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# First case of feline leishmaniosis caused by *Leishmania infantum* genotype E in a cat with a concurrent nasal squamous cell carcinoma

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## Abstract

**Case summary** This is the first clinical report of feline viscerocutaneous leishmaniosis caused by *Leishmania infantum* genotype E associated with an invasive squamous cell carcinoma (SCC) in a domestic cat from Portugal. Initially, the cat presented a single cutaneous lesion in the right nostril. A fine-needle aspiration was performed and *Leishmania* amastigotes were observed without the presence of cells compatible with neoplasia. Systemic treatment with allopurinol was started. One year later, the cat presented a crateriform non-encapsulated and badly delineated mass in the nasal planum, with naso-oral fistulation and nasal destruction. Histologically, the skin mass consisted on an ulcerative plaque-like lesion with a nasal SCC. *Leishmania infantum* MON-1 parasites were detected by histopathology, culture and PCR of the skin mass, submandibular and popliteal lymph nodes, liver and spleen. Restriction enzyme analysis revealed genotype E, previously identified in humans and dogs living in the same region.

**Relevance and novel information** This is, to the best of our knowledge, the first clinical report of feline viscerocutaneous leishmaniosis caused by *L. infantum* genotype E. The detection and isolation of parasites from a cat that are genetically identical to the ones obtained from humans and dogs with visceral leishmaniosis highlights the need to clarify whether cats play a role in the epidemiology of this parasitic zoonosis. From a clinical point of view, this case reinforces the importance of including leishmaniosis in the differential diagnoses of feline pathology, especially in cats with cutaneous lesions.

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## Introduction

Zoonotic visceral leishmaniosis caused by *Leishmania infantum* is a serious public health and veterinary problem in the Mediterranean basin. Although dogs are the major host for this parasite and the main reservoir for human infection, it has been proven that cats can be experimentally infected with strains of *Leishmania chagasi* (syn *L. infantum*).<sup>1</sup> In the past few years the number of feline leishmaniosis (FeL) case reports has increased in several countries where this zoonosis is endemic,<sup>2,3</sup> probably owing to higher awareness of veterinarians about the occurrence of this parasitosis in cats and to the development of more sensitive methods to detect it, such as PCR. In Portugal, one visceral and two cutaneous cases of FeL have been reported.<sup>4–6</sup>

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## Case description

The present report describes the first case of disseminated viscerocutaneous FeL caused by *L infantum* genotype E associated with an invasive squamous cell carcinoma (SCC) in a cat from Lisbon, Portugal. In January 2011, a 10-year-old unneutered domestic short-hair (European breed) female cat weighing 5 kg was presented for veterinary consultation. The cat lived on the street and was adopted after receiving treatment for multiple pelvic fractures. At that time, the animal presented with vaginal bleeding, abdominal bruising, pale mucous membranes, slow capillary refill time, severe pain in the lumbosacral region, absence of anal sphincter reflex and a small ulcerated lesion in the right nostril (2 mm). The cat tested negative for feline immunodeficiency and feline leukaemia viruses. In January 2012, clinical examination revealed a general good condition with a single ulcerated cutaneous lesion (1 cm in diameter) in the right nostril (Figure 1a). A fine-needle aspiration was performed and *Leishmania* amastigotes were observed in the cytoplasm of macrophages, as well as extracellularly, with the absence of neoplasia-compatible cells. The lesion was cauterised with silver nitrate and systemic treatment was established with allopurinol (30 mg/kg PO q24h until expected healing of the lesion), enrofloxacin (5 mg/kg PO q24h for 8 days), triamcinolone acetonid (1 ml/kg IM single dose) and the immunostimulant *Andrographis paniculata* (5 mg/kg PO q24h for 1 month). Two months later, the cat was presented with oral disease. The cat was anorectic and presented with sialorrhoea. After physical examination, a severe gingivitis on the right side was observed. The previous nasal lesion had a favourable evolution, as it was less inflamed. The lesion was re-cauterised with silver nitrate, and enrofloxacin (5 mg/kg PO q24h for 8 days) and meloxicam (0.05 mg/kg q24h for 10 days) were added to the treatment regimen. At the end of June 2012 the cat's owners stated that the animal was eating half the usual amount of food and appeared more prostrated with the falling of a crust from the nasal lesion (which occurred once a week). Abnormalities detected on physical examination were gingivitis and pain on palpation of the mouth on the right side next to the nostril, which was obstructed with secretions. The internal mucosa of the nostril was apparently normal at macroscopic examination. The owners did not allow a second aspirative biopsy or blood analysis. The cat was treated with enrofloxacin (5 mg/kg PO q24h for 10 days), meloxicam (0.05 mg/kg PO q24h for 15 days), famotidine (1 mg/kg PO q24h for 15 days) and mirtazapine (0.75 mg/kg PO q72h), and the dosage of allopurinol was increased to 50 mg/kg orally every 24 h. One month later allopurinol was discontinued owing to coprostitis. In August 2012, the owners reported that the cat had partially recovered its appetite but sometimes choked up and had a slight

discharge from the right eye. The crust had fallen and the lesion seemed less wide but deeper (Figure 1b). In September 2012, the animal was anorectic and the lesion had not evolved. The cat was treated with domperidone (0.5 mg/kg PO q24h for 1 month), famotidine (1 mg/kg PO q24h for 15 days) and a supplement of complex B vitamins. In November 2012, the cat presented with loss of appetite and a severe gingivitis, and its upper right canine tooth was shaking. The tooth was extracted and the animal was treated with clindamycin (10 mg/kg PO q12h for 8 days) and meloxicam (0.05 mg/kg PO q24h for 8 days). One month later, the bore of the tooth root was already filled with newly formed granulation/tumoural tissue. Although the nasal lesion seemed to be more superficial, the cat's face was becoming deformed.

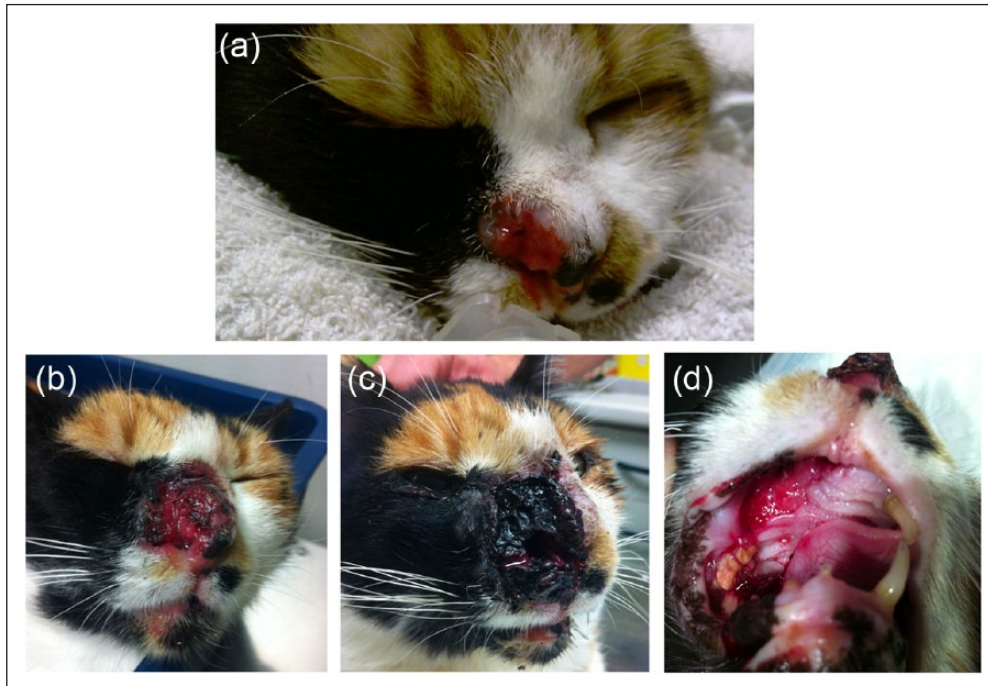
In January 2013, the owners reported apathy, dysphagia, anorexia and weight loss. On physical examination, a crateriform non-encapsulated and barely delineated mass was present in the nasal planum, with nasal destruction and naso-oral fistulation (Figure 1c,d). Submandibular lymphadenopathy was also present. Considering the poor prognosis, the owners requested euthanasia. A peripheral blood sample was collected for DNA extraction and for serology with an indirect fluorescence antibody test (IFAT).<sup>6</sup> Necropsy was performed and, skin mass, submandibular and popliteal lymph nodes, liver and spleen biopsies were processed for *in vitro* culture, DNA extraction and histology.

Macerates from all tissues were cultured and incubated as previously described.<sup>7</sup> Macerates were also used for DNA extraction using a commercial kit (PCR Template Preparation Kit; Roche Diagnostics) and submitted to PCR using primers designed from a kinetoplast DNA minicircle sequence of *L infantum*.<sup>7</sup> Tissue samples were routinely fixed (10% buffered formalin) and processed for paraffin inclusion. Sections (4 mm) were stained with haematoxylin and eosin for histopathological analysis.

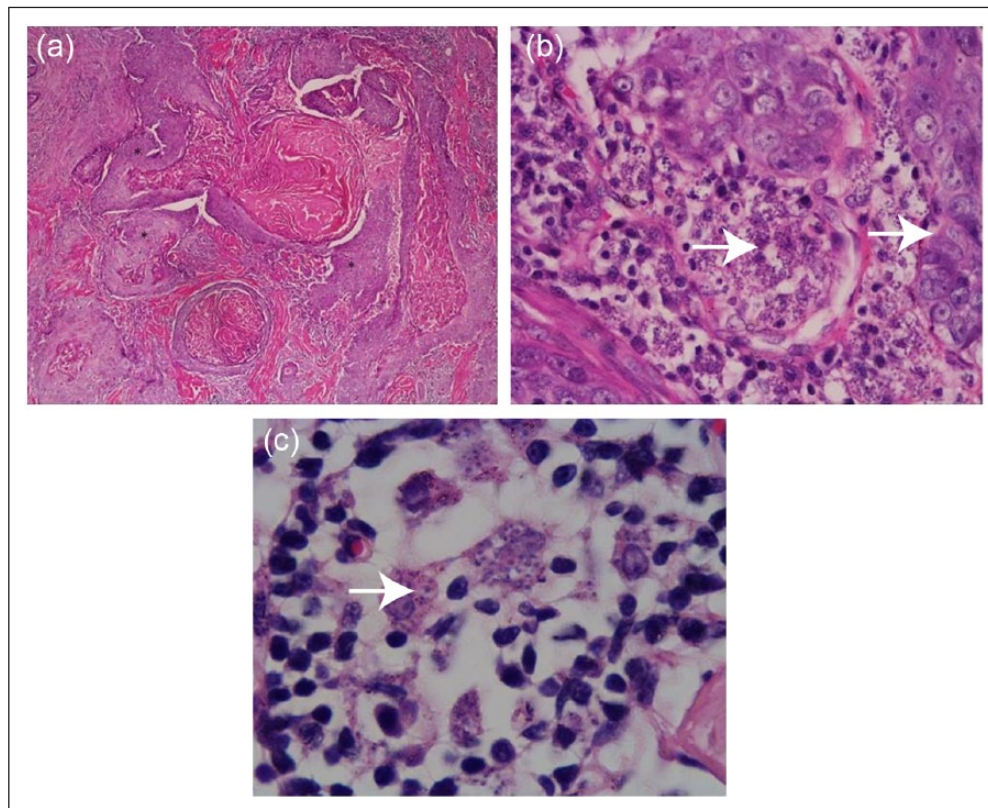
Histologically, the skin mass consisted of an ulcerative plaque-like lesion containing islands and trabeculae of squamous cell originated in the superficial epidermis and extending deep into the dermis. The epithelial structures showed progression from basal cells at the periphery to large, polygonal keratinised cells at the centre. Keratin pearls were present in the centre of larger islands (Figure 2a). Stromal fibroplasia was present and was severely infiltrated with macrophages, followed by lymphocytes and plasma cells. The macrophages contained variable numbers of round-to-oval amastigotes (1–2 µm in width and 2 µm in length). The amastigotes were present within macrophages and extracellularly (Figure 2b).

The submandibular and popliteal lymph nodes presented mild follicular atrophy and expansion of medullary cords filled with foamy macrophages containing amastigotes in the cytoplasm (see Figure 2c). The spleen





**Figure 1** (a) Ulcerated cutaneous lesion in the right nostril in January 2012 and (b) in August 2012; (c) crateriform non-encapsulated and barely delimited mass in the nasal planum in January 2013; (d) with nasal-oral fistulation



**Figure 2** (a) Nasal squamous cell carcinoma (SCC; haematoxylin and eosin,  $\times 40$ ). Ulcerative plaque-like lesion containing islands and trabeculae of squamous cell originating in the superficial epidermis and extending deep into the dermis (\*). (b) Nasal SCC (haematoxylin and eosin,  $\times 400$ ). Stromal infiltration of macrophages containing small ( $2\ \mu\text{m}$ ) unicellular basophilic microorganisms, compatible with *Leishmania* amastigotes (arrow), adjacent to islands of SCC. (c) Submandibular lymph node (haematoxylin and eosin,  $\times 400$ ) presented an infiltration of macrophages with unicellular basophilic microorganisms compatible with *Leishmania* amastigotes (arrow)

presented a low number of macrophages with *Leishmania* species in the red pulp, whereas the liver was unremarkable and no parasites were identified. Metastasis of the nasal carcinoma was not observed. A diagnosis of visceral with nasal squamous cell carcinoma (SCC) was made.

IFAT was positive, with a titre of 1024, suggesting that cats with clinical FeL develop a strong humoral response to the parasite.<sup>3,8</sup> Thus, the low seroprevalence obtained in previous epidemiological surveys performed in stray and domestic cats from Portugal was probably associated with the lack of clinical signs in the surveyed animals.<sup>9–12</sup> In fact, the development of clinical signs in FeL has been related to an impaired immune system due to a concurrent immunosuppressive infection/disease,<sup>3</sup> such as the nasal SCC reported in our case.

Coexistence of leishmania and neoplasia has been reported in humans, dogs and cats.<sup>3,8,13–15</sup> In human medicine, direct involvement of *Leishmania* species in the pathogenesis of cancer, specifically in skin and mucous membranes has been described.<sup>13</sup> In fact, owing to the adverse effects that they have in the activation and function of macrophages and dendritic cells, which might permit the escape of continuously produced clones of malignant cells from efficient immune destruction, recent findings have shown that *Leishmania* species parasites might favour the induction of carcinogenesis.<sup>13</sup> However, the available data on humans also suggest that leishmaniasis may manifest itself in a more severe clinical course in the setting of a co-existent malignancy. Although it can be hypothesised that in the present report *Leishmania* species infection occurred first, as no neoplastic cells were visualised in the cytology and might have facilitated the development of malignant cells, it can equally be hypothesised that the SCC, which do not always exfoliate well with fine-needle aspirates, preceded the leishmania and thus predisposed to the infection. As a skin biopsy was not undertaken at the time that the fine-needle aspiration was performed, the initial diagnosis cannot be definitively proven. Therefore, it is difficult to determine in the present report how many of the clinical signs (ie, skin ulceration, lymphadenopathy) observed in the cat were likely due to *Leishmania* species infection and how many were due to the SCC; however, the co-existence of both were responsible for the severe clinical presentation.

In addition, the immunosuppressive action of the carcinoma could be the reason why treatment with allopurinol, described to be clinically effective in FeL, was not efficacious in controlling leishmania infection.

*Leishmania* species DNA was amplified from lymph nodes, liver, skin and spleen, and a strain (MFEL/PT/13/IMT405) was isolated from cultured tissues and identified by isoenzymatic typing by the Centre National de Référence des *Leishmania*, Université de Montpellier,

as *L. infantum* MON-1, the most common zymodeme in dogs and humans with leishmaniasis in the Mediterranean basin.<sup>16</sup> In fact, *L. infantum* MON-1 is responsible for >96% of the cutaneous and visceral human cases and for 99% of canine leishmaniasis cases in Portugal.<sup>17</sup> Analysis of the enzymatic restriction of the PCR products showed that parasites belonged to genotype E, which has previously been identified among Portuguese *Leishmania* strains isolated from immunocompromised humans and from dogs.<sup>18</sup> Altogether, the visceral dissemination of the infection with the same species of *Leishmania*, zymodeme and genotype observed in humans and dogs with leishmaniasis reinforces the possible role of cats in the epidemiology of this zoonosis.<sup>2</sup>

## Conclusions

In the light of the increased number of clinical cases of FeL reported in the past few years in cats living in endemic areas of leishmaniasis, together with the considerable prevalence of *Leishmania* species infection observed in several epidemiological surveys, it is important to include this parasitosis systematically among the differential diagnoses of feline pathology. Nevertheless, from a public health perspective it is important to clarify whether this animal species is capable of sustaining and spreading *L. infantum* infection.

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**Conflict of interest** The authors do not have any potential conflicts of interest to declare.

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