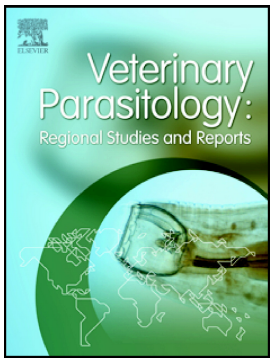

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Clinical leishmaniosis in a domestic ferret (*Mustela putorius furo*) treated with miltefosine plus allopurinol: serological and clinical follow-up

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ABSTRACT

The published information on the treatment of mustelid leishmaniosis is extremely scarce because there are only two case reports available. In one case, a domestic ferret (*Mustela putorius furo*) was treated with a combination of meglumine antimoniate plus allopurinol and, in the other case, a therapeutic regimen with allopurinol was administrated to a Eurasian otter (*Lutra lutra*). This article describes for the first time a combined therapeutic protocol with miltefosine (2 mg/kg once a day during 28 days *per os*), and allopurinol (10 mg/kg twice a day PO *sine die*) in a domestic ferret with splenomegaly, lymphadenomegaly and a facial pyogranulomatous dermatitis, with a moderate level of antibodies to *Leishmania infantum*.

Keywords: dermatitis; ferret; *Leishmania infantum*; leishmaniosis; *Mustela putorius furo*.

1. Introduction

Leishmaniosis caused by *Leishmania infantum* is a parasitic zoonotic disease in Southern Europe transmitted by phlebotomine sand flies. The domestic dog is the main reservoir host for *L. infantum* and canine leishmaniosis is an important and complex disease extensively studied (Solano-Gallego et al., 2011). However, other domestic mammals are likely to be in contact with the parasite and can also be potentially infected such as cats (Alcover et al., 2021) and other conventional household pets as ferrets (Giner et al, 2020a). Moreover, the reports that leishmaniosis affects many other animals besides dogs and cats are increasing, with a recent review published including other mammals (Cardoso et al., 2021).

The domestic ferret (*Mustela putorius furo*) belongs to the family Mustelidae, the largest family within the mammalian order Carnivora. Recently, the first notification of natural *L. infantum* infection detected by parasite culture in mustelids was described (Giner et al., 2020a). In the same way, it has been published the first treatments and follow-up clinical cases

of leishmaniosis in mustelids: a domestic ferret treated with a combined therapeutic protocol based on allopurinol and meglumine antimoniate (Giner et al., 2020b), and a captive Eurasian otter (*Lutra lutra*) with a therapeutic regimen based in allopurinol (Cantos-Barreda et al., 2020).

Different drugs are available as anti-*Leishmania* therapeutic protocols in dogs, including meglumine antimoniate, miltefosine y/or allopurinol (Solano-Gallego et al, 2011). Miltefosine is is considered as the only oral drug for the treatment of leishmaniasis in humans (Soto and Soto, 2006) and one of the drugs usually used for leishmaniosis treatment in dogs (Mateo et al., 2009; Manna et al., 2009). The combination of miltefosine plus allopurinol promoted better effects in comparison to miltefosine monotherapy (Dias et al., 2020).

2. Case Presentation

A 3-year-old intact female ferret from the Province of Valencia (39° 28′12.864”N,0° 22′36.48”W), on the eastern coast of Spain, was clinically evaluated because of the presence of an inflammatory and nonpruritic lesion on facial skin near the chin on April 2020. The ferret lived with other ferrets and a cat in a house with an outside lifestyle.

On physical examination, it was in good body condition, active, alert, normothermic and not dehydrated. The patient presented an erythematous, ulcerative, edematous and painful lesion on the margin of the right lower lip (Figure 1a, 1b, 1c), enlargement of loco-regional lymph nodes and splenomegaly with no other apparent clinical signs. A skin lesion sample and a lymph node sample were taken by fine needle aspiration and stained with Diff-Quick stain for cytological examination. Cytology results revealed a pyogranulomatous inflammation in which infectious agents were not visualized in the lesion sample and a reactive lymphoid hyperplasia in the lymph node sample. A complete blood cell count (LaserCyte Idexx, Westbrook, USA) and a biochemical profile (Catalyst One Idexx, Westbrook, USA) was performed with unremarkable results except a marked alteration of globulin levels (Table 1). An alteration of the electrophoretic profile of serum proteins, showing a polyclonal gammopathy, was detected (Figure 2).

Anti-*Leishmania* antibodies were determined by an in-house enzyme-linked immunosorbent assay (ELISA) using sonicated *L. infantum* antigens as described previously (Giner et al., 2020a). As a positive control, a serum from a seropositive ferret was included (Giner et al., 2020a) and as a negative control, serum from a healthy, non-infected ferret. The cutoff was set to 0.200 Optical Density units (OD units) (mean + 3 standard deviations of values from 40 healthy indoor ferrets). Medium levels of antibodies against *L. infantum* were detected in serum samples from this patient with an OD result of 0.45.

Equally, a full thickness incisional biopsy of the lesion was taken. Histopathological examination revealed a severe chronic pyogranulomatous dermatitis with fibrosis. No acid-fast organisms were identified by Zielh-Neelsen stain. A diagnosis of leishmaniosis was made based on clinical manifestations and clinicopathological findings including the detection of specific serum antibodies using a quantitative serological technique.

An anti-*Leishmania* therapeutic protocol was established with miltefosine (Milteforan®, Virbac Laboratories, Spain) at 2 mg/kg once a day during 28 days *per os* (PO) and allopurinol at 10 mg/kg twice a day PO sine die (Zyloric® 100 mg, Faes Farma, Spain). Marbofloxacin (Marbocyl® 5 mg, Vetoquinol, France) at 2 mg/kg twice a day PO was added to the treatment during the first 10 days of therapy to control possible secondary infections in the skin lesions detected. The pyogranulomatous lesions disappeared throughout the first month of treatment (Figure 1d) and there was no relapse of the clinical signs after 10 months. Equally, there were a significant decrease of spleen and lymph nodes size during the first three months of therapy. Follow-up visits to the attending veterinarian were made monitoring clinicopathological parameters including complete blood count, biochemistry, urine analysis and anti-*Leishmania* antibody levels by serology. A decrease in serum globulin levels over time was detected: July 2020 (4.4 g / dL), September 2020 (4.1 g / dL), December 2020 (3.6 g / dL) and February 2021 (3.5 g / dL) (Table 1). On the other hand, a serological follow-up of the response to treatment was carried out in which a reduction in anti-*Leishmania* antibody levels was observed over time: July 2020 (0.39), September 2020 (0.35), December 2020 (0.27), February 2021 (0.25)

and a decrease in serum globulin levels over time: July 2020 (4.4 g / dL), September 2020 (4.1 g / dL), December 2020 (3.6 g / dL) and February 2021 (3.5 g / dL) (Table 1).

3. Discussion and Conclusion

To the authors´ knowledge, this report describes the first clinical case of leishmaniosis in a domestic ferret (*Mustela putorius furo*) treated with a combination of miltefosine and allopurinol. Different therapeutic protocols are established for canine and feline leishmaniosis. Two different treatments protocols are described recently in mustelids (Giner et al., 2020b; Cantos-Barreda et al., 2020). In the case of the domestic ferret, the use of meglumine antimoniate during 8 weeks plus allopurinol during 4.5 months has been described with a clinical improvement 3 weeks after starting treatment, however, at 6 months after starting treatment, the presence of xanthinuria was observed. In the case report about the treatment of the Eurasian otter, it was based on the single use of allopurinol during 3 months, also observing a clinical improvement.

A combined therapeutic protocol based on miltefosine and allopurinol was well tolerated in our patient. Clinical improvement was observed in this ferret and pyogranulomatous dermatitis, splenomegaly and lymph nodes enlargement were resolved within a few weeks after treatment was initiated. In this case, after one year with allopurinol treatment, xanthinuria was not observed in urine sediment during the long-term administration of allopurinol. This finding suggests that urinary adverse effects of allopurinol treatment is variable depending on the individual response (Giner et al., 2020b).

Canine leishmaniosis is a systemic disease that may potentially involve any organ, tissue or body fluid and is manifested by nonspecific clinical signs (Villanueva-Saz et al., 2020). The diagnosis of clinical leishmaniosis in dogs and cats was based on the clinical manifestation and/or the laboratory abnormalities that were compatible with the disease as well as by the confirmation of *L. infantum* infection. In this sense, this patient presented a pyogranulomatous

dermatitis, lymphadenomegaly and splenomegaly. In ferrets, systemic coronavirus, atypical mycobacterias, *Pseudomona luteola* or *Criptococcus* spp. are pathogens that induce pyogranulomatous and granulomatous inflammation (Lucas et al., 2000; Garner et al., 2008; Morera et al, 2015; Baum et al., 2015). Splenic enlargement is a very common and nonspecific finding in adult ferrets and the causes are multiple, including extramedullary hematopoiesis, lymphosarcoma and other neoplasms such as hemangiosarcoma, cardiomyopathy or chronic infections. Equally, lymph nodes enlargement in ferrets is associated with chronic inflammation or chronic infections. Moreover, hyperglobulinemia is found in this species in many types of inflammation, determinate infections or certain neoplasms. Leishmaniosis could be a pathogen that cause those clinicopathological alterations commonly detected in ferrets as splenomegaly, lymphadenomegaly and hyperglobulinemia.

This report demonstrates that miltefosine plus allopurinol seems to be effective as anti-*Leishmania* treatment in a ferret with clinical leishmaniosis, as well as the possibility to detect the presence of anti-*Leishmania* antibodies over a long period of time.

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Conflict of interest statement

The authors have nothing to disclose.

CRediT author statement

Jacobo Giner: Conceptualization, Writing-Original Draft. **Sergio Villanueva-Saz:** Project administration, Writing-Original Draft, Reviewing and Editing. Conceptualization, Writing-Original Draft. **María Magdalena Alcover:** Resources.

Cristina Riera: Resources. **Roser Fisa:** Resources. **Maite Verde:** Supervision, Visualization. **Antonio Fernández:** Writing-Original Draft, Visualization. **Andrés Yzuel:** Writing-Original Draft.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure. 1: Different views of dermatologic lesions detected in this ferret (a, b, c) and improvement during anti-*Leishmania* treatment (d). a-c. Erythematous, ulcerative and edematous lesion on the margin of the right lower lip. d. Dermatological lesion in a follow-up visit 3 weeks after the initiation of treatment showing clinical response.

Figure. 2: Electrophoretogram using capillary zone electrophoresis. The electrophoresis revealed polyclonal gammopathy.

Table 1. Body weight, haematological and biochemical parameters determined in the leishmaniotic ferret at the first veterinary examination before treatment (April 2020) and during the follow-up.

Parameter	April 2020	July 2020	September 2020	December 2020	February 2021	Reference range
Body weight (g)	660	705	775	755	660	500-900
Haematology						
WBC (K/µL)	5.62	4.43	3.92	3.41	4.68	2-10
Neutrophils (K/µL)	3.30	1.41	1.68	1.47	1.99	0.62-3.30
Lymphocytes (K/µL)	1.26	2.08	1.33	1.25	1.62	1-8
Monocites (K/µL)	0.85	0.73	0.63	0.49	0.85	0.18-0.90
Eosinophils (K/µL)	0.15	0.18	0.24	0.17	0.18	0.10-0.60
Basophils (K/µL)	0.05	0.04	0.03	0.03	0.05	0.00-0.10
RBC (M/µL)	10.13	10.74	10.70	9.66	8.36	6.35-11.20
Haematocrit (%)	51.20	51.7	50.1	45.3	40.6	37.0-55.0
Haemoglobin (g/dL)	**18.6**	**18.9**	18.9	15.9	13.5	11.0-17.0
MCV (fL)	50.6	48.1	46.8	46.9	48.6	45.0-55.0
MCH (pg)	**18.4**	**17.6**	**17.7**	16.5	16.2	14.0-18.0
MCHC (g/dL)	**36.3**	**36.6**	**37.8**	35.1	33.2	32.0-35.0
RDW (%)	**16.0**	**16.0**	**16.8**	**16.1**	**15.8**	19.0-25.0
Platelets (K/µL)	565	478	623	566	348	270-880
Blood Chemistry						
ALT (U/L)	88	280	207	248	260	82-289
ALKP (U/L)	<10	43	48	38	33	9-84
Glu (mg/dL)	114	96	94	95	99	94-207
Crea (mg/dL)	0.4	0.8	0.8	0.6	0.5	0.4-0.9
BUN (mg/dL)	17	28	28	26	25	10-45

PT (g/dL)	**8.1**	**7.7**	7.1	6.5	6.3	5.2-7.3
Alb (g/dL)	3.1	3,3	3.1	2.8	2.7	2.6-3.8
Glob (g/dL)	**5**	**4.4**	**4.1**	**3.6**	**3.5**	1.8-3.1
Alb/Glob ratio	0.6	0.8	0.8	0.8	0.8	
ELISA (OD)	**0.45**	**0.39**	**0.35**	**0.27**	**0.25**	Cut-off: 0.20

Abbreviations: WBC White Blood Count, *RBC* Red Blood Count, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular haemoglobin concentration, *RDW* red *blood* cell distribution, ALT alanine amino-transferase, ALKP alkaline phosphatase, GLU glucose, TP total protein concentrations, Alb albumin, Glob globulins, CREA creatinine, BUN blood urea nitrogen, ELISA enzyme-linked immunosorbent assay, OD optical density units. Abnormalities are highlighted in bold.

HIGHLIGHTS

- Dermatological lesions are the most frequently detected clinical signs.
- Miltefosine plus allopurinol seems to be effective as anti-*Leishmania* treatment.
- Urinary adverse effects of allopurinol depend on the individual response.
- Anti-*Leishmania* treatment induces a decrease in specific antibody levels.

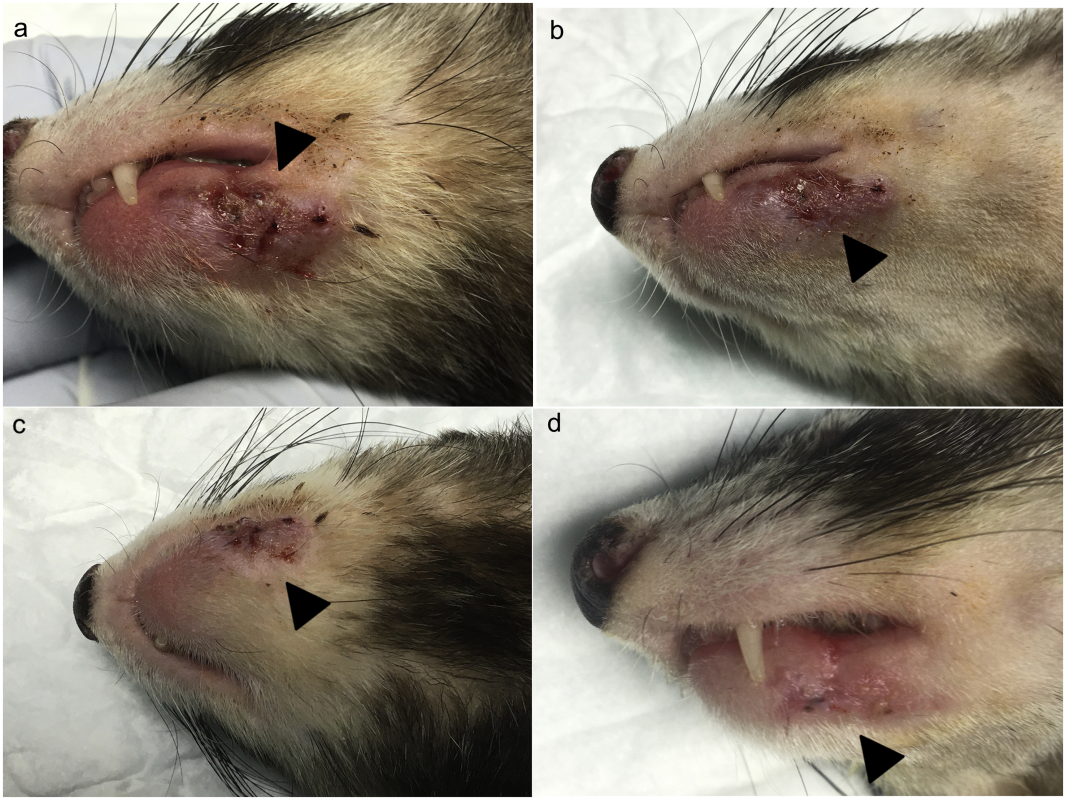

Figure 1

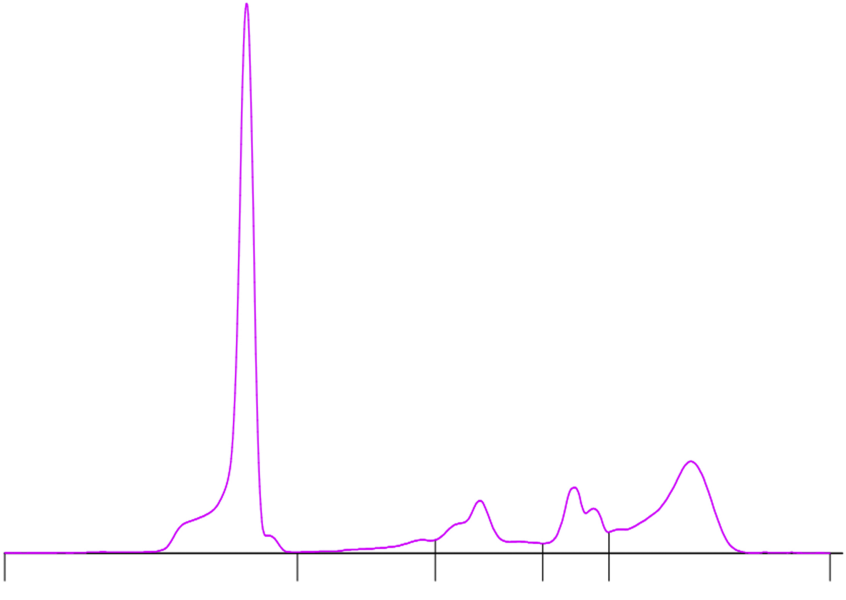

Figure 2