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Case Report

First clinical case of leishmaniosis due to Leishmania infantum in a domestic cat from Turkey

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ABSTRACT

A 12-year-old, female domestic cat was admitted with a history of suffering from pruritic, recurrent crusting-ulcerative skin lesions on neck for several years. Physical examination revealed lesions with hair loss, mild crusting and ulcerative plaques on the neck. Two weeks before the presentation, an attempt to treat the cat in a private clinic was unsuccessful. Based on history and dermatological signs, leishmaniosis was suspected. No *Leishmania* amastigotes were found on microscopical examination of the skin, blood and lymph node smears stained using Giemsa, and serological tests for detecting FeLV and FIV gave negative results. The presumptive diagnosis was confirmed by the detection of *L. infantum* DNA by C-nPCR in EDTA blood samples. Treatment with enrofloxacin and supporting drugs for two weeks provided complete recovery of skin lesions one month after the treatment, and the direct detection of pathogen DNA was negative. At a yearly follow-up for 3 years, no clinical recurrences were observed. To the authors' knowledge, this is the first clinical case of *L. infantum* infection diagnosed in a domestic cat in Turkey.

Keywords: Cat, Leishmania infantum, clinical case, Turkey

Türkiye'de bir kedide Leishmania infantum'dan ileri gelen ilk klinik leishmaniosis olgusu

ÖZET

Oniki yaşlı, dişi bir kedi kaşıntı ve boyun bölgesinde birkaç yıldır tekrarlayan kabuklu-ülseratif deri lezyonları şikayetiyle getirildi. Fiziksel muayenede boyun bölgesinde kıl dökülmesi, hafif kabuklanma ve ülseratif plaklarla karakterize lezyonlar belirlendi. Hasta getirilmeden iki hafta kadar önce özel bir veteriner kliniğinde uygulanan tedavi denemesinden sonuç alınmadığı belirtildi. Anamnez ve dermatolojik bulgular temelinde leishmaniosisden şüphelenildi. Giemsa ile boyanan deri, kan ve lenf yumrusu örneklerinin mikroskopik incelemesinde *Leishmania* amastigotlarına rastlanmadı; FeLV ve FIV'in belirlenmesine yönelik serolojik testler negatif sonuç verdi. Olası tanı EDTA'lı kan örneklerinde C-nPCR yöntemiyle *L. infantum* DNA'sının belirlenmesiyle doğrulandı. İki hafta süreyle enrofloksasin ve destekleyici ilaçlarla uygulanan tedaviden bir ay sonra deri lezyonlarında tamamen iyileşme sağlanırken, kanda etken DNA'sına rastlanmadı. Üç yıl boyunca gerçekleştirilen yıllık takipte klinik bulguların tekrarlamadığı görüldü. Yazarların bilgisine göre bu Türkiye'de *L. infantum* enfeksiyonu belirlenen bir kedide tanımlanan ilk klinik olgudur.

Anahtar kelimeler: Kedi, Leishmania infantum, klinik olgu, Türkiye

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Introduction

Leishmaniases are a group of arthropod-borne diseases including different clinical presentations caused by *Leishmania* spp. Leishmaniosis caused by *Leishmania (L.) infantum* is endemic in the countries of The Mediterranean Basin and dogs are accepted as the main reservoir of the parasite (Gramiccia, 2011; Baneth et al., 2012). In Turkey, *L. infantum* was determined as a causative agent of both cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) in humans (Toz et al., 2013). It is also primarily responsible for canine leishmaniosis (CanL) with a seroprevalence ranging between 3.6% and 25% in western Turkey (Toz et al., 2005).

In contrast to dogs, leishmaniosis in cats is sporadically reported in various parts of the world (Simoes-Mattos et al., 2004; Penissi et al., 2013, 2015), although the first record of infection by *Leishmania* spp. dates back to 1912 (Sergent et al., 1912). Recently, Pennisi et *al* (2015) have reviewed a total of 46 clinical cases of feline leishmaniosis (FeL) published between 1989 and 2014 from European countries, where the diagnosis of FeL was confirmed by serological and/or parasitological methods. Clinical cases are uncommonly in cats, but recent epidemiological investigations have confirmed that subclinical infections are not rare. The prevalence rates of *L. infantum* infection in cats in serological or molecular-based surveys range from 0 % to 68.5 % in CanL endemic regions of the Old World (Pennisi et al., 2015). Therefore, domestic cats are suggested as possible alternative reservoirs of *L. infantum* (Maia et al., 2010; Gramiccia, 2011; Pennisi et al., 2015).

Human VL and CanL caused by *L. infantum* are endemic in western Turkey and they have been investigated widely whereas information on FeL are very scarce. A recent study (Pasa et al., 2015) in the Aegean Region where CanL is endemic, revealed 13 (8.84%) out of the 147 cat blood samples positive for *Leishmania* by Real-Time (RT)-PCR (4 *L. major* and 9 *L. tropica*). Another seroprevalence study of *Leishmania* infection also revealed the molecular detection of *L. tropica* and *L. infantum* in stray cats of Izmir, Turkey (Can et al., 2016). To our knowledge, no clinical cases of FeL caused by *L. infantum* have been published to date in Turkey. Case reports of FeL are useful for gathering more data on the natural disease in this species. The aim of this study was therefore to present, for the first time, a clinical case of leishmaniosis due to *L. infantum* in a domestic cat in Turkey.

Case Description

In October 2012, a 12-year-old unneutered female domestic cat living in Kusadasi Town, western Turkey, was admitted to Adnan Menderes University Veterinary Teaching Hospital for further examination because of pruritic and recurrent ulcero-crusted skin lesions on the neck for several years. The owner noticed that the cat has been household with two dogs in which leishmaniosis had been diagnosed as CanL based on a positive IFAT and detection of amastigotes in lymph node two years ago. Two weeks before the presentation, the cat had been lastly treated with parenteral amoxicillin and dexamethasone, topical antifungal cream along with *Microsporium canis* vaccination in a private clinic. There was no response to this therapy. Conversely, the lesion became worse and ulcerative.

Physical examination was revealed the presence of pruritic skin lesions with diameters of 3x5 cm along with hair loss, mild crusting and ulcerative plaques on the neck (**Fig 1A, 1B**) without ocular, and systemic signs that have been reported in the cutaneous form of FeL (Pennisi, 2002; Poli et al., 2002; Gramiccia 2011; Pennisi et al., 2015). Emaciation was not observed, and there was no evidence of systemic disease. Based on the medical history and physical examination, FeL was suspected. In order to confirm the infection, the cat was submitted to laboratory evaluation.

Whole blood samples (EDTA anticoagulant) for complete blood cell (CBC) count and PCR testing, and serum samples for bio

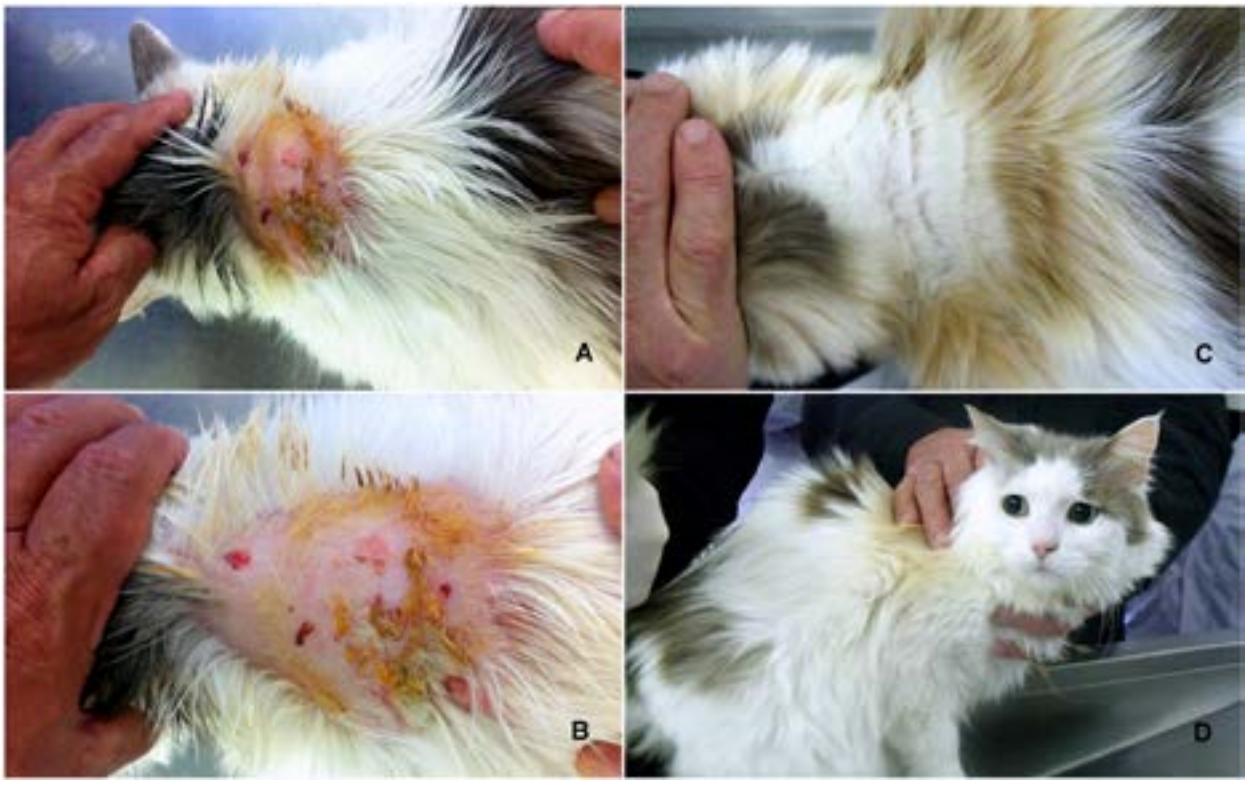

Figure 1 A, B. Ulcero-crusted skin lesions on neck of the cat.
Figure 1 C, D. Appearance of neck (C) and general appearance (D) of the cat one month after treatment.

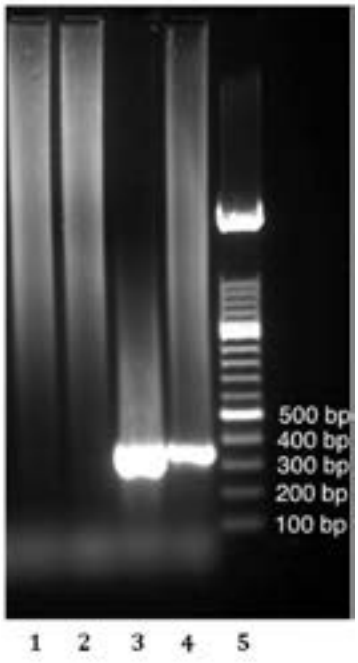

Figure 2. Results of C-nPCR identifying Leishmania spp. Lane 1, negative control; lane 2, one month after treatment; lane 3, before treatment; lane 4, Leishmania (L. infantum) positive control; lane 5, DNA marker 100 bp.

chemical parameters and investigation of the feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) were collected. Skin, blood and lymph node smears were prepared and stained using Giemsa to confirm the presence of *Leishmania* amastigotes, but no *Leishmania* spp. amastigotes were observed. Results of CBC and serum biochemical profile were within normal limits, and ELISA results for FeLV and FIV were negative.

DNA extractions were performed by Qiagen Dneasy Blood & Tissue kit using 200 µl of peripheral blood samples obtained before the treatment and one month after the treatment. Classic Nested PCR (C-nPCR) was performed using primers R221-R332 and R223-R333 as described before (van Eys et al., 1992). The target for amplification of the small subunit ribosomal RNA (SSU rRNA) gene was 358 bp. PCR products were visualized by 1.5 % agarose gel electrophoresis (**Fig 2**). Positive PCR product was sequenced commercially by Biyomer with ABI 3130 Genetic Analyser. Sequence analysis of the positive PCR products revealed a 99% similarity with *L. infantum* isolate KC347301 from Iran in BLAST. The GenBank accession number provided for the nucleotide sequence reported in this case is KM408430.

Treatment consisting of enrofloxacin (Baytril®, Bayer, 3 mg/kg, SC, SID), 0.1% rivanol solution (twice a day, topically) and cream containing pantothenic acid with chlorhexidine HCl (Bepanthene Plus®, Bayer, twice a day, topically) was applied for two weeks. One month after the treatment, the skin lesions of the cat improved completely **(Fig 1C, 1D)** and the C-nPCR test was negative. At a yearly follow-up for 3 years, no clinical recurrences were observed.

Discussion

So far, clinical leishmaniosis in cats has never been reported in Turkey and this case, therefore represents the first clinical report of a domestic cat naturally infected with *L. infantum*. The cat was born and stayed permanently in Turkey; therefore, *L. infantum* infection has to be considered as sporadic autochthonous appearance. Clinical leishmaniosis is uncommon in cats although significant numbers of cats might be subclinically infected in the same areas where the disease occurs in dogs or humans (Maia et al., 2010; Gramiccia, 2011; Pennisi et al., 2015). Kusadasi town located in western Turkey, where the infected cat described herein was identified, is an endemic region for CanL with a seroprevalence rate reaching up to 9.1% and autochthonous human and/or canine cases of leishmaniosis have been reported previously (Toz et al., 2005). The cat presented herein lived in the same house where two dogs were affected by *L. infantum*. Therefore, these dogs are suspected to play a reservoir role for the presented cat.

Leishmaniosis due to *L. infantum* in cats can cause both cutaneous and visceral forms of the disease, although the cutaneous form is recorded most frequently (Simoes-Mattos et al., 2004; Gramiccia, 2011; Pennisi et al., 2013, 2015). Ulcerative, crusty, nodular, or scaly dermatitis found mainly on the head and neck and alopecia with unspecific clinical characteristics are the most frequent clinical manifestation (Hervás et al., 1999; Poli et al., 2002; Spickler et al., 2010; Gramiccia, 2011; Pennisi et al., 2013, 2015), and sometimes the only finding on physical examination (Pennisi et al., 2013, 2015). The cat with possible FeL in this report presented clinical signs compatible with cutaneous abnormalities described in the literature (Spickler et al., 2010; Gramiccia, 2011; Pennisi et al., 2013, 2015). Clinical disease and visceral signs developing in some cats seem to be related to concurrent immunosuppressive viral infections such as FIV and FeLV (Poli et al., 2002; Pennisi et al., 2013), although this association has not been confirmed (Leiva et al., 2005). In our case, it can be suspected that a concomitant immunosuppressive event might have caused clinical disease. FIV and FeLV testing yielded negative findings, but the cat was treated several times with systemic glucocorticoids, the immunological status may have been impaired (Pennisi et al., 2013, 2015). Information on clinicopathological abnormalities are FeL is scarce. Mild nonregenerative anemia, thrombocytopenia, and hyperproteinemia are reported as the main laboratory findings (Pennisi, 2002; Leiva et al., 2005) which were not detected in our case.

Diagnosis of FeL is fairly difficult since clinical signs can mimic other infections in cats and culture is not routinely available (Poli et al., 2002; Simoes-Mattos et al., 2004; Gramiccia, 2011; Pennisi et al., 2013, 2015). However, FeL should be suspected in cats living or traveling in endemic areas with clinical presentation of skin, ocular, or systemic signs (Pennisi et al., 2015). The serological diagnosis usually confirms the direct diagnosis but it is not standardized in cats as for dogs (Maia et al., 2010). Many problems of serology are circumvented by the use of PCR that enables detection and accurate quantification of the parasite (Gramiccia, 2011; Chatzis et al., 2014; Pennisi et al., 2015). Definitive diagnosis can also be made by the detection of *Leishmania* amastigotes in different tissues by cytological examination (Pennisi et al., 2013), although the sensitivity of this method may not be high enough (Maia et al., 2010; Chatzis et al., 2014). The diagnosis in this case at presentation was suspect leishmaniosis because of information on case history and unspecific clinical findings. Thereafter, C-nPCR reaction confirmed the suspected diagnosis, and the etiologic agent was identified as *L. infantum* (**Fig 2**), a *Leishmania* species that had been previously reported to infect cats from The Mediterranean Basin (Ozon et al., 1998; Hervas et al., 1999; Pennisi, 2002; Poli et al., 2002).

Treatment of FeL has not been standardized due to the small number of reported cases. Successful treatment of cats with clinical recovery can be achieved with allopurinol (Leiva et al., 2005; Pennisi et al., 2013, 2015) and meglumine antimonate (Hervas et al., 1999). Both fatal cases and spontaneous cures have also been reported (Spickler, 2010). In the present study,

the skin lesions recovered completely and *L. infantum* DNA was not detected from the blood, as documented by PCR after one month of the treatment. Clinical and parasitological improvement of the case could be explained by the efficacy of enrofloxacin as described early in CanL (Bianciardi et al., 2004). Alternatively, the cat could experience spontaneous clearance of infection, as evidenced by the negative PCR assays in blood tested; similar to what has been documented previously in dogs (Koutinas and Mylonakis, 2010). Lastly, the negative PCR status of the treated cat in the present study may be due to the suppression of tissue *Leishmania* loads to a level not detectable by the applied assays. This possibility was demonstrated in dogs (Francino et al., 2006). The cat presented herein is still living healthy.

FeL has to be included in the differential of ulcerative and crusty dermatitis for cats living in *L. infantum*-endemic areas. Further studies are necessary to demonstrate the possible epidemiological role of domestic cats in leishmaniosis in Turkey.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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