Tropical Biomedicine 43(1): 60-64 (2026)
https://doi.org/10.47665/tb.43.1.008

CASE REPORT

Cutaneous leishmaniasis case caused by the *Leishmania donovani* complex in northern Cyprus

Ruh, E.[1,2*], Ozkayalar, H.[3], Nalcaci, M.[4], Toz, S.[5], Mocan, G.[6], Taylan-Ozkan, A.[7]

[1]Department of Medical Microbiology and Clinical Microbiology, Faculty of Medicine, Near East University, Nicosia, Northern Cyprus
[2]DESAM Research Institute, Near East University, Nicosia, Northern Cyprus
[3]Department of Pathology, Dr. Burhan Nalbantoglu State Hospital, Nicosia, Northern Cyprus
[4]Department of Biology, Graduate School of Natural and Applied Sciences, Ege University, Izmir, Türkiye
[5]Department of Parasitology, Faculty of Medicine, Ege University, Izmir, Türkiye
[6]Department of Pathology, Faculty of Medicine, Near East University, Nicosia, Northern Cyprus
[7]Department of Medical Microbiology, Faculty of Medicine, Cyprus International University, Nicosia, Northern Cyprus
*Corresponding author: emrah.ruh@neu.edu.tr

ARTICLE HISTORY

Received: 27 October 2025
Revised: 28 November 2025
Accepted: 16 February 2026
Published: 31 March 2026

ABSTRACT

The island of Cyprus is located in the Eastern Mediterranean, where leishmaniasis is endemic. Although human visceral and cutaneous leishmaniasis (VL and CL) cases have already been documented on the island, there are limited data on the *Leishmania* species in northern Cyprus. In this report, we present a CL case diagnosed by both microscopic examination and quantitative real-time PCR (qPCR). The patient, a 79-year-old man residing in northern Cyprus, developed an ulcerative lesion on his left leg. The lesion was surgically excised for histopathological examination, and tissue sections were stained with hematoxylin and eosin (H&E). Microscopic examination of H&E-stained tissue sections revealed *Leishmania* amastigotes. To confirm the diagnosis and identify *Leishmania* species at the molecular level, DNA was extracted from the paraffin-embedded tissue sections. Following deparaffinization, qPCR targeting the *Leishmania*-specific Internal Transcribed Spacer 1 region (located between SSU and 5.8S rRNA genes) was performed. In the qPCR assay, the infecting agent was identified as a member of the *L. donovani* complex, presumptively *L. infantum*, based on the melting curve analysis. Our findings provide molecular evidence for the presence of leishmaniasis in northern Cyprus and contribute to addressing the lack of molecular data in the region. Our study also suggests that, due to the zoonotic nature of the identified pathogen, continuous vector and reservoir control programs should be implemented in the region to prevent the spread of the disease.

Keywords: Cutaneous leishmaniasis; *Leishmania donovani* complex; real-time PCR; northern Cyprus.

INTRODUCTION

Leishmaniasis is an endemic disease in various parts of the world, including the Mediterranean Region (Ruh & Taylan Özkan, 2019). The island of Cyprus, situated in the eastern part of the Mediterranean Sea, has a typical Mediterranean climate with hot, dry summers and mild winters (Ruh *et al*., 2017). Due to the favorable climatic conditions, sandflies that transmit *Leishmania* spp. are present on the island (Demir *et al*., 2010; Mazeris *et al*., 2010; Töz *et al*., 2013a). Moreover, studies have detected *Leishmania* spp. in *Phlebotomus* sandflies (Ergunay *et al*., 2014; Yetişmiş *et al*., 2022), and reported the existence of canine leishmaniasis (CanL) (Mazeris *et al*., 2010; Toz *et al*., 2013a; Beyhan *et al*., 2016; Çanakçı *et al*., 2016; Güler *et al*., 2025), as well as human leishmaniasis cases in both northern (Sayili *et al*., 2016; Ruh *et al*., 2017; Özdoğaç *et al*., 2022; Güler *et al*., 2025) and southern Cyprus (Antoniou *et al*., 2008; Koliou *et al*., 2014).

Although studies identified the detailed molecular characteristics of the parasite species in southern Cyprus (Antoniou *et al*., 2008; Koliou *et al*., 2014), molecular data from northern Cyprus are limited to a few publications. In a 1990 study, Leishmanin skin test positivity was found 10% in Kyrenia and 35% in Lapithos in northern Cyprus (Figure 1). The infecting agent was reported as *L. infantum* by DNA hybridization (Deplazes *et al*., 1998). More than two decades later, in 2016, the agent of three infantile visceral leishmaniasis (VL) cases was identified as *Leishmania infantum* in northern Cyprus (Sayili *et al*., 2016).

Considering that transmission of leishmaniasis still occurs on the island, it is essential to elucidate the infecting parasite species at the molecular level. For this purpose, we present a case of cutaneous leishmaniasis (CL) in a 79-year-old man from northern Cyprus and report the molecular identification of the causative agent.

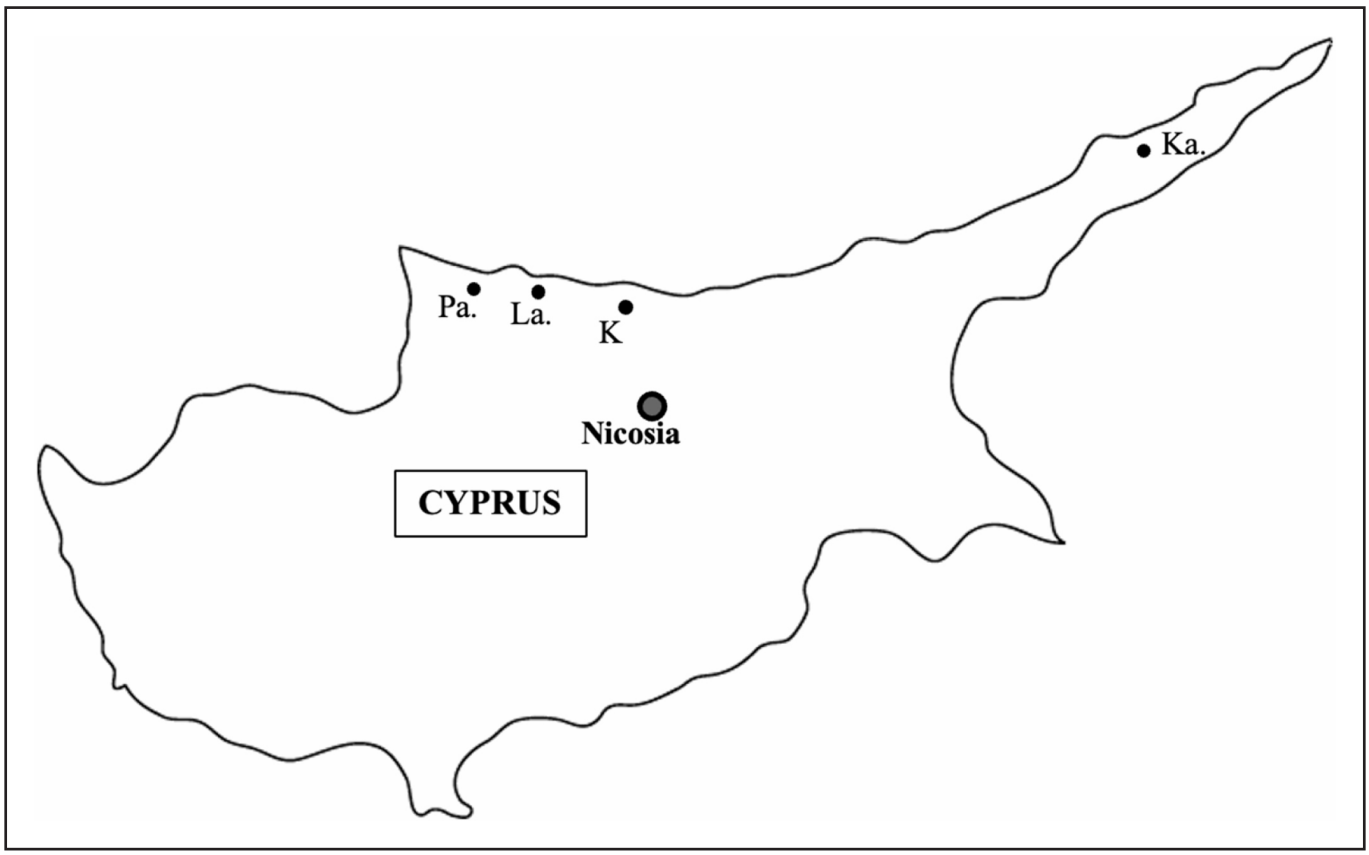

Figure 1. Map of Cyprus, showing the capital Nicosia, Kyrenia (K), Lapithos (La.), Panagra (Pa.), and Karpasia Peninsula (Ka.) in northern Cyprus (the map was adapted from: http://www.worldatlas.com/webimage/countrys/europe/outline/cy.htm).

CASE REPORT

The patient was a 79-year-old British man living in the Kyrenia district of northern Cyprus. He had an ulcerative lesion approximately 1 cm in diameter on his left leg. The lesion was surgically removed, and the tissue was excised for histopathological examination. Tissue sections were stained with hematoxylin and eosin (H&E), and the slides were examined microscopically for *Leishmania* amastigotes.

To confirm the microscopic diagnosis and identify the infecting *Leishmania* species, paraffin sections were analyzed by molecular methods. Genomic DNA was isolated directly from formalin-fixed paraffin-embedded (FFPE) tissue sections. Briefly, tissue sections were obtained from the paraffin block, deparaffinized with xylene, and rehydrated through a graded ethanol series. Genomic DNA was extracted using the DNeasy Blood & Tissue isolation kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocols. DNA was eluted in a final volume of 50 µl and stored at -20°C until use. The quality and quantity of extracted DNA were assessed using a NanoDrop spectrophotometer (Thermo Fisher, Japan).

Molecular diagnosis of the disease and identification of the causative *Leishmania* species were performed using quantitative real-time PCR (qPCR). This method targeted the ITS-1 located between the small subunit (SSU) and the 5.8S ribosomal RNA (rRNA) genes. Specific primers and probes were designed for this region to facilitate accurate detection (Toz *et al*., 2013b). In this genomic DNA-based method, the LightCycler FastStart DNA Master HybProbe (Roche Diagnostics GmbH, Mannheim, Germany) kit was used in the qPCR reaction. The primer and probe set were used as indicated in Toz *et al*. (2013b). The generation of melting curves and quantification in qPCR assay were performed using control strains *L. infantum* (MHOM/TR/01/EP59), *L. tropica* (MHOM/SY/14/EP200), and *L. major* (MHOM/SU/1973/5ASKH), as well as nucleic acid-free molecular-grade ultrapure water (Qiagen GmbH, Hilden, Germany) as a negative control.

Histopathological examination of H&E-stained slides revealed *Leishmania* amastigotes in the patient's tissue sections (Figure 2). Subsequently, in the qPCR assay, the causative agent was identified as a member of the *L. donovani* complex, presumptively *L. infantum*, based on the melting curve analysis (Figure 3).

DISCUSSION

In this report, we presented a CL case developed by *L. donovani* complex in northern Cyprus. Previously, CL cases were documented in the region by different studies. In a publication from 2017, seven of 249 participants had a history of CL diagnosis, and three of the CL cases (1.2% of the whole study population) were found to be positive by serological tests. All these cases were reported from Kyrenia district. In that study, molecular tests did not detect any positive result among the participants (Ruh *et al*., 2017). Another report documented that a tourist who returned from Lapithos, Kyrenia district, developed CL six months after his return to his country, and the agent was identified as *L. donovani* complex (de Silva *et al*., 2015). More recently, in a female pediatric patient who was living in Nicosia, *L. donovani* was detected as the agent of CL (Güler *et al*., 2025). Apart from CL cases, VL was also documented in northern Cyprus. In 2016, three infantile VL cases (one in Kyrenia district and the other two in Karpasia Peninsula) were reported, and the infecting agent was detected to be *L. infantum* (Sayili *et al*., 2016).

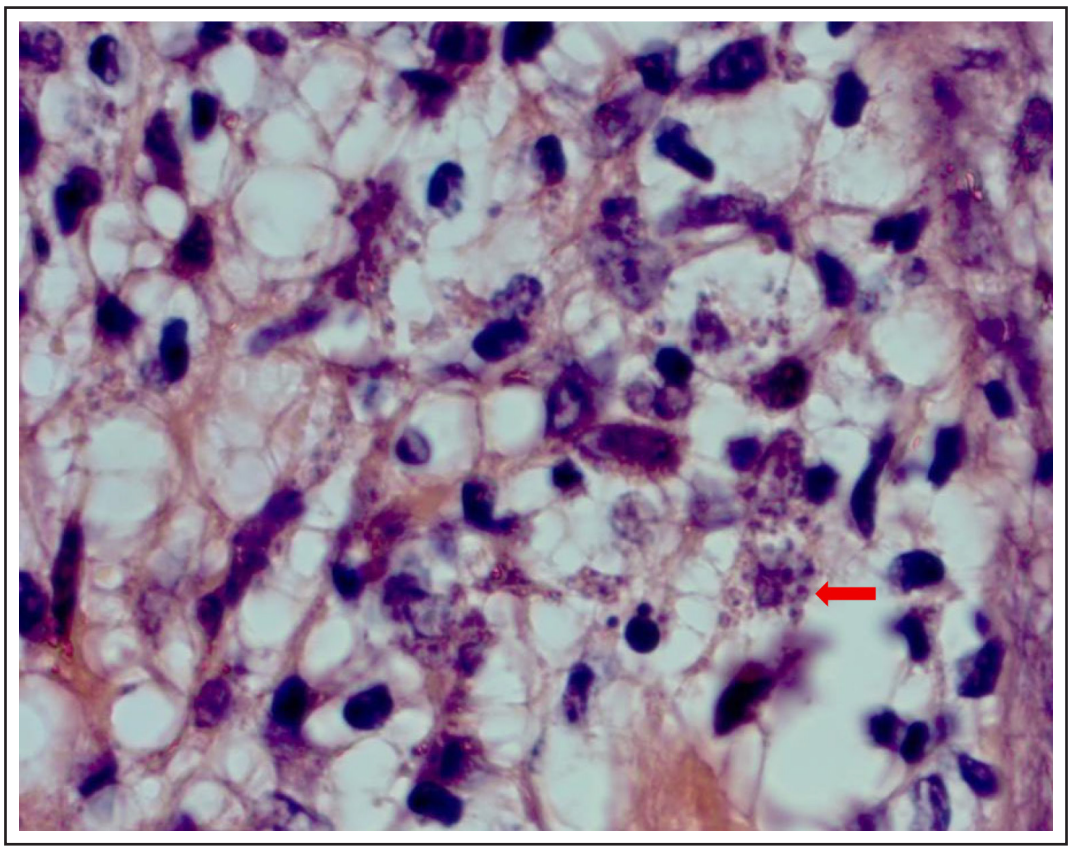

Figure 2. The H&E-stained tissue sections revealed *Leishmania* amastigotes (red arrow) at 100× magnification.

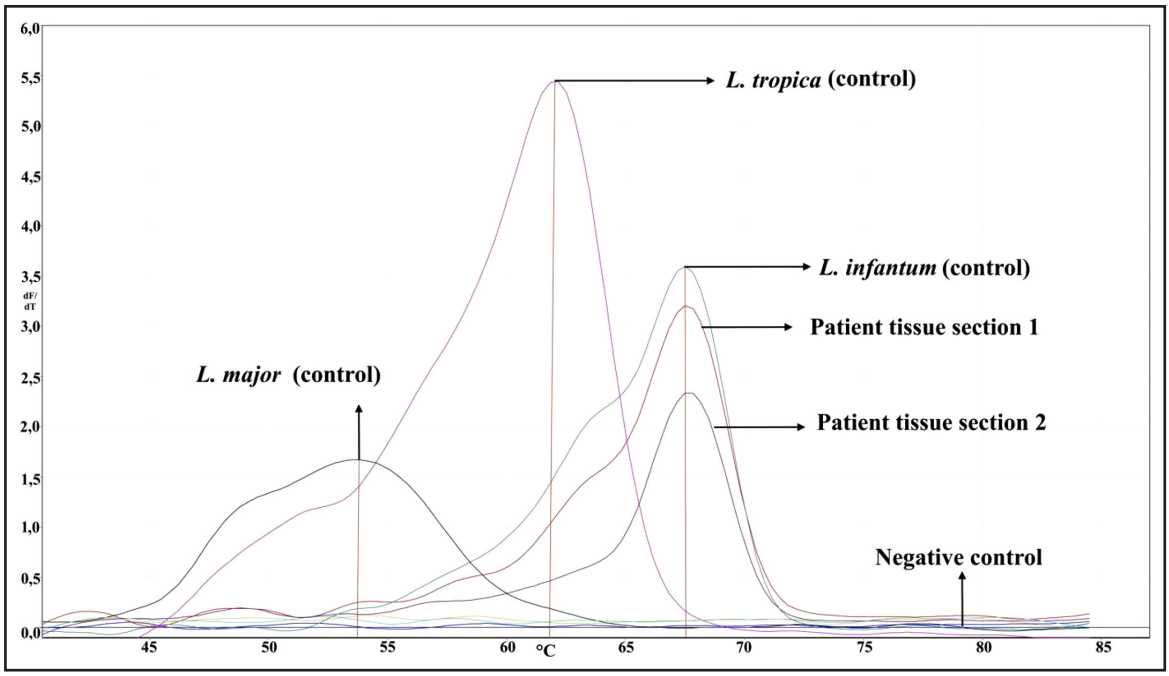

Figure 3. Melting curve analysis of the qPCR assay identified the infecting species as a member of the *L. donovani* complex, presumptively *L. infantum*.

It is noteworthy that Kyrenia district (the northern coastal region) is a focus for leishmaniasis in northern Cyprus, which can be attributed to several reasons. Firstly, Lapithos in Kyrenia district was one of the two residential places where *Phlebotomus* spp. diversity was detected at the highest levels in northern Cyprus (Demir *et al*., 2010). In another study, *Phlebotomus tobbi* was identified as the dominant species among *Phlebotomus* and *Sergentomyia* spp. collected in Kyrenia and Lapithos (Toz *et al*., 2013a). Furthermore, *L. infantum* was detected in *P. tobbi* pools collected from Lapithos and a nearby village, Panagra (Ergunay *et al*., 2014). The clustered leishmaniasis cases in the Kyrenia district can also be explained by the presence of CanL infection in this region (Toz *et al*., 2013a). While CanL seropositivity was detected across different localities in northern Cyprus, the highest seroprevalence (13.2%) was observed in Kyrenia district (Çanakçı *et al*., 2016).

Although ITS-1 melting curve analysis cannot definitively distinguish between *L. infantum* and *L. donovani* without sequencing, the environmental and climatic conditions of northern Cyprus strongly support *L. infantum* as the most probable agent related to the high presence of the vector sandfly species. The Kyrenia district is a well-documented focus for canine leishmaniasis (CanL), with seroprevalence rates reaching 13.2%. Given that CanL in the Mediterranean basin is primarily caused by *L. infantum* (Ferdes *et al*., 2025), and *L. infantum* DNA was identified in local *Phlebotomus tobbi* pools (Ergunay *et al*., 2014), the current CL case most likely reflects a zoonotic transmission cycle characteristic of this region. While alternative markers such as heat shock protein 70 (*hsp70*) and cysteine proteinase b (*cpb*) (Di Muccio *et al*., 2026) were initially considered for species-level differentiation, these low-copy-number genes failed to yield detectable amplicons in the present study. This was attributed to formalin-induced DNA fragmentation inherent in FFPE specimens and a potentially low parasite load. Consequently, the multi-copy ITS-1 region was prioritized for its high sensitivity, enabling reliable detection of the *L. donovani* complex despite the challenges posed by the sample matrix.

Leishmania infantum is traditionally associated with VL, but cases of CL have been reported in the Mediterranean basin (Crowe *et al*., 2014). The emergence of *L. infantum* as a skin lesion rather than its typical visceral form may be linked to various factors related to both the parasite's tropism and the host's immune response. One hypothesis is that strain differences within *Leishmania* species affect the clinical phenotype (Roca-Geronès *et al*., 2024). Another is that effective Th1-mediated cellular immunity may limit the parasite's systemic spread and lead to a localized skin lesion (Dubie & Mohammed, 2020). Additionally, factors such as parasite inoculum size and tissue parasite load may influence the clinical presentation; lower parasite loads and specific components of sandfly saliva can shape the course of infection, favoring cutaneous rather than visceral manifestations (Rohousová & Volf, 2006; Loeuillet *et al*., 2016).

Due to the retrospective nature of this study, detailed information on the patient's symptoms, the clinical appearance of the skin lesion, as well as the treatment outcome, were unavailable, which is a limitation of the study. Despite this, our findings contribute to the understanding of leishmaniasis across Cyprus by highlighting the island's heterogeneous epidemiological structure. In the southern part of the island, *L. donovani* (MON-37) emerges as the dominant agent, particularly in CL and VL foci within the Greek Cypriot community. At the same time, our data confirm the persistent impact of the *L. donovani* complex on human infections across the island. As proposed by Koliou *et al*. (2014) in their dual transmission cycle hypothesis, the human-focused anthroponotic cycle and the dog-reservoir-based zoonotic cycle align completely with our case. Presence of CanL and infected vector populations previously identified in the Kyrenia region reinforces the epidemiological evidence that this case originated from a regional zoonotic cycle. Consequently, it is essential to implement integrated molecular surveillance activities covering both human and veterinary cases to monitor and control the island's concurrent and distinct transmission dynamics.

CONCLUSION

Our findings will form the basis for future molecular studies to identify infected *Leishmania* species and determine the genetic relationship between parasite strains. We emphasize that protective measures must be implemented to interrupt transmission among the canine reservoir, requiring strict management of infected dogs. In this context, identification of all infected dogs, whether symptomatic or asymptomatic, is critical to the success of disease prevention strategies. Furthermore, presence of the vector sandfly species in the region underscores the risk of disease transmission. Therefore, future epidemiological studies will be crucial for providing information on the current status of leishmaniasis and for contributing to vector and reservoir control strategies in northern Cyprus.

Informed Consent:
This report was prepared in compliance with ethical standards. Verbal informed consent was obtained from the patient for the study and the publication of the data presented. The authors are grateful to the patient for his cooperation.

Conflict of Interest:
The authors declare that they have no conflict of interest.

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