (4.8)	0.001
Cure, *n* (%)	13 (100)	25 (100)	1

Discussion

This study describes the clinical and epidemiological characteristics of paediatric VL on the Spanish Mediterranean coast. The incidence was consistently low and characteristic of a hypo-endemic pattern over the study period, although there were isolated spikes (though not outbreaks) for reasons we were unable to ascertain. There *was* an important outbreak of VL in Madrid from January 2010 to December 2012 [19–21], representing the largest reported community outbreak of leishmaniasis in Europe [19]. Of the 103 cases reported, 24 were in children under 16 [19]. The surveillance system for canine leishmaniasis did not detect any increase in prevalence during the period. Environmental control measures were undertaken to contain the outbreak, including improvements in sanitation, disinfection of the risk areas and control of the overpopulation of leporidae (rabbits and hares), as xenodiagnosis studies have shown that hares play an active role as reservoirs [19]. However, the Madrid outbreak did not coincide with the spikes in incidence seen in Alicante.

This report in Spanish children is consistent with other studies in the Mediterranean region in terms of age [7,10], with three-quarters of cases being children under the age of 5. In contrast, a study in Ethiopia reported that only a quarter of paediatric VL cases were in the under-five age group [22].

The clinical presentation in this study was similar to that observed in other series [8–10,23–25], although there were some differences in clinical presentation and laboratory results according to age. Children aged <24 months presented lower levels of haemoglobin and higher aminotransferase levels.

In view of reports of VL in children who have travelled to Spain and the particular popularity of Valencia, especially Alicante (with *c*. 8.9 million tourists in 2017) [26], the findings of this study are of international interest. In a recent report of VL related to travel in endemic regions of Europe, three of ten reported cases were acquired in

Spain, mostly after short trips (<30 days) [13]. Apart from the Alicante area, the Balearic Islands are also an endemic part of Spain [3–5].

LAmB is used widely to treat VL with excellent results in children and adults [25,27,28]. This study confirms the superiority of LAmB over MA with shorter treatment, hospital stay and febrile period. It also seems to be safe in children. The reported case-fatality rate for paediatric VL in the literature generally relates to hospital-based mortality and ranges from 1.5 to 7.2% [1,7,27,29]. The mortality rate in this series was zero thanks to high clinical suspicion and early treatment.

Different studies attest to the *Leishmania*-specific nested PCR (Ln-PCR) in BM aspirates being the most sensitive test for diagnosing VL, with 100% sensitivity [10,30]. Conversely, blood PCR is easier to perform and can be the first diagnostic step, but a negative result does not exclude infection [8,10,16,30]. As Ln-PCR was performed only in the latter period of the study, there were too few cases to draw any definitive conclusions on its accuracy. The rapid rK39 strip test indicating anti-*Leishmania* antibodies is also useful in the diagnosis of paediatric VL [31]; however, this non-invasive test was not used in this study.

In making the diagnosis, it is important to remember that paediatric VL can mimic more common diseases such as leukaemia, viral infections or auto-immune diseases owing to polyclonal B-cell activation and other mechanisms that lead to multiple positive serological tests. Thus, positive serology for *Leishmania* can be helpful in diagnosing VL in children from non-endemic areas [1,23–25]. In this study, 89% of serological tests were positive.

HLH is a potentially fatal hyperinflammatory syndrome characterised by histiocyte proliferation and haemophagocytosis [32]. *Leishmania* parasites are the most common protozoan trigger of acquired HLH [32], with the VL-associated variety of HLH accounting for 2.1–41.1% of these cases [33,34]. In this study, the proportion of VL-associated HLH in children was 32%. A favourable outcome was achieved by treating the underlying VL.

As with all retrospective investigations, this study has several limitations. The total number of cases was small. However, it was sufficient to allow comparison of age groups (<24 months *vs* ≥24 months). In this multicentre study, some information was not properly recorded on patients' charts so data for several variables were missing.

Paediatric VL in this region affect mainly children under five. Children under 24 months presented with lower haemoglobin and increased ferritin levels. LAmB therapy reduced the duration of treatment, time to becoming afebrile and length of hospital stay.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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