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# Hyperbetaglobulinemia, anaemia and thrombocytopenia in a domestic ferret (*Mustela putorius furo*) associated to *Leishmania infantum*

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ABSTRACT

Leishmaniosis in domestic ferrets is a vector-borne disease caused in Europe by the protozoan parasite *Leishmania infantum*. There is limited information on clinical signs and laboratory abnormalities in ferrets due to leishmaniosis. This clinical case report described a domestic ferret (*Mustela putorius furo*) with severe hyperbetaglobulinemia, anaemia, thrombocytopenia, and abnormal renal parameters. A good clinical response following an anti-*Leishmania infantum* treatment protocol was achieved. However, the presence of pain at the site of injection was the main side effect due to meglumine antimoniate administration. Xanthine crystalluria was not observed in urine sediment with no other urine alterations detected by urine analysis during the follow-up. Initially, clinical signs noted in this ferret could not initially be attributed to leishmaniosis. However, no causes were found that could have caused the hyperglobulinemia in this patient. A reduction of the levels of anti-*L. infantum* serum antibodies and the concentrations of beta-globulin fraction was detected in this patient after anti-*Leishmania* treatment administered as well as the disappearance of thrombocytopenia. To extent of the knowledge of leishmaniosis in ferrets, this is the fourth case report of leishmaniosis documented in this species.

## 1. Introduction

Leishmaniosis is a vector-borne disease caused by L. *infantum*, with different clinical signs in dogs and cats. In ferrets, these manifestations are poorly described, the first cases in this species were published only recently. In this sense, there is a case report of leishmaniosis in a pet ferret from Sicily, Italy, in which the diagnosis was confirmed by PCR from blood, skin and bone marrow (Brianti et al., 2005). The epidemiological role of this species in areas where L. *infantum* infection is endemic is unknown; however, a recent epidemiological study performed in an endemic area of canine leishmaniosis in Spain detected a seroprevalence from 9.0 to 25.5% based on enzyme-linked immunosorbent assay (ELISA) and western blot, respectively. The seroprevalence of L. *infantum* infection, based on a positive result in any serological test, was 28.4% in apparently healthy ferrets (Alcover et al., 2022). The presence of sick ferrets due to L. *infantum* has been described with the presence of clinical signs such as piogranulomatous skin lesions and papular dermatitis (Halck et al., 2023), splenomegaly and/or renal changes together with hyperglobulinemia (Giner et al., 2020a, 2020b, 2021).

Hyperglobulinemia is the most common laboratory finding seen in ferrets with clinical leishmaniosis (Villanueva-Saz et al., 2021). In this sense, leishmaniosis should be included in the differential diagnosis of hyperglobulinemia in ferrets. This laboratory abnormality is usually associated with a variety of infections in this species, including Aleutian disease, systemic coronaviruses, distemper virus, and certain systemic neoplasms or mycoses (e.g., blastomycosis and coccidiomycosis) (Melillo, 2013). Serum protein electrophoresis in ferrets with clinical disease shows typical polyclonal hyperglobulinemia as seen in canine and feline leishmaniosis (Solano-Gallego et al., 2011; Pennisi et al., 2015).

Mild to moderate thrombocytopenia is often detected in dogs with leishmaniosis (Meléndez-Lazo et al., 2018). However, cases of thrombocytopenia associated with clinical leishmaniosis have not been reported in domestic ferrets to date. This clinical case describes a domestic ferret with nervous and intestinal signs with thrombocytopenia and

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monoclonal hyperglobulinemia due to an increase in beta-globulin fraction.

## 2. Case presentation methods

A 1-year and 6-month-old intact male domestic ferret (*Mustela putorius furo*) with a body weight of 850 g was presented to the consultation after the owners noted soft stools of 7 days and a tilt of the head along with loss of balance and maladaptive movements in the last 48 h. The ferret had not been vaccinated and feeds on a natural diet based on prey animals and ferrets' special food. On physical examination, the ferret had a body condition of 2/5 and had a normal heart rate, respiratory rate, temperature, and systolic blood pressure. The patient was active and alert and showed changes in movement and a tilt of the head to the left, manifestations characteristic of vestibular syndrome (~~Fig. 1~~).

Complete blood tests including blood cell count and serum biochemical profile (Idexx, Westbrook, USA) and radiographs of the head were performed. The main radiological finding was the presence of an opacity in the left bulla tympanica, which indicated a possible accumulation of contents in the bulla tympanica and was a possible cause of the vestibular syndrome in this patient (Fig. 1a). After evaluation of the blood count, serum biochemistry, and serum protein electrophoresis, the main findings were mild thrombocytopenia with hypoalbuminemia and hyperbetaglobulinemia (Fig. 2a) (Table 1). The other biochemical parameters were within the reference ranges.

A parasitological examination of the stool was performed with negative results. A bacterial and mycological stool culture was processed detecting a high concentration of *Escherichia coli* and an alpha-hemolytic *Streptococcus*. The antibiogram showed sensitivity to lincosamides and fluoroquinolones.

To rule out possible infectious causes for the observed neurologic changes, we performed serum antibody tests against several pathogens, including canine distemper virus, *Toxoplasma gondii*, *Neospora caninum*, and *Cryptococcus* spp. All serological tests were negative. Likewise, blood molecular test for Aleutian disease was performed with a negative result. Cytology performed with on the otic sample obtained by swabbing the left external atrial pavilion showed no changes consistent with possible infection or inflammation of the ear canal.

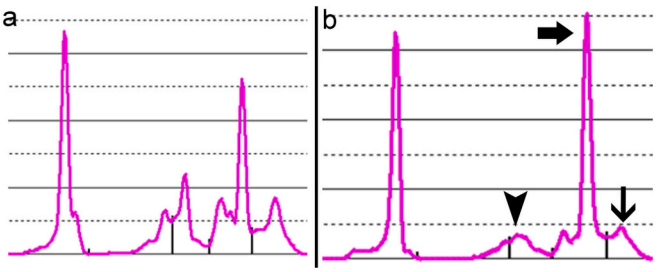


**Fig. 2.** Capillary electrophoretograms of serum proteins of the ferret before starting anti-*Leishmania* treatment during the first visit (a) and during the second visit, four weeks later (b). Electrophoretic changes are marked (arrows).

Although radiographic changes such as a thickened, irregular bulla and soft tissue opacities within the bulla lumen are commonly seen in otitis media (Garosi et al., 2003; Rohleder et al., 2006), the owner was advised to perform a Computed Tomography (CT) scan of the skull of this ferret. However, since the owner ruled out the possibility of performing a CT scan to confirm the presence of otitis media, as well as performing a myringotomy to obtain a specimen for culture and antibiogram and cytology of any contents of the tympanic membrane, it was decided to perform a conservative drug treatment with maropitant 8 mg/kg once daily and the administration of a combination of enrofloxacin 5 mg/kg BID and clindamycin 10 mg/kg BID orally with methylprednisolone 0.5 mg/kg BID, finding a rapid improvement of the patient's neurological and intestinal signs. Similarly, complete regression of radiological changes was noted after 4 weeks of treatment (Fig. 1b).

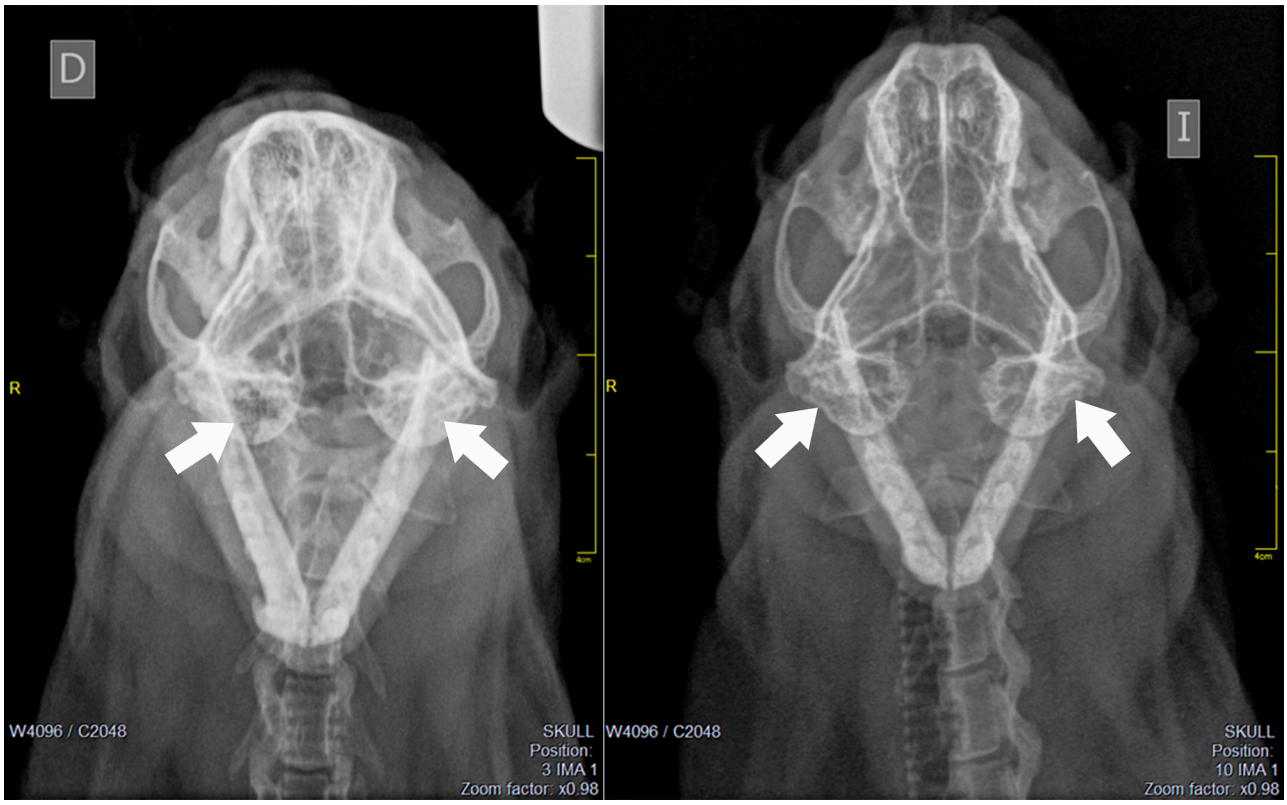


**Fig. 1.** Radiographs of the head including before (left) and after (right) antibiotic treatment. Bulla tympanica of each radiograph is marked (arrows).

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

| Parameter | Before anti-*Leishmania* treatment | | After anti-*Leishmania* treatment | | |
|---|---|---|---|---|---|
| | First Visit | Second visit (Four weeks later) | Third visit (Nine weeks later) | Fourth visit (Thirteen week later) | Reference Range |
| **Body weight (g.)** | 720 | 710 | 810 | 850 | 500–900 |
| *Haematology* | | | | | |
| WBC (K/µl) | 3.83 | 2.25 | 8.93 | 2.33 | 2.00–10.00 |
| Neutrophils (K/µL) | 2.05 | 1.20 | **6.58** | 1.54 | 0.62–3.30 |
| Lymphocytes (K/µL) | 1.04 | **0.54** | 1.18 | 0.23 | 1.00–8.00 |
| Monocytes (K/µL) | 0.44 | 0.30 | 0.78 | 0.40 | 0.18–0.90 |
| Eosinophils (K/µL) | 0.25 | 0.18 | 0.34 | 0.12 | 0.10–0.60 |
| Basophils (K/µL) | 0.05 | 0.03 | 0.05 | 0.04 | 0.00–0.10 |
| RBC (M/µL) | 7.80 | **5.91** | 7.86 | 6.83 | 6.35–11.20 |
| Haematocrit (%) | 38.50 | **25.20** | **31.80** | 37.90 | 37.00–55.00 |
| Haemoglobin (g/dL) | 12.00 | **10.70** | 12.10 | 13.20 | 11.00–17.00 |
| MCV (fL) | 46.1 | 42.5 | 45.5 | 41.8 | 45.0–55.0 |
| MCH (pg) | 15.4 | 14.5 | 15.3 | 16.0 | 14.0–18.0 |
| MCHC (g/dL) | 33.1 | 32.5 | 33.1 | 34.2 | 32.0–35.0 |
| Plateles (K/µL) | **252** | **232** | 615 | 357 | 270–880 |
| *Blood Chemistry* | | | | | |
| Alanine aminotransferase (U/L) | **38** | **59** | **38** | **49** | **82–289** |
| Alkaline phosphatase (U/L) | <10 | 17 | <10 | 43 | 9–84 |
| Total Bilirubin (mg/dL) | 0.1 | 0.15 | 0.14 | 0.3 | 0.1–1.0 |
| Cholesterol(mg/dL) | 124 | 179 | 167 | 124 | 64–296 |
| Glucose (mg/dL) | 173 | 102 | 100 | 93 | 94–207 |
| Creatinine (mg/dL) | 0.5 | **1.2** | 0.8 | 0.6 | 0.4–0.9 |
| Blood Urea Nitrogen (mg/dL) | 26 | **59** | 34 | 22 | 10–45 |
| Phosphorus (mg/dL) | 5.5 | 7.7 | 7.2 | 7.6 | 4.8–8.9 |
| Calcium (mg/dL) | 8.3 | 9.2 | 8.6 | 8.1 | 8.0–11.8 |
| Gamma Glutamyltransferase (U/L) | 2 | 5 | 4 | 0 | 0.2–14 |
| *Electrophoretograms of serum proteins (AGE)* | | | | | |
| Total protein (g/dL) | 6.6 | **8.5** | 7 | 7.2 | 4.9–7.3 |
| Albumin (g/dL) | 2.5 | 3.3 | **1.9** | 2.5 | 2.3–3.6 |
| Globulins (g/dL) | **4.1** | **5.2** | **5.1** | **4.7** | 1.8–3.1 |
| Alpha 1 globulins (g/dL) | 0.38 | 0.5 | 0.4 | 0.1 | 0.1–0.6 |
| Alpha 2 globulins (g/dL) | 0.73 | **1.7** | 1.4 | 0.5 | 0.4–0.9 |
| Beta globulins (g/dL) | **2.0** | **2.9** | 1.5 | 1 | 1.0–1.9 |
| Gamma globulins (g/dL) | **1.0** | **0.1** | **1.7** | **3.1** | 0.3–0.9 |
| A/G | 0.62 | 0.65 | 0.38 | 0.54 | |
| *Serology* | | | | | |
| One dilution (EU) | 0.85 | 0.66 | 0.47 | 0.39 | ≥0.180 |

Abbreviations: A/G albumin:globulin ratio, MCH mean corpuscular haemoglobin, MCHC mean corpuscular haemoglobin concentration, MCV mean corpuscular volume, RBC red blood count, RDW red blood cell distribution, WBC white blood count. Abnormalities are highlighted in bold.

In contrast, low body condition was observed at this time point (2/5). The ferret showed apathy and partial anorexia, and blood tests revealed normocytic normocromic anaemia with persistent thrombocytopenia. No platelet aggregation was detected in a blood smear from the patient. Biochemistry analysis detected and increase of blood urea nitrogen and creatinine levels (Table 1), and persistently elevated hyperbetaglobulinemia detected by serum protein electrophoresis performed with capillary technique (Fig. 2b). Urinalysis revealed low urine density (1.015 (reference interval: 1.026–1.060)) with normal urine protein creatinine ratio.

At this time, a complete radiological and ultrasound examination was performed to detect possible changes suggestive of masses, without significant findings. Given the presence of hyperglobulinemia, thrombocytopenia, and an increase of renal parameters usually seen in canine patients with clinical leishmaniosis, it was decided to determine the level of anti-*Leishmania* antibodies in the serum sample.

A quantitative serology based on in-house ELISA technique was performed to detect the presence of anti-*Leishmania* antibodies (Alcover et al., 2022). The cut-off was set to 0.180 optical density units (OD) (mean + 3 standard deviations of values from 30 indoor ferrets from northern Spain), and results above this value were considered positive. In this sense a positive result was obtained with a OD value of 0.85.

Due to the severity of the laboratory alterations detected in this ferret and no other causes responsible for these abnormalities, it was decided to establish an anti-*Leishmania* treatment protocol based on the presence of laboratory findings compatible with leishmaniosis and the detection of a positive result by a confirmatory serological technique.

Anti-*Leishmania* treatment protocol was administered based on meglumine antimoniate at a dosage of 50 mg/kg BID subcutaneously for five weeks and allopurinol at 10 mg/kg BID PO sine die. In the case of antimoniate meglumine was initially administered from 25 mg/KG BID the first week to 40 mg/kg BID subcutaneously in the third, fourth and fifth weeks BID subcutaneously. During meglumine antimoniate treatment, the patient experienced episodes of hyperthermia along with erythema, hardening and pain at the injection site (Fig. 3) with evidence of piogranulomatous panniculitis associated with subcutaneous administration of this drug confirmed by fine needle aspiration and subsequent cytology.

After cessation of meglumine antimoniate administration, the animal showed continuous improvement in body condition and disappearance of clinical signs noted as apathy and partial anorexia. Previously observed laboratory abnormalities such as anaemia and thrombocytopenia were not detected after completion of meglumine antimoniate treatment. In addition, laboratory renal parameters were within interval reference, and beta-globulin fraction decreased significantly in the analysis performed (Table 1).

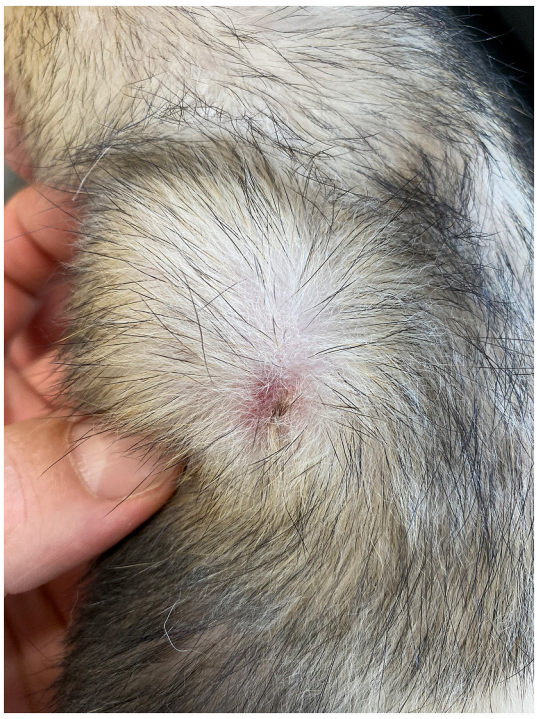

**Fig. 3.** Local side reaction at the site of injection with the presence of erythema and induration in dorsal area.

After 4 months of continuous treatment with allopurinol, xanthine crystals were no longer detected in the monthly urine tests. Finally, a significant decrease in anti-*Leishmania* antibodies was observed in this patient, proving the efficacy of the treatment administered to the patient. Unfortunately, follow-up of the patient was not possible.

## 3. Discussion and conclusion

This case represents the first case of clinical leishmaniosis in a ferret in which non-regenerative anaemia and thrombocytopenia have been detected, laboratory abnormalities frequently detected in dogs with laboratory abnormalities of leishmaniosis (Solano-Gallego et al., 2011).

Clinicopathologic abnormalities in dogs and cats with leishmaniosis are usually nonspecific and usually depend on the tissue involved. The clinicopathological alterations of canine leishmaniosis include the development of non-regenerative normochromic normocytic anaemia, thrombocytopenia, or changes in the leukogram. Serum biochemistry and urinalysis may indicate renal dysfunction (such as azotemia, decreased urine specific gravity, and proteinuria) as well as an inflammatory/immune response (Navarro et al., 2022). However, other mechanisms have been described in dogs, including a decrease in erythropoietin synthesis and the production of autoantibodies to the erythrocytes (Paltrinieri et al., 2016). Similar mechanisms are responsible for anaemia in cats (Pereira and Maia, 2021).

In our case, we found the presence of thrombocytopenia. This finding is uncommon in sick dogs and occurs very rarely. In dogs, it is associated with the presence of vector-borne pathogens, and if coinfections have been excluded and thrombocytopenia is still present, other possible causes such as decreased platelet concentration should be investigated. The pathogenesis of this condition in dogs with leishmaniosis could be attributed to several mechanisms, including immune-mediated destruction of circulating platelets due to the presence of anti-platelet antibodies, hypercoagulability due to decreased concentration of antithrombin III, and suppression of platelet production in the bone marrow (Paltrinieri et al., 2016). In the case of sick cats, this alteration has been frequently detected (Pereira and Maia, 2021). This is the only reported case of thrombocytopenia associated with leishmaniosis in ferrets. It is difficult to determine the pathologic mechanism responsible for the thrombocytopenia, although it is most likely similar to the dog.

On the other hand, this patient was found to have polyclonal hyperglobulinemia with a marked peak in the beta-globulin fraction. The most common electrophoretic serum pattern detected in dogs with leishmaniosis is polyclonal hypergammaglobulinemia, followed by oligoclonal hypergammaglobulinemia, while occasionally the monoclonal type can be observed. Recently, the presence of a polyclonal spike in the beta fraction was described in a dog with leishmaniosis (Villanueva-Saz et al., 2022b). The presence of this peak in the beta fraction is related to the migration of IgM and some acute phase proteins to this region (Paltrinieri et al., 2016). In the case of feline leishmaniosis, the available scientific information on the electrophoretic profile is limited. Some authors detected monoclonal gammapathy in cats with clinical leishmaniosis (Villanueva-Saz et al., 2022c).

Canine leishmaniosis is a common cause of glomerulonephritis, which can cause proteinuria and may progress to renal failure. Initially, asymptomatic infected dogs with renal involvement show moderate to severe proteinuria without azotemia (González et al., 2023; Navarro et al., 2022; Roura et al., 2021). As the disease progresses, tubulointerstitial lesions and azotemia develop, ultimately leading to end-stage renal failure, which remains the most significant cause of death in canine leishmaniosis (Roura et al., 2021). Possible kidney chronic disease in this patient was not attributable to leishmaniosis due to the absence of proteinuria.

A common situation due to anti-*Leishmania* treatment in dogs and cats is the possibility of the presence of adverse drug reaction. In dogs, during antimoniate meglumine treatment different systemic adverse reactions could be observed such as general weakness, myalgia, reduced food intake up to anorexia, vomiting and diarrhoea. By contrast, meglumine administration could case potentially cause local side reaction such as pain at the site of injection, cutaneous abscesses and/or cellulitis (Baneth and Shaw, 2002; Solano-Gallego et al., 2011). It is important to remark that the individual factor is very important with an unknown information about the frequency and severity of the adverse reactions (Bravo et al., 1993). Miltefosine is the second line treatment in canine leishmaniosis, but reaction adverse is also possible including transient digestive disorders such as vomiting, anorexia and apathy (Solano-Gallego et al., 2011). In cats, miltefosine could cause a decreased life span of red blood cells due to the presence of propylene glycol as an excipient of the miltefosine oral formulation and a worsened azotaemia (Pennisi et al., 2015).

This case report of leishmaniosis in ferrets, the only adverse effect found in the patient was the presence of inflammatory reactions at the inoculation point of meglumine antimoniate that gradually disappeared during treatment. In our case, a good clinical response to the treatment was detected after initiating the anti-*Leishmania* treatment. This fact could clearly establish the cause (*L. infantum*)–effect (clinicopathological findings) relationship.

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## Author contributions

All authors made substantial contributions. Jacobo Giner and Diego López-Sahuquillo managed the case. Diana Marteles and Antonio Fernández performed the clinical laboratory analyses. Sergio Villanueva-Saz, María Eugenia Lebrero and Jacobo Giner drafted the manuscript. Andrés Yzuel and Alex Gómez reviewed and edited the article. All authors participated in critically appraising the manuscript and revising if for intellectual content. All authors gave final approval of the completed manuscript.

## Ethics approval

The ferret was sampled with the owner's consent and for clinical reason. No additional ethical approval was required.

## Consent for publication

The owner gave their written informed consent for publication by signing our official client acceptance form.

## Declaration of Competing Interest

The authors have nothing to disclose.

## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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