



CASE REPORT

The First Molecular Characterization of Pediatric Visceral Leishmaniasis in Jordan

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Abstract

Leishmaniasis is a neglected endemic, vector-borne tropical disease that poses a substantial public health threat in Jordan, yet data on visceral leishmaniasis, particularly in human cases, remain limited. To our knowledge, this article is the first peer-reviewed report describing the clinical and molecular aspects of the locally acquired pediatric visceral leishmaniasis cases in Jordan. Current literature associated with the disease's emergence in the region is also reviewed. Three pediatric cases presenting symptoms of visceral leishmaniasis were clinically evaluated. Diagnosis was confirmed according to bone marrow aspirates, cytology, molecular techniques, and sequencing to identify the causative *Leishmania* species. Two cases were caused by *L. infantum*, and one case was caused by *L. tropica*. The sequences of the studied samples showed a high similarity to regional isolates instead of the isolated strains showed close genetic similarity to regional isolates. The identification of autochthonous human visceral leishmaniasis cases in Jordan is a critical public health concern. The emergence of such cases in new geographic areas might strain healthcare services, particularly for vulnerable populations. Improved surveillance, research, and crisis management plans for both human and canine leishmaniasis in Jordan are urgently needed.

Keywords: Jordan, visceral, cutaneous, leishmaniasis, *L. infantum*, *L. tropica*, zoonotic

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INTRODUCTION

Leishmaniasis is widespread globally and is caused by protozoan parasites of the genus *Leishmania* [1]. Various species of *Leishmania* cause a wide range of clinical disease forms, ranging from asymptomatic infections to skin to a lethal visceral form of the disease, depending on the parasite and host characteristics [2,3]. The main disease burden is found in tropical and sub-tropical regions, and 2–3 million cases of leishmaniasis have been estimated to occur annually worldwide [4]. Leishmaniasis has been reported in more than 100 countries [4]. More than 20 *Leishmania* species

cause three clinical forms of leishmaniasis worldwide: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis, and visceral leishmaniasis (VL) [5]. CL is the most common presentation in Jordan and other parts of the Old World, such as the Middle East and North Africa [6]. *Leishmania major* has been reported to be the predominant species causing CL in multiple regions of Jordan [7]. To date, information on VL in Jordan is lacking in the literature. The most representative *Leishmania* species causing VL in the Mediterranean Basin and North Africa is *L. infantum* [8]. *L. infantum* infection can present a wide range of clinical signs, primarily according

to the interactions among the parasite, the host, and the environment. In a 2023 World Health Organization (WHO) report on leishmaniasis country profiles, Jordan is listed as an endemic area for VL, and two cases were recorded and cited by the Jordanian Ministry of Health [9]. To our knowledge, no peer-reviewed articles have described the clinical and molecular aspects of the locally acquired human VL cases in Jordan. Therefore, this study provides the first documented pediatric and molecularly confirmed cases of VL in Jordan. Health authorities require additional information regarding the actual incidence of VL cases in Jordan and similar ecosystems. Moreover, health authorities must invest in public outreach and education, to increase public awareness of the extent of the disease and to prevent its spread.

CASE SERIES: VISCERAL LEISHMANIASIS IN PEDIATRIC PATIENTS—CLINICAL AND HEMATOLOGICAL PROFILES

Herein, we describe a case series of three pediatric patients diagnosed with VL, characterized by fever of unknown origin and hematological abnormalities. The diagnosis was confirmed by bone marrow cytology revealing *Leishmania* amastigotes. These cases were clinically diagnosed between 2023 and 2024. Leishmaniasis was acquired in two locations in Jordan: Northern Shunah and Deir Alla (Fig 1). Both regions are located in the same geographical area, the Jordan Valley, where medically important sand-fly species are present [10].

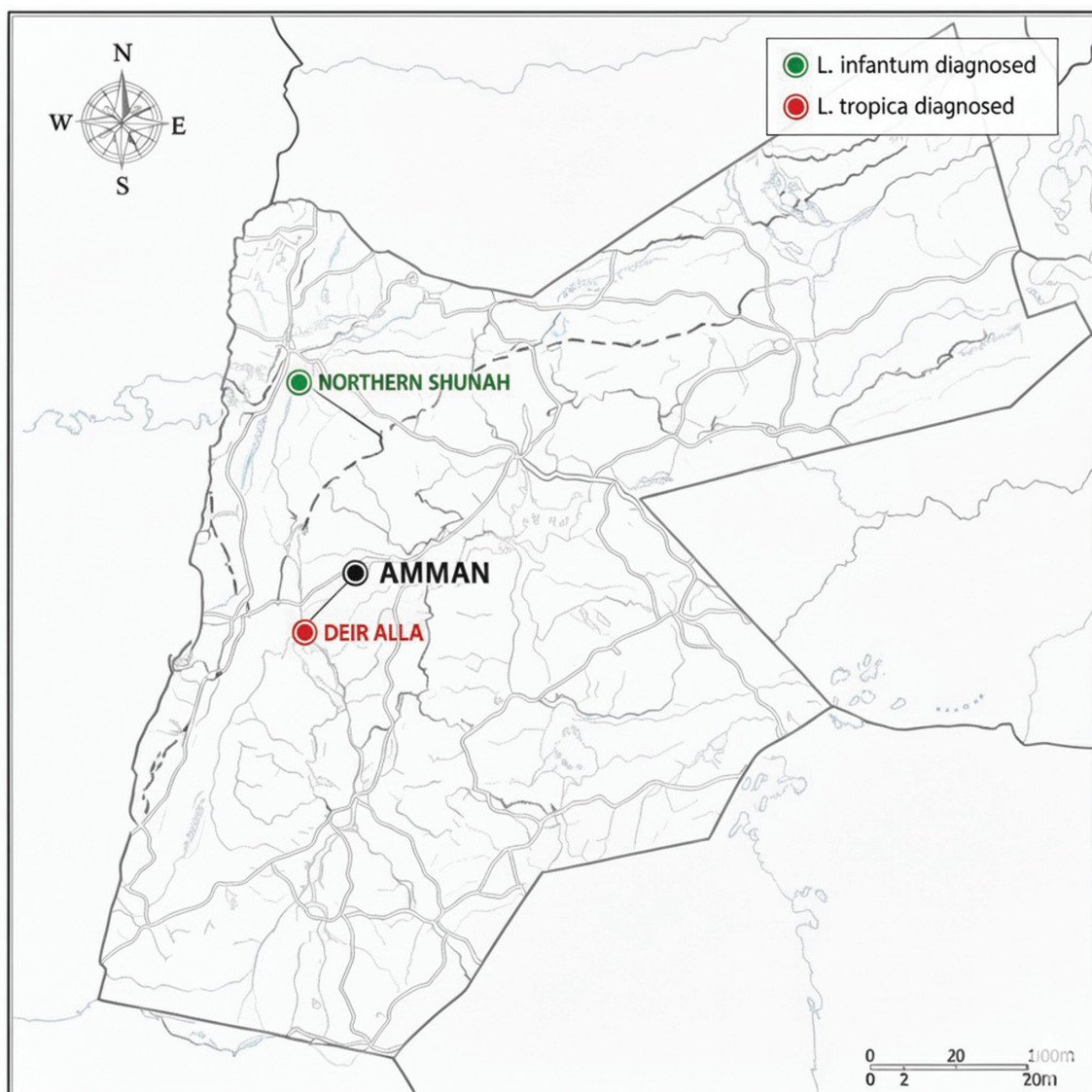


FIGURE 1 | Locations of visceral leishmaniasis in Jordan, 2023–2024. The map shows the two primary foci of visceral leishmaniasis identified in the Jordan Valley. Northern Shunah is a focus for *L. infantum*, and Deir Alla is a focus for *L. tropica*.

CASE 1: 8-YEAR-OLD GIRL

Clinical presentation

Case 1 was in an 8-year-old girl presenting with a 3-week history of pyrexia, occasional cough, a distended abdomen, a cushingoid facial appearance, hypertension, and urinary tract infection. On examination, she was found to have hepatosplenomegaly. Her complete blood count (CBC) showed moderate microcytic hypochromic anemia, leukopenia with neutropenia and lymphopenia, and a normal thrombocyte count. Her biochemical findings revealed mild elevations in liver enzymes (ALT, AST, and LDH), hyperglycemia, hypercalcemia, mild hyperproteinemia, and high ferritin levels. An abdominal CT scan showed splenomegaly. Bone marrow cytological findings revealed normocellularity and increased erythroid precursors with megaloblastic changes, thus suggesting possible nutritional deficiencies. Her granulocytic precursors were adequate and showed left shifted maturation. Her megakaryocytes were adequate and showed normal morphology. Her histiocytes were elevated. Several intracytoplasmic and extracellular inclusions consistent with *Leishmania* amastigotes were observed. They were round and 1–4 μm in diameter, and had round to oval nuclei and round purple kinetoplast (Fig 2).

CASE 2: 2-YEAR-OLD BOY

Clinical presentation

A 2-year-old boy presented with a primary concern of pyrexia of unknown origin, and with massive splenomegaly but no lymphadenopathy. His CBC revealed pancytopenia characterized by normocytic normochromic anemia, leukopenia with severe neutropenia, and severe thrombocytopenia. His biochemical findings included hypoalbuminemia, hyponatremia, hypocalcemia, elevated C-reactive protein, a high erythrocyte sedimentation rate, elevated ferritin, and hypertriglyceridemia, thus indicating systemic inflammation. Cytological bone

marrow examination revealed hypercellularity with an estimated cellularity of 95%. His erythroid precursors were quantitatively adequate. His granulocytic precursors were quantitatively adequate, and showed normal maturation and differentiation. His megakaryocytes were adequate. His histiocytes were elevated, and showed intracellular and extracellular inclusions consistent with *Leishmania* amastigotes. They were round and 1–4 μm in diameter, and had round to oval nuclei and round purple kinetoplast. The clinical and laboratory findings indicating hyperinflammatory markers prompted concern for associated hemophagocytic lymphohistiocytosis, a known complication of VL in young children.

CASE 3: 5-YEAR-OLD GIRL

Clinical presentation

A 5-year-old girl showed a more subtle presentation, with fever as the sole concern. Unlike the other two children, she had unremarkable abdominal ultrasound findings and showed no visible hepatosplenomegaly. However, her CBC showed microcytic hypochromic anemia, leukopenia characterized by neutropenia, and severe thrombocytopenia. Her bone marrow cytology indicated low cellularity. Low numbers of erythroid and myeloid precursors were present. Several histiocytes were seen and contained intracytoplasmic inclusions consistent with *Leishmania* amastigotes. They were oval in shape, 1–6 μm in diameter, had a round purple nucleus and a bar-shaped kinetoplast. Although she did not show overt organomegaly, her hematological and bone marrow findings supported the diagnosis of VL.

In summary, all three cases showed fever, cytopenia, and bone marrow infiltration with *Leishmania* amastigotes, thus meeting the diagnostic criteria for VL. Bone marrow aspiration was conducted in all three pediatric patients. Microscopic examinations of Giemsa-stained smears revealed the presence of *Leishmania* amastigotes either

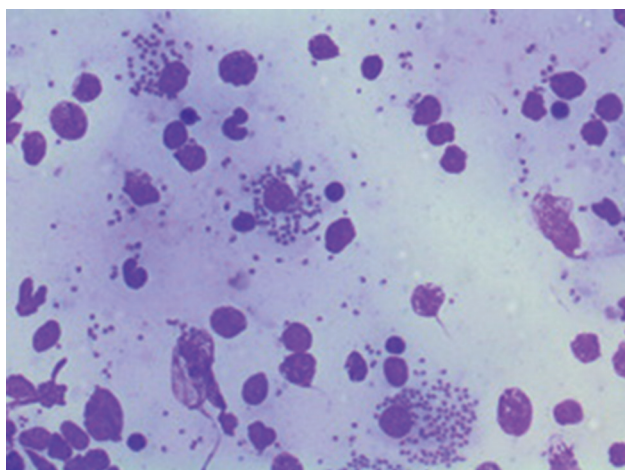


FIGURE 2 | Bone marrow aspirate. Several intracytoplasmic and extracellular inclusions consistent with *Leishmania* amastigotes are seen. They are round and 1–4 μm in diameter, and have round to oval nuclei. Magnification 400 $\times$.

within macrophages or freely within the smear, thus confirming the diagnosis. The bone marrow aspiration procedure remains a gold-standard diagnostic method for VL and was essential for definitive identification in these cases [11]. However, the cases differed in their severity; clinical presentation; and hematological (Table 1), biochemical, and bone marrow findings. Case 1 was complicated by metabolic features and megaloblastic changes in the erythroid cell line; case 2 represented a classic severe presentation, possibly progressing to hemophagocytic lymphohistiocytosis; and case 3 presented bone marrow suppression without organomegaly. These differences underscore the heterogeneity of VL and the need for individualized diagnostic and management approaches in pediatric patients.

Bone marrow biopsies and blood from all cases were subjected to polymerase chain reaction to detect *Leishmania* infection with four different primer pairs sets. The first reaction targeted the *ITS1* and *5.8S rRNA* region in *Leishmania* spp.; the second and third reactions targeted the *cytochrome oxidase subunit II (COXII)* gene in *L. major* and *L. tropica*; and the final reaction targeted *cysteine protease cpb (E and F)* of *L. infantum/donovani*. Summary information for each PCR is presented in Table 2. The positive amplicons were sent to Macrogen (South Korea) for purification and gene sequencing through a commercial Sanger sequencing service.

TABLE 1 | Complete blood counts of the three pediatric patients.

Parameter	Case 1	Case 2	Case 3	Reference range
WBC $\times 10^3/\mu\text{l}$	2.30	3.30	3.22	3.8–10.4
RBC $\times 10^6/\mu\text{l}$	3.70	2.41	4.14	4.1–5.2
HB g/dl	7.9	6.5	8.8	11.5–14.3
HCT %	23.8	19.1	27.1	34–42
MCV fL	64.4	79.0	65.5	77.8–91.1
MCH pg.	21.3	27.1	21.3	25–30
MCHC g/dl	33.0	34.3	32.5	31–34
PLT $10^3/\mu\text{l}$	191	49	44	187–400
RDW-SD fL	36.3	49.0	46.5	35–46
RDW-CV %	16.0	17.6	20.1	11.4–13.5
MPV fL	8.8	8.2	////	
Neutrophils %	46.6	19.3	13.7	
Neutrophils $\times 10^3/\mu\text{l}$	1.1	0.6	0.44	1.5–6.5
Lymphocytes %	45.9	70.0	76.7	
Lymphocytes $\times 10^3/\mu\text{l}$	1.1	2.3	2.47	1.4–3.9
Monocytes %	7.5	9.8	9.3	
Monocytes $\times 10^3/\mu\text{l}$	0.2	0.3	0.3	0.2–0.8
Eosinophils %	0	0	0	

The tested samples were positive for *ITS1* and *5.8S rRNA* PCR, and negative for species-specific PCRs. On the basis of the alignment of the provided sequences against BLAST database sequences, we determined that one human sample comprising L-1 (blood) and L-2 (tissue) shared 99.59% and 98.11% similarity with *L. tropica*, respectively (Fig 3). The other human samples, M and Ma, showed high similarity to *L. infantum* and *L. donovani* species (99.62% and 100%, respectively), as confirmed by *cysteine protease cpb* PCR (Fig 4).

The NJ phylogenetic tree was constructed with 1000 bootstraps and a Kimura 2-parameter model for the *ITS1* and *5.8S rRNA* sequenced amplicons, including reference isolates reported in the surrounding countries. The L-1 and L-2 human samples clustered with *L. tropica* reference sequences and showed high similarity to the Turkish and Saudi Arabian isolates (MH347938.1 and ON872294.1). Moreover, the M and Ma human samples best matched the Cypriot and Turkish *L. infantum* isolates (KC998879.1 and PP034050.1) and *L. donovani* isolates from Georgia and Eastern Sudan (KT438675.1 and AJ276259.1) (Fig 5).

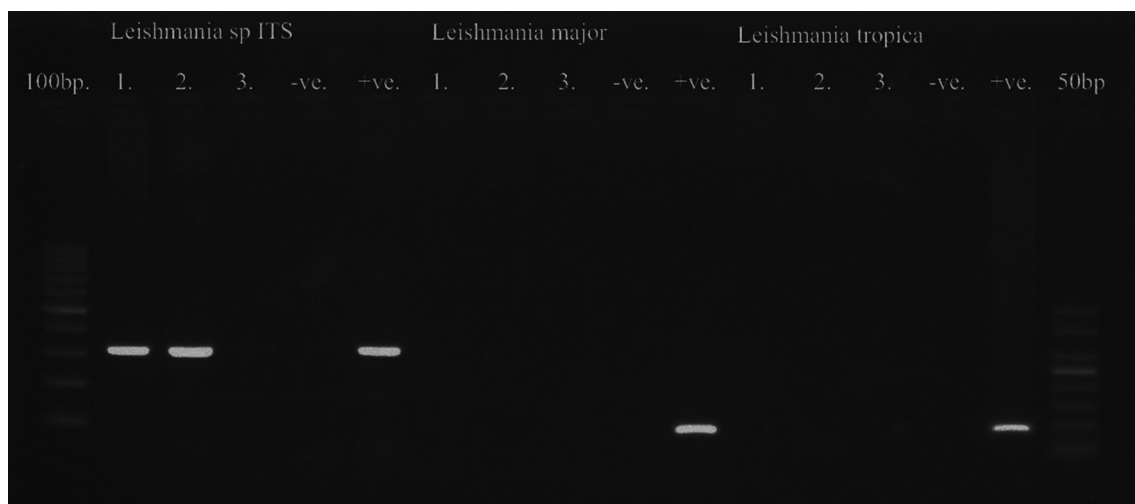
DISCUSSION

VL is a neglected tropical disease caused primarily by the *L. infantum* parasite in the Middle East and the Mediterranean Basin. Although the WHO classifies Jordan as an endemic area for VL, and the Ministry of Health reported two official cases [9], no reports in peer-reviewed journals providing clinical or molecular confirmation of local (indigenously acquired) cases have been published, to our knowledge. Therefore, this study addresses a critical gap by documenting the first molecularly confirmed pediatric cases of VL in Jordan. Although VL was previously reported in Jordan [15], the case was not from Jordan but from Bal'a, Palestine, and was caused by *L. donovani* in a patient admitted and diagnosed in Jordan in 1995. Herein, two human cases of *L. infantum* and one case of *L. tropica* were first clinically and molecularly diagnosed in pediatric patients in Jordan. The patients had no travel history, thus confirming the autochthonous nature of these infections. These cases occurred in rural settings, where individuals are potentially regularly exposed to bites from affected sandfly species, which can act as vectors. The first detection of *L. infantum* in Jordan was concerning, because the established presence of both competent sandfly vectors and potential animal reservoirs would create favorable conditions for the parasite's transmission and persistence. This finding has substantial public health implications, by suggesting the potential for endemic transmission cycles to become established, particularly in ecologically suitable areas. The sandfly species *P. perfiliewi* and *P. syriacus*, known vectors of *L. infantum*, have been reported in the Levant region, thus indicating a biological ability for local transmission [16]. Moreover, a serological and molecular survey conducted in the West Bank, Palestine, has detected *Leishmania* parasites in apparently healthy dogs,

TABLE 2 | PCR primer sequences according to *Leishmania* species and target genes. Related information: annealing temperatures, product sizes, and references.

Parasite	Target gene	Primer sequences (5' to 3')	Tm (°C)	Product size	Reference
<i>Leishmania</i> sp.	<i>ITS1</i> and <i>5.8S rRNA</i>	LITSR (CTGGATCATTTCGATG) L5.8S (TGATACCACTATCGCACTT)	52	300–350 bp*	[12]
<i>L. major</i>	<i>Cytochrome oxidase subunit II (COXII)</i>	L. major-F (GCATGTTTATTAGTGTCGAAA) L. major-R (ACCTCCAATAATGAATCAATACA)	52	97 bp	[13]
<i>L. tropica</i>	<i>Cytochrome oxidase subunit II (COXII)</i>	L. tropica-F (ACGGCATCAAAATTTATTGA) L. tropica-R (GGCCAAAATATAATAACAAACATAA)	52	97 bp	[13]
<i>L. infantum/L. donovani</i>	<i>Cysteine protease cpb (E and F)</i>	cpbEF-F (CGTGACGCCGGTGAAGAAT) cpbEF-R (CGTGCACTCGGCCGTCTT)	63	<i>L. infantum</i> 702 bp (<i>cpbE</i>) <i>L. donovani</i> 741 bp (<i>cpbF</i>)	[14]

*Product size varies by target species.

**FIGURE 3** | *Leishmania* species detection through PCR targeting three genes: the *ITS1* and *5.8S rRNA* regions of *Leishmania* spp.; species-specific PCR of the *COXII* gene of *L. major*; and species-specific PCR of the *COXII* gene of *L. tropica*. VC DNA ladders: 100 and 50 bp. Lane 1: isolate M. Lane 2: isolate Ma. Lane 3: isolate L. Lane +ve: positive controls for each PCR (*L. tropica* Syrian isolate and *L. major* DNA construct). Lane -ve: nuclease free water (NFW). The expected amplicon size for general PCR was 300–350 bp (according to the species), and those for species-specific PCRs were 97 bp for *L. major* and *L. tropica*. Gel electrophoresis (1.5%) of three PCRs is shown.

thereby underscoring dogs' role as a silent reservoir in the region [17]. Those findings highlight the potential for domestic dogs to contribute to the maintenance and spread of VL in Palestinian communities.

These cases have epidemiologic implications pertaining to public health, and concerns have been raised regarding the ability of *L. infantum* to spread through vectors [18]. The detection of *L. infantum* in the Jordan Valley was not unexpected, because the area is considered a hotspot for medically important sandfly species, primarily *P. papatasi*, which is the most abundant [10]. Furthermore, *P. sergenti* and *P. alexandri* have also been recorded. These findings underscore the sandfly's epidemiological importance in leishmaniasis transmission in the Jordan Valley. These cases highlight the importance of public vigilance and the adoption of immediate and effective surveillance care mechanisms to control the disease in Jordan. Because this species was found in a clinical setting, previously developed

measures to monitor and control leishmaniasis might need to be reconsidered. This previously neglected species currently requires greater focus. Overall, the finding of *L. infantum* in the area of the Jordan Valley broadens the scope of understanding of leishmaniasis in the region and sounds an alarm for present and future public health efforts.

The transmission of *L. infantum* relies on various species of sandflies, particularly *P. syriacus*, which is found throughout the Middle East [19]. The discovery of *L. infantum* in Jordan parallels findings in neighboring areas such as Palestine and Lebanon [20,21]. For example, CL due to *L. infantum* has been documented in Israel, thus suggesting that this area facilitates vector-host dynamics that would enable the spread of this species [20]. The need to monitor a subsequent rise in leishmaniasis incidence due to sandfly bites is therefore crucial.

Notably, the cases documented herein were in children, in agreement with observations from previous studies, in

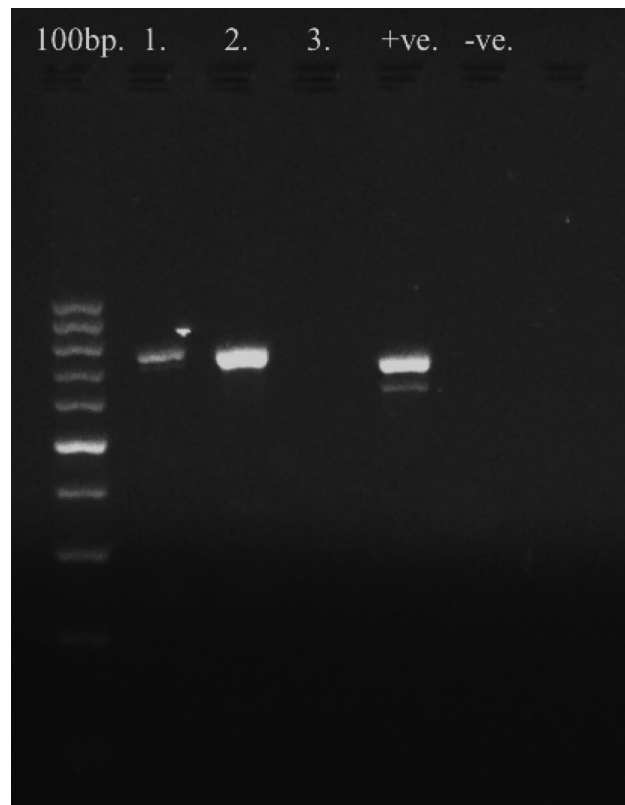


FIGURE 4 | Gel electrophoresis (1.5%) of species-specific PCR of the cysteine protease *cpb* (*E* and *F*) region of *L. infantum/donovani*. Lane M: VC 100 bp DNA ladders (Vivantis). Lane 1: human isolate M. Lane 2: human isolate Ma. Lane 3: human isolate L. Lane –ve: nuclease-free water (NFW). The expected amplicon sizes were 702 bp for *L. infantum* and 741 bp for *L. donovani*.

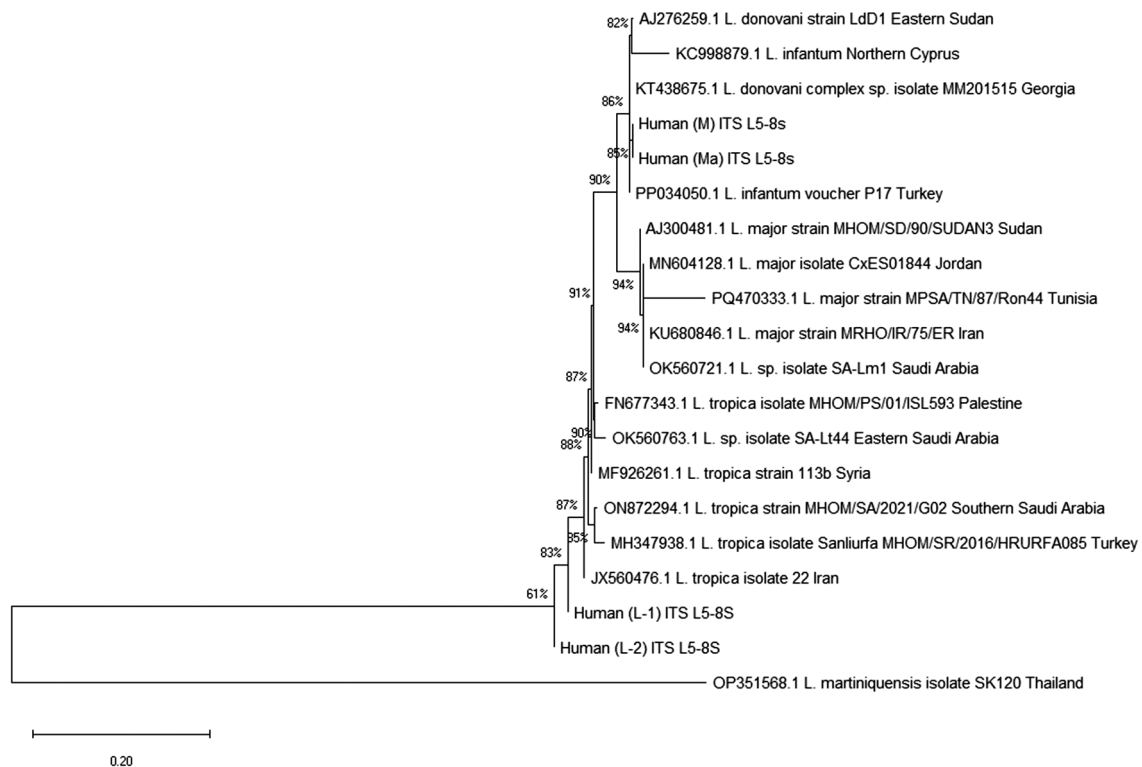


FIGURE 5 | Neighbor-joining (NJ) phylogenetic tree for ITS and the 5.8S rRNA gene. The tree was constructed with the Kimura 2-parameter model with 1000 bootstraps and the maximum composite likelihood method. The figure includes reference isolates from BLAST, and human isolates (M, Ma, and L) are reported.

which most VL cases have been found to occur in children younger than 10 years [8,22]. In Iraq, 90% of VL cases are diagnosed in children younger than 5 years [22]. Between 1993 and 2007, the Hebron district of the West Bank, Palestine, reported 76 cases of VL [23]. These cases were found exclusively in children, all of whom were younger than 9 years. Similar results have been reported in Syria, where most cases of VL are diagnosed in children [24]. Children are the most vulnerable group to VL, because of their immature immune responses and greater prevalence of childhood malnutrition compared to adult [8]. Early recognition and treatment are therefore essential to prevent morbidity and mortality in this age group.

Despite Jordan's geographical location in the endemic belt of leishmaniasis, data concerning *Leishmania* spp. remain limited. The current understanding of both human and canine leishmaniasis in the country is fragmented, and only several scattered reports have addressed disease occurrence, vector distribution, and reservoir hosts. This paucity of data poses a major challenge to the implementation of effective surveillance and control strategies. Human cases of CL have been reported in Jordan, and attributed primarily to *L. major* and *L. tropica*, but molecular confirmation is rare, and VL remains underreported and probably underdiagnosed. More concerning, the status of canine leishmaniasis, particularly involving *L. infantum*, has not been reported in Jordan to date. The lack of systematic epidemiological studies obscures the true burden of the disease and limits understanding of its transmission dynamics, including the roles of vectors such as *P. alexandri* and potential canine reservoirs. Given the regional context, in which neighboring areas, such as Syria and Palestine, have reported endemic VL, the situation in Jordan warrants urgent and comprehensive investigation to inform evidence-based public health interventions.

CONCLUSION

In conclusion, although the WHO has acknowledged the presence of VL in Jordan through surveillance data, the absence of detailed published case reports has hindered both clinical awareness and scientific understanding of the disease in the country [9]. To our knowledge, this study presents the first clinically and molecularly confirmed cases of VL among children in Jordan, and highlights the need to strengthen diagnostic capacity, physician awareness, and systematic epidemiological studies. Future work should aim to clarify the geographic distribution of transmission; identify sandfly vectors; and assess canine reservoirs, which are known to play a key role in sustaining *L. infantum* transmission across the region.

Health authorities require additional information regarding the actual incidence of *L. infantum* cases in Jordan and similar ecosystems. Moreover, health authorities must invest in public outreach and education, to increase public awareness of the extent of the disease and to prevent its spread. Effective control of CL and VL in humans requires

close collaboration between health authorities and the scientific community, and consideration of hosts, vectors, and reservoirs.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are contained within the published article. Further data may be obtained from the corresponding author upon reasonable request.

ETHICS STATEMENT

This report was reviewed and approved by the Royal Medical Services Directorate of Technical Training and Planning, Institutional Review Board (approval number: 31/2023). The requirement for informed consent was waived by the IRB, because of the retrospective and anonymized nature of the data.

AUTHOR CONTRIBUTIONS

WH conceptualized the study and writing the original draft. RA prepared the case series and conducted the laboratory investigations. RA interpreted the hematological and biochemical data. MM conducted the clinical evaluation and patient management. RH collected the Data and participated in patient management. RM interpreted the molecular data and phylogenetic analysis. HH executed the methodology, PCR analysis and sequencing validation. NA supervised the clinical diagnosis and contributed to writing and editing the manuscript. All authors contributed to the writing and interpretation of results, reviewed the manuscript, and approved the final version.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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