Treatment and follow-up of a domestic ferret (*Mustela putorius furo*) with clinical leishmaniosis caused by *Leishmania infantum*

Jacobo Giner[a,b1], Sergio Villanueva-Saz[b,c*1], María Magdalena Alcover[d], Cristina Riera[d], Roser Fisa[d], Asier Basurco[b,e], Andrés Yzuel[b], Michele Trotta[f], Caterina Fani[f], María Teresa Verde[b,e], A. Fernández[b,e]

[a] *Menescalia Veterinary Clinic, Ismael Merlo Actor, 5, 46020 Valencia, Spain*

[b] *Clinical Immunology Laboratory, Veterinary Faculty, University of Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain*

[c] *Department of Pharmacology and Physiology, Veterinary Faculty, University of Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain*

[d] *Laboratory of Parasitology, Pharmacy Faculty, University of Barcelona, Avda. Joan XXIII s/n, 08028 Barcelona, Spain*

[e] *Animal Pathology Department, Veterinary Faculty, University of Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain*

[f] *CD Vet Laboratorio Analisi Veterinarie, Via Ernesto Monaci, 21, 00161 Roma Rome, Italy*

*Corresponding author: Department of Pharmacology and Physiology and Clinical Immunology Laboratory, Veterinary Faculty, University of Zaragoza, Miguel Servet 177, 50013-Zaragoza, Spain. Telephone: (+34) 679 72 72 85. E-mail: svs@unizar.es

[1]These authors have contributed equally to the study.

ABSTRACT

Leishmania infantum infection including treatment and follow up in domestic animals other than dogs and cats has not been described at this moment. This article describes the anti-*Leishmania* treatment and follow-up of a ferret (*Mustela putorius furo*) with leishmaniosis. A combined therapeutic protocol established for the patient, not yet approved for ferrets, was a combination of meglumine antimoniate plus allopurinol. A follow-up was established monthly during the first year in order to monitor the health condition of the patient. Six months after commencing allopurinol therapy, xanthine crystalluria was observed in urine sediment with no other urine alterations detected by urine analysis. The ferret worsened progressively with diarrhoea and weight loss after cohabiting with another ferret diagnosed with cryptosporidiosis. *Cryptosporidium parvum* was isolated in faecal samples from the patient detected by three different methods including Ziehl-Neelsen staining, a qualitative test to detection of *C. parvum* antigens and finally a specific molecular analysis to characterize the species. To the best of the authors´ knowledge, this is the first report providing information about anti-*Leishmania* protocol therapy used and follow-up in a domestic ferret with clinical leishmaniosis. Veterinarians practicing in endemic areas should be aware of this infection in ferrets at risk and their susceptibility especially when immunosuppressive conditions are present.

Keywords: Cryptosporidium parvum; ferret; *Leishmania infantum*; PCR; serology; treatment.

1. Introduction

Zoonotic leishmaniosis due to *Leishmania infantum* is a vector-borne disease endemic in Southern Europe, Asia, North Africa and South America. Dogs are the main reservoir for this infection and the disease can be fatal some circumstances if not treated in people and dogs (Solano-Gallego et al., 2011). It has been estimated, based on seroprevalence studies from Italy, Spain, France and Portugal, that 2.5 million dogs in these countries are infected by *L. infantum* (Moreno and Alvar, 2002). In European Mediterranean countries, canine and human

leishmaniosis are mainly caused by the same zymodeme MON-1 (Baneth et al., 2008). In areas where *L. infantum* is transmitted to dogs, other animals such as cats, and other non-conventional household pets are likely to be in contact with the parasite and can also be potentially infected (Pennisi, 2015), although with lesser impact in comparison to the role of the dog in the epidemiology of this infection. It has been suggested that cats may act as a peridomestic reservoir and not only as accidental hosts (Solano-Gallego et al., 2007). In the same way, wild mammals have been considered as potential silent reservoirs of *L. infantum* in the Mediterranean area, suggesting the presence of chronic subclinical infection without evidence of clinical signs and they may contribute to maintaining the zoonotic cycle in an area where the presence of the dog is limited (Alcover et al., 2020). However, one of the common problems in the identification of alternative reservoirs is the lack of reagents to detect the infection, furthermore specific techniques such as xenodiagnosis is not always possible to be applied.

In endemic areas with high canine leishmaniosis prevalence, the detection of the *Leishmania* infection in other household pets like cats has been described, although information concerning the epidemiology and clinical picture is scarce. Moreover, the proportion of infected cats is inferior in comparison to dogs in the same endemic area. Immunosuppressive conditions in this species have been involved with promoting factors to increase the susceptibility to develop clinical disease (Solano-Gallego et al., 2011; Pennisi and Persichetti, 2018).

Allopurinol and meglumine antimoniate are the two main drugs used for the treatment of *L. infantum* in dogs (Solano-Gallego et al., 2011) and cats (Pennisi et al., 2015). Nevertheless, the use of these two drugs is off-label and there is no information in cats being empirically evidence-based (Pennisi and Persichetti, 2018).

The domestic ferret (*Mustela putorius furo*) is a small mustelid considered to be the same species as the European polecat and was domesticated between 2000-3000 years ago (Talbot et al., 2013). The present report, which describes therapeutically findings observed in a domestic ferret with leishmaniosis along with follow-up data thus provides information on the disease in ferrets, becomes highly relevant owing to the increasingly popularity of ferret as household pets

since about the 1970´s across Europe and the United States of America (Shepherd, 2006; Talbot et al., 2013), with an official registered population of 20.000 pet-ferrets in Spain (Ministerio de Agricultura, Alimentación y Medio Ambiente, Spanish Government, 2015), which could be considered potential natural host for *Leishmania*.

2. Methods

A 4-year-old intact female ferret from Valencia (39° 28' 12.864"N, 0° 22' 36.48"W), on the east coast of Iberian Peninsula, was clinically evaluated in February 2019 because of a presence of a nonpruritic dermal lesion in right pinna. This ferret was adopted at the age of two years with unknown previous history. The patient lives in an apartment with other ferrets and has outdoor terrace access. The owner provides the pet-ferret an environmental enrichment with mental stimulation and outlets for its activity needs and an indoor housing appropriate for agile active, ferrets with climbing levels and multiple sleeping areas. Furthermore, ferret care is supply with a highly digestible diet consisting of a high-quality animal protein and fat, with minimal carbohydrate and fiber commercial ferret diet. It was under chronic medical management with prednisolone 0,5 mg/kg *Per os* (PO), twice a day (BID), and cyclosporine 7 mg/kg, PO once a day (SID) because of inflammatory bowel disease diagnosed one year earlier and also it was diagnosed a suppurative cholangitis six months prior to presentation.

On physical examination, the ferret presented an erythematous and edematous papular painless lesion with a diameter of 5 mm in the right ear pinna (Figure 1a) with no other apparent clinical signs.

A skin lesion sample was taken by fine needle aspiration and stained with Diff-Quick stain for cytological examination. Cytology results revealed pyogranulomatous inflammation in which infectious agents were not visualized. Topical therapy with was iniciated with marbofloxacin 3 mg/clotrimazole 10 mg/dexamethasone plus 0.9 mg per ml suspension (Marbodex®, Ecuphar, Belgium) BID. Equally, the animal was treated with marbofloxacin

(Marbocyl® 5mg, Vetoquinol, France) 2 mg/kg PO BID during 3 weeks (Figure 1b, 1c). A follow-up exam three weeks later revealed little improvement of the dermatitis.

A full thickness biopsy of the lesion was taken under short general anesthesia. The lesion was completely excised, fixed in 10% neutral-buffered formalin, and submitted for histological examination. A severe chronic diffuse pyogranulomatous dermatitis with proteinaceous edema, serocellular crusts, and intramacrophagic oval organisms with eccentrical nuclei and pale cytoplasm, approximately 3 to 4 µm in size, interpreted as protozoas (*Leishmania* spp.) or fungal organisms (*Histoplasma* spp.), was observed microscopically (Figure 2).

Anti-*Leishmania* treatment was not initiated because lesion was completely excised and any anti-*Leishmania* therapeutic protocol for ferrets was available or documented in that moment. Immune-modulating drugs dosage were reduced to avoid possible drug immunosuppression (prednisolone 0.5 mg/kg PO SID and cyclosporine 7 mg/kg PO SID) and oral nutritional supplements based on S-adenosyl methionine 31.25 mg, silybin 3.75 mg and vitamin E 1.5 mg (Hepatosil Plus®, Opko health, Spain) SID were added to the treatment (Figure 1d, 3a, 3b). During follow-up, four weeks later, a new papular dermatitis was detected on cicatricial edge from previous surgical incision on the right ear pinna (Figure 3c). A sample from the cutaneous lesion was taken by needle aspiration for parasite culture in Novy-MCNeal-Nicolle medium (NNN), which tested positive for *Leishmania* parasites (Figure 3d). Additional diagnostic procedures included detection of parasite DNA by PCR from peripheral blood sample and Whatman filter paper number 3 with aspirated material from the perilesional excised area, *i*mmunohistochemistry specific against *L. infantum* and anti-*Leishmania* antibodies detected by western blot, immunofluorescence indirect test and enzyme-linked immunosorbent assay (Giner et al., 2020). Delayed type hypersensitivity (DTH) reaction to leishmanin was evaluated using an inactivated suspension of 3×10^8 *L. infantum* promastigotes (MHOM/FR/78/LEM75) per ml in 0.2% phenol-saline, with a protein content of 30 µg/ml. The solution (100µl) was intradermally injected in the skin of the groin. Skin reactions were recorded after 72 hours and an induration or eritematous area > 5 mm in diameter was considered positive.

An anti-*Leishmania* therapeutic protocol was established with allopurinol at 10 mg/kg BID PO *sine die* (Zyloric® 100 mg, Faes Farma, Spain) and meglumine antimoniate (Antishmania®, Fatro, Italy) during three weeks at increasing doses every week to control possible drug adverse effects from 25 mg/kg BID the first week to 50 mg/kg BID the third week subcutaneously (Figure 4a and 4b). Anticoagulated blood sample was analysed by an automated haematology analyser (LaserCyte Idexx, Westbrook, USA) to perform a complete cells blood count. Clinical biochemistry was analysed with an automatic analyser (Catalyst One Idexx, Westbrook, USA) including the following parameters: alanine amino-transferase (ALT), alkaline phosphatase (ALKP), serum gamma glutamyl transferase (GGT), total bilirubin (TBil), total cholesterol (CHO), glucose (GLU), total protein concentrations (TP), creatinine (CRE), blood urea nitrogen (BUN), calcium (Ca), inorganic phosphorus (P). Urine analysis was performed including urine specific gravity (USG) and urine protein to creatinine ratio (UPC). Serum protein electrophoresis was run manually with agarose gels (Sebia, Evry, France) and densitometer (Shimadzu CS-9000, Kyoto, Japan) was used for scanning the electrophoretograms.

Cryptosporidium infection was detected by faecal smear by modified Ziehl-Neelsen staining method according to World Organization for Animal Health (OIE) (OIE, 2018). Later, a rapid immunochromatographic test for the qualitative detection of *Cryptosporidium spp*. antigens in faeces (FASTest® CRYPTO Strip, MEGACOR Diagnostik GmbH, Hörbranz, Austria) was used to confirm the diagnosis. Finally, molecular analysis was performed to characterize and determine the species of *Cryptosporidium* parasite. For this purpose, total genomic DNA was extracted from 180 – 200 mg of stool samples from the ferret using a QIAamp® Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Purified DNA was stored at 4°C until used in polymerase chain reaction (PCR). Real-time PCR amplification specific for *Cryptosporidium parvum* DNA was performed according to Jothikumar et al. (2008). Real-time PCR amplifications were performed using a LightCycler96 instrument (Roche, Basel, Switzerland).

3. Results

A follow-up visit one month since the treatment was initiated demonstrated clinical response and it was observed that the ear pinna lesions had almost disappeared (Figure 4c). At any rate, a new sample was taken by needle aspiration for parasite culture in NNN which was again positive for *Leishmania* promastigotes. The same biochemical abnormalities were observed as initially with persistent alteration of protein electrophoresis with hypergammaglobulinemia and elevated serum enzyme activities (ALT, ALKP and GGT).

Because the anti-*Leishmania* protocol therapy was tolerated well with no renal compromise or clinically apparent side effects noted, treatment with allopurinol at 10 mg/kg BID PO *sine die* and meglumine antimoniate at 50 mg/kg BID was continued. Three weeks following completion of the treatment, blood PCR was negative for *L. infantum* and new sample for parasite culture in NNN was taken by needle aspiration from affected area which was negative for *Leishmania* parasites. Equally, on this follow-up the ferret was appeared healthy and haematological and biochemical tests were repeated observing a partial resolution of the alteration in protein´s electrophoresis (Tables 1 and 2). For these reasons, meglumine antimoniate treatment was discontinued after 56 days. The ferret continued to be treated with allopurinol at the same dose for five months. Follow-up visits (Figure 4d) to the attending veterinarian were made every month monitoring clinicopathological parameters including complete blood count (CBC), biochemistry, urine analysis, anti-*Leishmania* antibody levels by serology and PCR (Table 3). On a follow-up visit six months since treatment was initiated, xanthinuria was observed in urine sediment and no other urine alterations were detected by urine analysis (Figure 5). The presence of xanthine crystals was associated to the allopurinol treatment. Abdominal ultrasound before starting allopurinol was performed and no urinary tract alterations were observed.

Because serology and protein electrophoresis (Figure 6) revealed an improvement of the animal status, allopurinol therapy was discontinued temporarily to prevent xanthine urolithiasis. Xanthine crystals were not persisted after withdrawal of allopurinol two weeks later observed by urine analysis. Response to treatment after withdrawal of allopurinol was considered as effective way to evaluate if the xanthinuria was or not persistent.

Seven months after leishmaniosis diagnosis was made, the ferret was examined because of severe weight loss, apathy, diarrhoea and dyspnoea with tachypnea. The ferret´s owner reported that the patient lived with two new ferrets diagnosed with cryptosporidiosis. A blood sample was collected and submitted for follow-up CBC and serum biochemical profile. The CBC showed neutrophilia and marked monocytosis. Serum biochemical profile revealed increases in ALT, GGT, ALKP activities, and TBil (10.3 mg/dl) concentration Table 1, November 2019). Equally, hyperproteinemia and alteration of electrophoresis with hypergammaglobulinemia and a reduced albumin/globulin ratio was detected. Thoracic radiographs revealed a diffuse broncointerstitial pattern with alveolar infiltrates compatible with a pulmonary parenchyma disorder such an infectious pneumonia (bacterial, fungal, viral protozoal or parasitic in origin) or neoplasia. *Cryptosporidium* infection was detected by faecal smear stained by modified Ziehl-Neelsen, a rapid immunochromatographic test for the qualitative detection of *Cryptosporidium spp.* antigens in faeces (Figure 7) and a positive result obtained in molecular analysis. Immunocompromised hosts are more likely to develop clinical signs of cryptosporidiosis (Scorza and Tangtrongsup, 2010; Kumar et al., 2016). Therefore, immune-modulating drugs were discontinued because they were considered a possible cause of immunosuppression in this patient. DTH reaction to leishmanin was negative and no induration or eritematous area was observed. Azithromycin (EFG, Cinfa, Spain) was administered at 10 mg/kg PO once a day for one month. Because immunosuppressive status is one of the strongest risk factors for overt clinical disease and serology in the patient still remained positive, allopurinol 5 mg/kg PO BID *sine die* was added to the therapy. Respiratory constants and stools were normal within a week of initiating therapy. Currently, the animal´s general clinical status is stable and the ferret continues with the same oral daily dose of allopurinol.

4. Discussion

The present study represents the first report describing leishmaniosis treatment and one-year follow-up with routine physical examination, laboratory tests, serology and PCR in a domestic ferret (*Mustela putorius furo*) with a well-maintained quality of life.

Parasitic virulence, nutritional status, age, the host genetic and response factors in canine leishmaniosis are known to contribute to the disease although the progression of the disease after primary infection is only partly understood. Infection in dogs may be subclinical or manifested as a self-limiting disease, or a severe, and sometimes, fatal illness (Solano-Gallego et al., 2009). Subclinical infection is not necessarily permanent and factors such as immunosuppression or concomitant diseases lead to the progression of clinical disease in dogs (Solano-Gallego et al., 2011) as in cats (Pennisi and Persichetti, 2018). Leishmaniosis has been associated in cats with an impaired immunocompetence due to several factors, including retroviral infections, immunosuppressive treatments and concomitant debilitating diseases, thus suggesting that these conditions may act as promoting factors (Pennisi and Persichetti, 2018; Fernández-Gallego et al., 2020). In humans, whereas most immunocompetent individuals will not develop disease after *Leishmania* infection, immunosuppression is a well-established risk factor for the development of the disease, as in patients with human immunodeficiency virus (HIV) mainly and a wide range of non-HIV-related immunosuppressive states falling under the realm of transplantation medicine, rheumatology, hematology, and oncology medicine (Van Griensven et al., 2014).

Immunosuppression induced by immunomodulating drugs such cyclosporine in dogs with atopic dermatitis have been described (Navarro et al., 2008). Although ferrets often are relatively resistant to the immunosuppressive effects of prednisolone (Chen, 2008; Chen, 2010), this clinical case reports a ferret with a suggested immunosuppression condition initially associated with immune-modulating drugs therapy (prednisolone and cyclosporine). Furthermore, *Leishmania* infection probably could increase the immunosuppression status of the patient because an intestinal cryptosporidiosis and supposed pulmonary cryptosporidiosis was detected seven months since *Leishmania* infection was diagnosed. *Cryptosporidium spp.* are protozoa that inhabit the respiratory and intestinal tract epithelium though the disease is usually

238 self-limiting and subclinical (Patterson and Fox, 2007; Powers, 2009). *Cryptosporidium*
239 infections of the ileum are the most common, although gastric, respiratory, and conjunctival
240 infections have been reported in immunosuppressed people (Scorza and Tangtrongsup, 2010).

241 According to a recent feline retrospective leishmaniosis study, median survival time in a
242 group of cats treated specifically for leishmaniosis without concomitant diseases was longer than
243 in another group with concomitant diseases or known immunosuppression status; however, no
244 statistical differences were seen between groups (Fernández-Gallego et al., 2020).

245 A negative result in DTH test is observed in acute cases of visceral leishmaniasis in
246 humans (Sundar and Rai, 2002) and dog with clinical leishmaniosis (Solano-Gallego et al., 2005),
247 whilst, a positive result can be detected in human cases where kala-azar has been cured (Sundar
248 and Rai, 2002) and in dogs that improve after anti-*Leishmania* treatment (Solano-Gallego et al.,
249 2001). In this ferret, a negative result detected by DTH could be interpreted as potentially no
250 resistant to *Leishmania* infection.

251 Laboratory findings revealed high serum enzymes activities (ALT, ALKP, GGT) on each
252 follow up serum chemistry evaluation performed. Various hepatic diseases have been reported in
253 ferrets; the most common liver disorders encountered in this species are inflammatory, infectious
254 and toxic hepatic diseases; and less commonly hepatic lipidosis, and hepatic neoplasia. Owing to
255 most previously mentioned liver diseases remained often subclinical may lead to difficulties in
256 diagnosing those conditions accurately (Huynh et al., 2013). Hepatobiliary failure due to *L.*
257 *infantum* has been documented in dogs experimentally as well as in natural infection, recording
258 laboratory alterations associated with liver injury and histopathological changes in the liver which
259 are characterized by the presence of a mononuclear inflammatory cell infiltration (Valladares et
260 al., 1997; Rallis et al., 2005; Moreira et al., 2016). Bile culture was performed in the ferret of this
261 report with negative results and abdominal ultrasound did not revealed biliary tree or liver
262 parenchyma abnormalities. The cause of ALT, ALKP and GGT elevations in the ferret, and if
263 these were related to leishmaniosis, cannot be determined because liver biopsies could not be
264 obtained.

Anti-*Leishmania* treatment selection between meglumine antimoniate versus miltefosine was based on the better clinical efficacy of meglumine antimoniate in long term clinical setting in dogs (Manna et al., 2014). Different treatment protocols with variable survival time have been described in cats with clinical leishmaniosis: allopurinol alone, a combination meglumine antimoniate plus allopurinol, a combination miltefosine plus allopurinol, and finally meglumine antimoniate alone with very variable survival time between anti-*Leishmania* treatments (Fernández-Gallego et al., 2020).

Although combined therapeutic protocol based on allopurinol and meglumine antimoniate was well tolerated in this patient, information is lacking on pharmacokinetic and pharmacodynamic characteristics of these drugs in ferrets and also about their safety. Clinical improvement was observed in this ferret and papular dermatitis was resolved within a few weeks after treatment was initiated. In the same way, protein electrophoresis alterations were partially resolved until *Cryptosporidium* infection was detected in roughly seven months since anti-*Leishmania* treatment was initiated. The prolongation of the anti-*Leishmania* combined treatment by 2-3 weeks has been described in dogs and may be considered if patient improvement is inadequate (Solano-Gallego et al., 2011). In our patient, the clinical decision to increase the anti-*Leishmania* combined treatment for four more weeks was based on positive results in parasite culture of a new material aspirated from the previous lesion site.

However, xanthinuria was observed in urine sediment during a follow-up six months after long-term administration of allopurinol. Presence of xanthine crystals is a result of the inhibition of a specific enzyme, xanthine oxidase, which is part pathway of purine degradation and usually occurs secondary to therapy with allopurinol influenced by several variables including the dosage of allopurinol, quantify of dietary purine precursors, the rate of degradation and hepatic function. Our findings suggest that the presence of xanthinuria can be found during allopurinol therapy in ferrets. Treatment of leishmaniosis in this ferret was not based on scientific evidence reports. At any rate, the drugs protocol used in this animal seems to be effective and safe with prolonged survival time.

This report demonstrates that meglumine antimoniate plus allopurinol seems to be effective as anti-*Leishmania* treatment in a ferret with clinical leishmaniosis. In general, close clinical and analytical monitoring should be performed to detect drugs side effects such as xanthinuria associate with allopurinol administration. Veterinarians practicing in endemic areas should be aware of this infection in ferrets at risk and their susceptibility especially when immunosuppressive conditions are present.

Acknowledgements

We thank Maria Ángeles Lostao (University of Zaragoza) for their excellent technical assistance in performing this study.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The authors have nothing to disclose.

References

Alcover, M.M., Ribas, A., Guillén, M.C., Berenguer, D., Tomás-Pérez, M., Riera, C., Fisa, R., 2020. Wild mammals as potential silent reservoirs of *Leishmania infantum* in a Mediterranean area. Prev. Vet. Med. 23,175:104874.

Baneth, G., Koutinas, A.F., Solano-Gallego, L., Bourdeau, P., Ferrer, L., 2008. Canine leishmaniosis - new concepts and insights on an expanding zoonosis: part one. Trends Parasitol. 24,324-30.

Chen, S., 2008. Pancreatic endocrinopathies in ferrets. Vet. Clin. North Am. Exot. Anim. Pract. 11,107–123.

Chen, S., 2010. Approaches and current medical management of insulinomas and adrenocortical disease in ferrets (*Mustela putorius furo*). Vet. Clin. North Am. Exot. Anim. Pract. 13,439–452.

Fernández-Gallego, A., Feo Bernabe, L., Dalmau, A., Esteban-Saltiveri, D., Font, A., Leiva, M., Ortuñez-Navarro, A., Peña, M.T., Tabar, M.D., Real-Sampietro, L., Saló, F., Lloret, A., Bardagi, M., 2020. Feline leishmaniosis: diagnosis, treatment and outcome in 16 cats. J. Feline Med. Surg. 13:1098612X20902865.

Giner, J., Basurco, A., Alcover, M.M., Riera, C., Fisa, R., López, R.A., Juan-Sallés, C., Verde, M.T., Fernández, A., Yzuel, A., Villanueva-Saz, S., 2020. First report on natural infection with *Leishmania infantum* in a domestic ferret (*Mustela putorius furo*) in Spain. Vet. Parasitol. Reg. Stud. Reports 19: 100369.

Huynh, M., Laloi, F., 2013. Diagnosis of liver disease in domestic ferrets (*Mustela putorius*). Vet. Clin. North Am. Exot. Anim. Pract. 16, 121-44

Jothikumar, N., da Silva, A.J., Moura, I., Qvarnstrom, Y., Hill, V.R., 2008. Detection and differentiation of *Cryptosporidium hominis* and *Cryptosporidium parvum* by dual TaqMan assays. J. Med. Microbiol. 57,1099-1105.

335 Kumar, H., Singh, V.B., Meena, B.L., Agrawal, J., Beniwal, S., Swami, T., 2016.
336 Pulmonary cryptosporidiosis in an immunocompetent host treated successfully with
337 nitazoxanide. Lung India. 33,69-71.

338 Manna, L., Corso, R., Galiero, G., Cerrone, A., Muzi, P., Gravino, A.E., 2014. Long-term
339 follow-up of dogs with leishmaniosis treated with meglumine antimoniate plus
340 allopurinol versus miltefosine plus allopurinol. Parasit. Vectors. 8, 289.

341 Ministerio de Agricultura, Alimentación y Medio ambiente. Análisis y caracterización
342 del sector de los animales de compañía, 2015. Ministerio de Agricultura,
343 Alimentación y Medio ambiente, Gobierno de España [WWW Document]. URL
344 https://www.mapa.gob.es/es/ganaderia/temas/produccion-y-mercados
345 ganaderos/20160222_informeestudioparapublicar_tcm30-104720.pdf. (Accessed
346 29 April 2020).

347 Moreno, J., Alvar, J., 2020. Canine leishmaniasis: epidemiological risk and the
348 experimental model. Trends Parasitol. 18,399-405.

349 Moreira, P.R., Franciscato, D.A., Rossit, S.M., Munari, D.P., Vasconcelos, R de O., 2016.
350 Influence of apoptosis on liver and spleen resistance in dogs with visceral
351 leishmaniosis. Rev Bras Parasitol Vet. 25,342-347.

352 Navarro, L., Verde, M.T., Ortúñez, A., Basurco, A., 2008. Leishmaniasis reactivation in
353 an atopic dog treated with cyclosporine A. Vet. Dermatol. 19 (Suppl. 1): 65–66.

354 OIE, 2018. Manual of diagnostic tests and vaccines for terrestrial animals Chapter 2.9.4.
355 Cryptosporidiosis p.1193 http://www.oie.int/standard-setting/terrestrial-manual/
356 (Accessed 29 April 2020).

357 Patterson, M., Fox, J.G., 2007. Parasites of ferrets. In: Baker DG, editor. Flynn's parasites
358 of laboratory animals. 2nd edition. Ames (IA): Blackwell Publishing ; p. 501-508

359 Pennisi, M.G., 2015. Leishmaniosis of companion animals in Europe: an update. Vet.
360 Parasitol. 208,35-47.

361 Pennisi, M.G., Cardoso, L., Baneth, G., Bourdeau, P., Koutinas, A., Miró, G., Oliva, G.,
362 Solano-Gallego, L., 2015. LeishVet update and recommendations on feline
363 leishmaniosis. Parasit. Vectors. 8,302.

364 Pennisi, M.G., Persichetti, M.F., 2018. Feline leishmaniosis: is the cat a small dog? Vet.
365 Parasitol. 251,131–137.

366 Powers, L., 2009. Bacterial and Parasitic Diseases of Ferrets. Vet. Clin. North. Ame. Exot.
367 Anim. 12,531–561.

368 Rallis, T., Day, M.J., Saridomichelakis, M.N., Adamama-Moraitou, K.K., Papazoglou,
369 L., Fytianou, A., Koutinas, A.F., 2005. Chronic hepatitis associated with canine
370 leishmaniosis (*Leishmania infantum*): a clinicopathological study of 26 cases. J.
371 Comp. Pathol. 132, 145-152.

372 Scorza, V., Tangtrongsup, S., 2010. Update on the diagnosis and management of
373 *Cryptosporidium spp* infections in dogs and cats. Trop. Companion. Anim. Med.
374 25; 163-169.

375 Shepherd, A.J., 2008. Results of the 2006 AVMA survey of companion animal ownership
376 in US pet-owning households. J. Am. Vet. Med. Asso. 232,695-696.

377 Solano-Gallego. L., Riera, C., Roura, X., Iniesta, L., Gallego, M., Valladares, J.E., Fisa,
378 R., Castillejo, S., Alberola, J., Ferrer, L., Arboix, M., Portús, M., 2001. *Leishmania*
379 *infantum*-specific IgG, IgG1 and IgG2 antibody responses in healthy and ill dogs

from endemic areas. Evolution in the course of infection and after treatment. Vet. Parasitol. 96,265-276.

Solano-Gallego, L., Llull, J., Ramis, A., Fernández-Bellon, H., Rodríguez, A., Ferrer, L., Alberola, J., 2005. Longitudinal study of dogs living in an area of Spain highly endemic for leishmaniasis by serologic analysis and the leishmanin skin test. Am. J. Trop. Med. Hyg. 722,815-818.

Solano-Gallego, L., Koutinas, A., Miró, G., Cardoso, L., Pennisi, M.G., Ferrer, L., Bourdeau, P., Oliva, G., Baneth, G., 2009. Directions for the diagnosis, clinical staging, treatment and prevention of canine leishmaniosis. Vet. Parasitol. 165,1-18.

Solano-Gallego, L., Miró, G., Koutinas, A., Cardoso, L., Pennisi, M.G., Ferrer, L., Bourdeau, P., Oliva, G., Baneth, G., 2011. LeishVet guidelines for the practical management of canine leishmaniosis. Parasit. Vectors. 4,86.

Sundar, S., Rai, M., 2002. Laboratory diagnosis of visceral leishmaniasis. Clin. Diagn. Lab. Immunol. 9,951-958.

Talbot, S., Freire, R., Wassens, S., 2013.Effect of captivity and management on behaviour of the domestic ferret (*Mustela putorius furo*), 2013. Applied Animal Behaviour Science. 151, 94-101.

Valladares, J.E., Riera, C., Pastor, J., Gállego, M., Portús, M., Arboix, M., 1997. Hepatobiliar and renal failure in a dog experimentally infected with *Leishmania infantum*. Vet Rec. 141:574-575.

Van Griensven, J., Carrillo, E., López-Vélez, R., Lynen, L., Moreno, J., 2014. Leishmaniasis in immunosuppressed individuals. Clin. Microbiol. Infect. 200,286-299.

Figure captions

Figure 1: Right ear pinna from the affected ferret with dermatological lesion associate to *L. infantum.* a Edematous papular lesion 5 mm in diameter before the lesion was excised. b Right ear pinna after full thickness biopsy for histopathological evaluation. c Cicatricial edge 10 days after lesion was excised. d Papular dermatitis detected on cicatricial edge from previous surgery during a follow-up one-month after surgical incision (lateral view).

Figure 2: Timeline of the treatment periods and following up visits.

Figure 3: Clinical lesion detected in the ferret before anti-*Leishmania* treatment was initiated. a-b Clinical sign observed at the relapse; medial view (a) and dorsal view (b). c Fine needle aspiration from papular lesion for parasite culture. d *Leishmania* promastigotes forming in rossetes from parasite culture in NNN medium. Giemsa stain (40x).

Figure 4: Dermatological clinical sign improvement during anti-*Leishmania* treatment. a-b Different right ear pinna views on a follow-up visit one month after the initiation of treatment showing clinical response. c Fine needle aspiration from affected area for a new parasite culture in NNN medium. d Ear pinna four months since anti-*Leishmania* therapy was initiated.

Figure 5: A photomicrograph of urine sediment xanthine crystals. Unstained sediment, x40 objective.

Figure 6: Agarose gel electrophoretograms of serum proteins of the ferret before starting anti-*Leishmania* treatment (a) and during the follow up (b, c, d, e, f, g, h). b One-month follow-up (June 2019). c Two-month follow-up (July 2019). d Three-month follow-up (August 2019). e Four-month follow-up (September 2019). f Five-month follow-up (October 2019). g Six-month follow-up (November 2019). h Eight-month follow-up (January 2020).

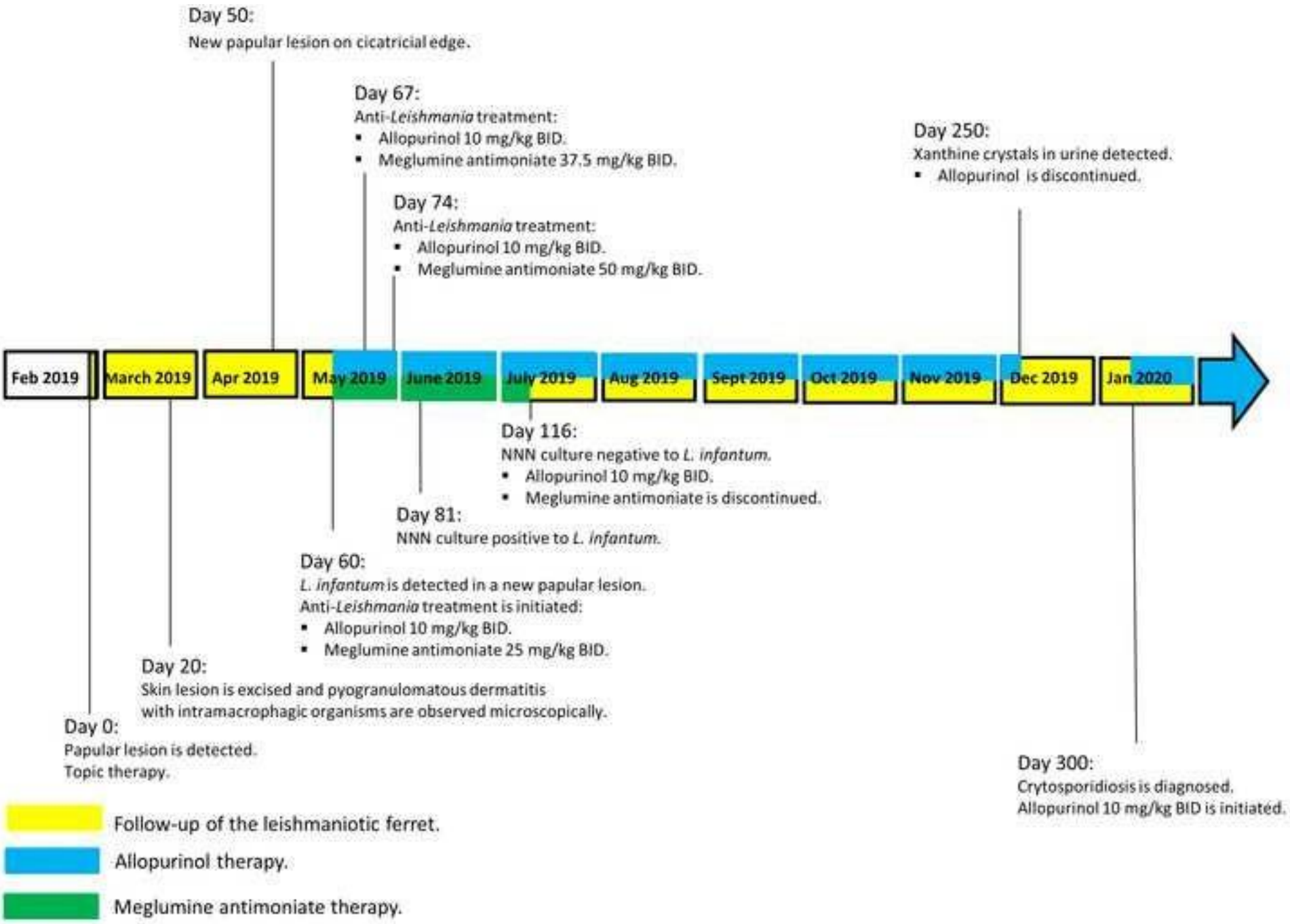
Figure 7: a Oocysts of *Cryptosporidium spp.* Ziehl Neelsen, 40×. b Qualitative detection of *Cryptosporidium* antigens by a rapid test-kit. A red test line indicates a positive result whilst a blue test line indicates that the test has been performed properly.

Figure

Click here to download Figure Figure 1.tif

Figure 2

Click here to download Figure Figure 2 rev.tif

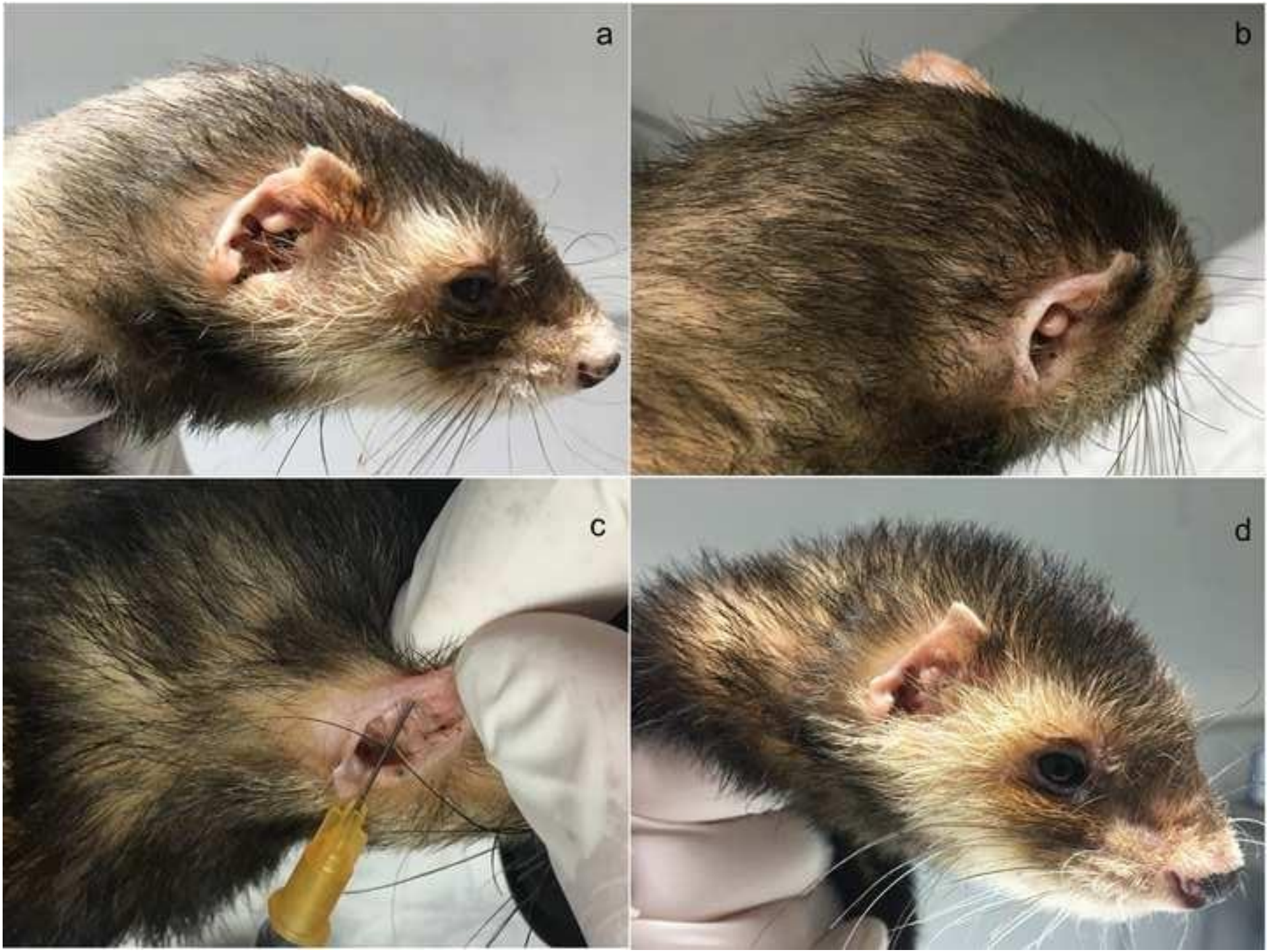


Figure

Click here to download Figure Figure 3.tif

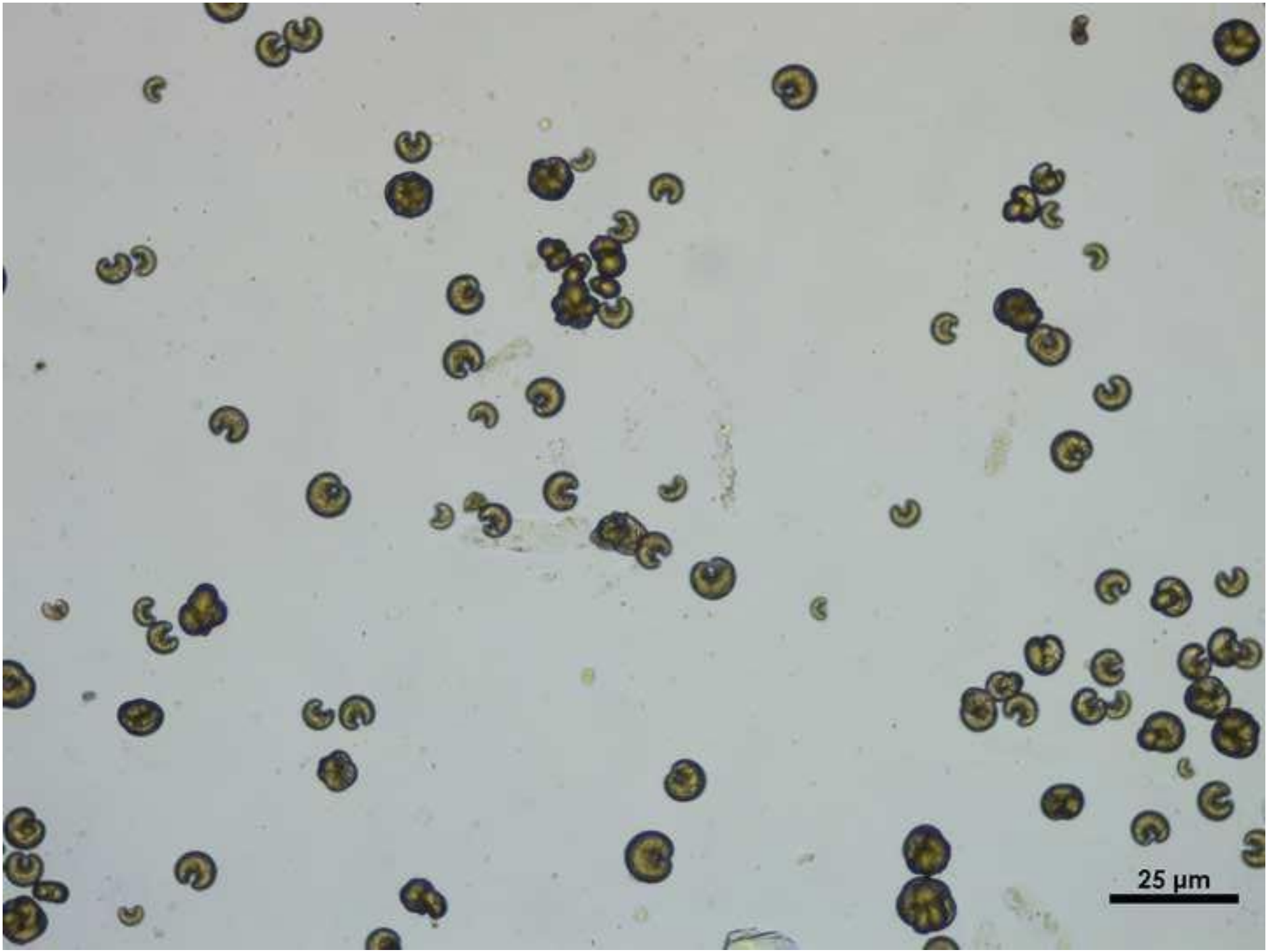
Figure

Click here to download Figure Figure 4.tif



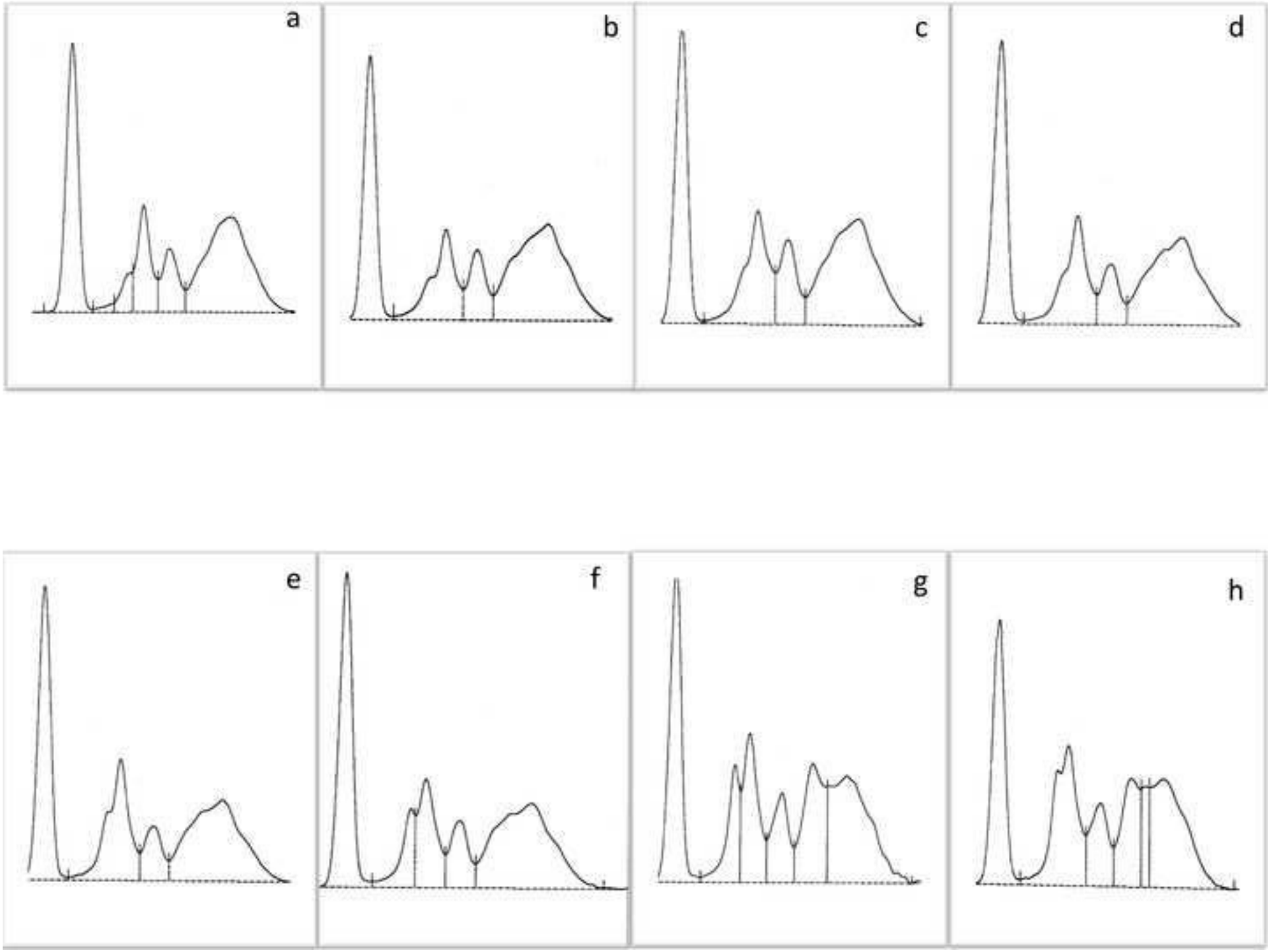
Figure

Click here to download Figure Figure 5.tif



Figure

Click here to download Figure Figure 6.tif



Figure

Click here to download Figure Figure 7. definitiva.tif

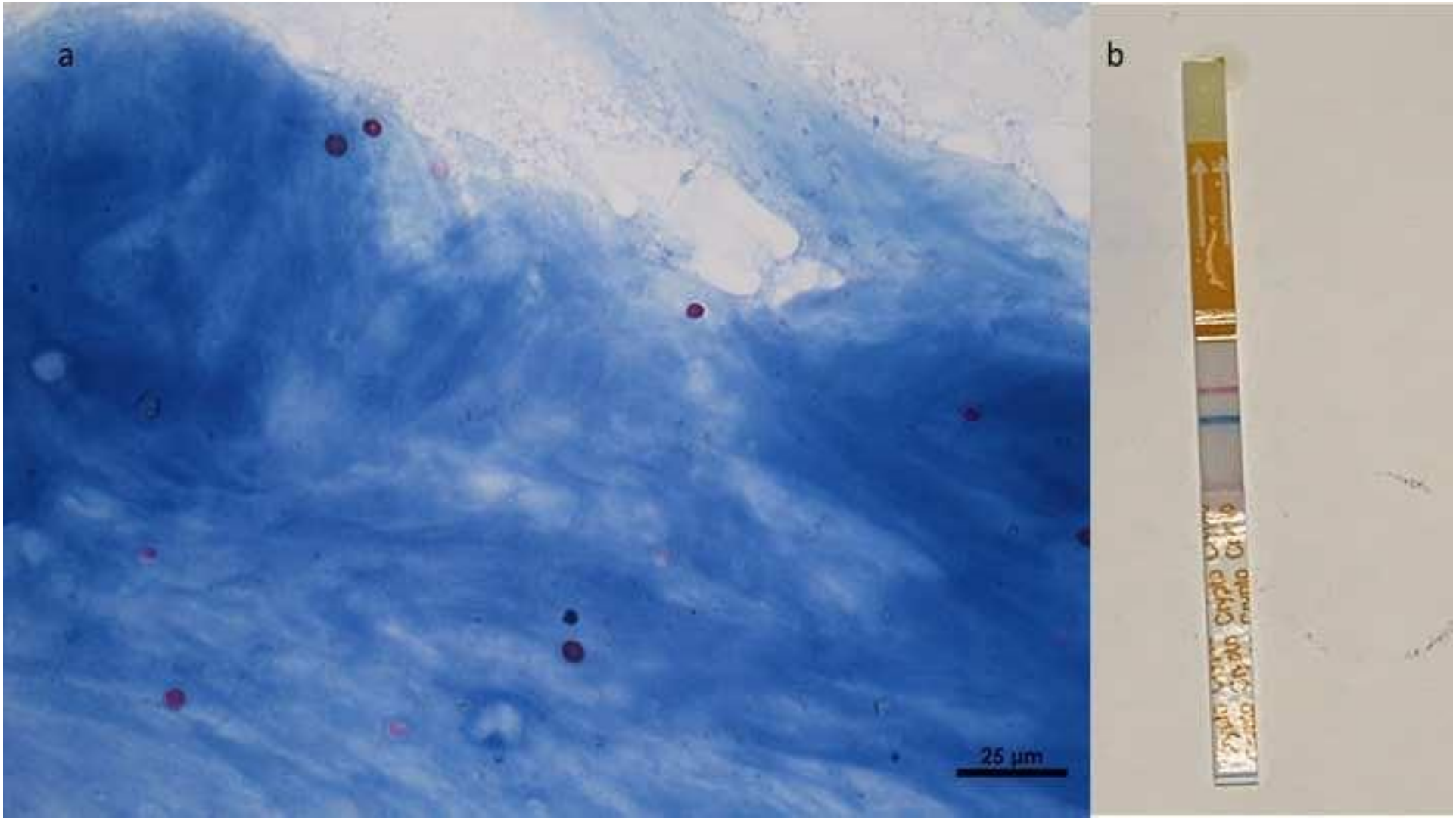


Table 1. Body weight, haematological and biochemical parameters determined in the leishmaniotic ferret at the first veterinary examination before treatment (March 2019) and during the follow-up.

Parameter	Dates											Reference range
	March 2019	April 2019	May 2019	June 2019	July 2019	August 2019	September 2019	October 2019	November 2019	December 2020	January 2020	
Body weight (g)	625	635	665	620	640	530	505	520	500	**420**	**405**	500-900
Haematology												
WBC (K/µL)	4.46	5.56	5.34	4.97	4.91	5.88	3.57	5.99	6.25	nd	**15.29**	2-10
Neutrophils (K/µL)	**3.35**	**3.41**	**3.80**	**3.54**	2.61	**4.91**	2.37	**4.48**	**4.55**	nd	**11.05**	0.62-3.30
Lymphocytes (K/µL)	**0.37**	1.16	**0.32**	**0.34**	1.37	**0.17**	**0.41**	**0.36**	**0.53**	nd	**0.61**	1-8
Monocites (K/µL)	0.61	0.83	**0.95**	0.89	0.79	0.51	0.66	0.90	**0.92**	nd	**3.12**	0.18-0.90
Eosinophils (K/µL)	0.11	0.12	0.22	0.12	0.11	0.21	**0.08**	0.16	0.25	nd	0.41	0.10-0.60
Basophils (K/µL)	0.02	0.05	0.05	0.07	0.03	0.08	0.05	0.09	0.01	nd	0.10	0.00-0.10
RBC (M/µL)	9.89	8.17	9.28	**11.43**	8.82	8.57	8.42	7.59	9.27	nd	6.65	6.35-11.20
Haematocrit (%)	47.6	44,1	43.2	**59.9**	47.2	**57.0**	45.3	43.5	49.9	nd	**35.2**	37.0-55.0
Haemoglobin (g/dL)	13.9	14.3	14.7	15.2	15.2	12.4	15.6	16.0	**18.9**	nd	11.8	11.0-17.0
MCV (fL)	48.1	54.0	46.5	52.4	53.5	**66.5**	53.8	**57.3**	53.8	nd	52.9	45.0-55.0
MCH (pg)	14.1	17.5	15.9	**13.3**	17.2	14.5	**18.5**	**21.1**	**20.4**	nd	17.7	14.0-18.0
MCHC (g/dL)	**29.3**	32.5	34.1	**25.4**	32.1	**31.8**	34.4	**36.7**	**38.0**	nd	33.4	32.0-35.0
RDW (%)	**17.0**	**16.9**	**18.1**	**17.7**	**16.6**	**17.5**	**17.1**	**16.4**	**16.8**	nd	**17.3**	19.0-25.0
Platelets (K/µL)	357	327	278	**230**	**207**	**102**	271	**171**	309	nd	306	270-880
Blood Chemistry												
ALT (U/L)	**>1000**	**>1000**	**634**	**>1000**	**657**	**370**	**>1000**	**>1000**	**>1000**	nd	**700**	82-289
ALKP (U/L)	**239**	**255**	**193**	**154**	**169**	**194**	**389**	**538**	**649**	nd	**748**	9-84
GGT (U/L)	**250**	**205**	**216**	**137**	**119**	**436**	**735**	**876**	**752**	nd	**952**	0.2-14
TBil (mg/dL)	**1.2**	0.6	0.8	0.6	0.6	0.9	0.8	0.9	**10.3**	nd	**1.4**	0.1-1.0
Cholesterol (mg/dL)	**369**	**379**	**377**	**321**	228	296	**365**	**360**	**362**	nd	**257**	64-296
Glu (mg/dL)	131	152	107	113	119	126	102	100	155	nd	112	94-207
Crea (mg/dL)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	nd	0.4	0.4-0.9
BUN (mg/dL)	21	20	34	19	32	31	28	19	34	nd	31	10-45
P (mg/dL)	5.8	7.7	7.0	5.9	5.6	7.5	6.7	7.0	7.8	nd	6.0	4.8-8.9
Ca (mg/dL)	9.4	9.7	9.3	9.2	8.2	8.4	8.9	8.7	8.8	nd	8.1	8.0-11.8

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 width *nd* not determined. Abnormalities are highlighted in bold.

Table 2. Electrophoretograms of serum proteins and urine analysis determined in the leishmaniotic ferret at the first veterinary examination before treatment (March 2019) and during the follow-up.

Parameter	Dates											Reference range
	March 2019	April 2019	May 2019	June 2019	July 2019	August 2019	September 2019	October 2019	November 2019	December 2020	January 2020	
Electrophoretograms of serum proteins												
Total protein (g/dl)	**8.7**	**10.5**	**9.7**	**7.9**	**7.8**	7.0	**8.4**	**8.9**	**7.4**	nd	7.3	4.9-7.3
Albumin (g/dl)	3.54	**3.78**	3.43	2.91	2.53	**2.26**	3.21	3.10	2.79	nd	2.58	2.30-3.60
Total globulins (g/dl)	**5.16**	**6.72**	**6.27**	**4.99**	**5.27**	**4.74**	**5.19**	**5.80**	**4.60**	nd	**4.72**	1.8-3.1
Alpha 1 globulins (g/dl)	0.22	0.24	**0.74**	0.21	0.18	0.14	0.19	0.40	**0.93**	nd	**0.99**	0.10-0.60
Alpha 2 globulins (g/dl)	**1.11**	0.75	0.55	0.75	**0.97**	0.56	**1.19**	**1.00**	0.44	nd	0.40	0.40-0.90
Beta globulins (g/dl)	1.63	1.26	**0.75**	**0.91**	**2.71**	1.23	1.70	**2.30**	1.27	nd	1.18	1.00-1.90
Gamma globulins (g/dl)	**2.20**	**4.47**	**4.23**	**3.12**	**3.24**	**2.81**	**2.11**	**2.10**	**1.96**	nd	**2.15**	0.30-0.90
A/G	0.69	0.56	0.55	0.58	0.48	0.48	0.62	0.53	0.61	nd	0.54	
Urine analysis												
UPC	nd	nd	nd	nd	nd	nd	0.21	nd	nd	0.35	0.41	<0.5
USG	1.029	1.028	1.029	1.035	1.035	1.030	1.028	1.035	1.032	1.030	1.035	1.026-1.060
Sediment	-	-	-	-	-	-	-	-	-	**Xanthine crystals**	-	

Abbreviations: A/G albumin:globulin ratio, *UPC* urine protein to creatinine ratio, *USG* urine specific gravity, *nd* not determined, - negative.

Abnormalities are highlighted in bold.

Table 3 Results of *Leishmania* infection confirmation tests recorded during the follow-up.

Leishmania infantum confirmation tests			March 2019	April 2019	May 2019	June 2019	July 2019	August 2019	September 2019	October 2019	November 2019	January 2020
Parasitological methods	Cytology		-	nd	nd	nd	nd	nd	nd	nd	nd	nd
	H&E		+	nd	nd	nd	nd	nd	nd	nd	nd	nd
	IHQ		nd	+	nd	nd	nd	nd	nd	nd	nd	nd
	Culture		nd	+	nd	+	-	-	nd	nd	nd	nd
Molecular (qPCR)	Peripheral blood		nd	+	-	-	-	-	-	-	-	-
	Paraffin block		nd	+	nd	nd	nd	nd	nd	nd	nd	nd
	Whatman filter paper from the skin lesion		nd	+	nd	nd	nd	-	nd	nd	nd	nd
Serology	ELISA (OD)	Cut-off: 0.137	nd	nd	+ (0.599)	+ (0.443)	+ (0.485)	+ (0.684)	+ (0.619)	+ (0.522)	+ (0.455)	+ (0.237)
	IFAT(titer)	Cut-off: 1:20	nd	nd	+ (1:160)	+ (1:80)	+ (1:80)	+ (1:160)	+ (1:160)	+ (1:80)	+ (1:40)	+ (1:20)
	WB (bands)	Cut-off: 14 and/or 16 kDa	nd	nd	+ (16 kDa)	+ (16 kDa)	+ (16 kDa)	+ (16 kDa)	+ (16 kDa)	+ (16 kDa)	+ (16 kDa)	+ (16 kDa)
Method for detecting cellular response against *L. infantum*	DTH test	An induration or eritematous area >5 mm in diameter was considered positive	nd	nd	nd	nd	nd	nd	nd	nd	nd	-

Abbreviations: DTH delayed type hypersensitivity,*H&E* hematoxylin and eosin staining, *IFAT* immunofluorescence antibody test, *IHQ* specific immunohistochemistry for *Leishmania* detection, *kDa* kilodaltons, *ELISA* enzyme-linked immunosorbent assay, *WB* western blot, + positive, − negative, *nd* not determined.