Veterinary Parasitology: Regional Studies and Reports 1–2 (2015) 65–69

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr

Case Report

Feline leishmaniosis in Portugal: 3 cases (year 2014)

Paulo Pimenta [a,*], Sofia Alves-Pimenta [a], João Barros [a], Pedro Barbosa [a], Ana Rodrigues [a], Maria João Pereira [a], Luís Maltez [b], Adelina Gama [b,c], José Manuel Cristóvão [d], Lenea Campino [d,e], Carla Maia [d,f], Luís Cardoso [b,c]

[a] Hospital Veterinário de Trás-os-Montes, Rua de La Lys 7, 5000-056 Vila Real, Portugal
[b] Department of Veterinary Sciences, School of Agrarian and Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD), Quinta de Prados, 5000-801 Vila Real, Portugal
[c] Animal and Veterinary Research Centre (CECAV), UTAD, Quinta de Prados, 5000-801 Vila Real, Portugal
[d] Global Health and Tropical Medicine, Unidade de Parasitologia Médica, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Rua da Junqueira 100, 1349-008 Lisboa, Portugal
[e] Departamento de Ciências Biomédicas e Medicina, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
[f] Faculdade de Medicina Veterinária, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749-024 Lisboa, Portugal

ARTICLE INFO

Article history:
Received 14 November 2015
Received in revised form 6 February 2016
Accepted 10 February 2016
Available online 12 February 2016

Keywords:
Cat
Leishmania infantum
Feline leishmaniosis
Portugal

ABSTRACT

Leishmaniosis caused by *Leishmania infantum* is a zoonotic disease endemic in many countries of America, Asia and Europe, including Portugal. Dogs are the major reservoir of *L. infantum*, but domestic cats may also be infected. Three clinical cases of feline leishmaniosis are described, with ocular clinical signs as the only manifestation of the disease. A case had bilateral anterior uveitis and a granulomatous conjunctivitis, another one presented keratitis and the third case had a nodular blepharitis. All the affected cats had high serum titres of antibodies to *L. infantum*, while polymerase chain reaction results were positive in two of the cats. Although all cats in the present study improved after treatment with meglumine antimoniate and/or allopurinol, one of them died 6 months later apparently due to a systemic *L. infantum* infection. The prevalence of disease may be underestimated in cats, because leishmaniosis is often not considered in the differential diagnosis of feline diseases. Feline leishmaniosis should be suspected in cats with ocular clinical signs and in those living in or traveling to areas where the zoonosis is endemic.

1. Introduction

Several cases of feline leishmaniosis (FL) have been reported in cats living in areas where *Leishmania infantum* is endemic, including Portugal (Pennisi et al., 2015). Cutaneous forms are the most frequent clinical manifestations of FL and may consist of ulcerative, crusty, nodular or scaly dermatitis and alopecia (Ozon et al., 1998; Hervás et al., 1999; Navarro et al., 2010). The visceral form of the disease involves organs and tissues such as the spleen, liver, lymph nodes, bone marrow, kidneys, mouth and eyes (Pennisi et al., 2015). Uveitis, nodular blepharitis and panophthalmitis are the most commonly reported ocular lesions (Leiva et al., 2005; Richter et al., 2014). A natural resistance of cats to the development of leishmaniosis has been suggested, primarily due to a cellular immune competency (Maia et al., 2010). Most cats that develop the disease are suspected of having an impaired immune response due to concurrent feline immunodeficiency virus (FIV) or feline leukemia virus (FeLV) infections, squamous cell carcinoma, diabetes mellitus, autoimmune diseases (pemphigus foliaceus), demodectic mange and treatment with corticosteroids or other immunosuppressive drugs (Poli et al., 2002; Rüfenacht et al., 2005; Sobrinho et al., 2012; Migliazzo et al., 2015; Pennisi et al., 2015).

Although the diagnosis of FL can be challenging, it is usually confirmed by direct methods such as cytology, histology, in vitro culture, polymerase chain reaction (PCR) or immunohistochemical techniques on samples of skin, lymph nodes, blood, bone marrow and other affected tissues (Poli et al., 2002; Dalmau et al., 2008; Migliazzo et al., 2015; Pennisi et al., 2015). Serological methods such as the indirect immunofluorescence antibody test, enzyme-linked immunosorbent assay, western blot, direct agglutination (DAT) or indirect hemagglutination tests usually confirm the direct diagnosis, but they are not standardized as for canine leishmaniosis (CanL) (Poli et al., 2002; Martín-Sánchez et al., 2007; Solano-Gallego et al., 2007; Cardoso et al., 2010). In fact, antibody levels can be very low or even negative in some affected cats (Poli et al., 2002; Pennisi et al., 2015). Treatment of FL has been based on a few drugs out of those used for CanL, namely meglumine antimoniate, allopurinol and ketoconazole (Leiva et al., 2005; Rüfenacht et al., 2005; Richter et al., 2014).

This paper describes three clinical cases of FL with ocular clinical signs, in which the ocular abnormalities were the only manifestation of the disease in all the cats.

2. Methods

For cytology, fine needle aspirates of lesions or smears from the cornea were performed and stained with DiffQuick® (cases 1 and 2). During necropsy (case 1) representative organ samples were collected,

* Corresponding author.
E-mail address: ppimenta@hvtm.pt (P. Pimenta).

http://dx.doi.org/10.1016/j.vprsr.2016.02.003
2405-9390/

fixed in 10% buffered formalin, paraffin-embedded, sectioned at 3 μm thick sections and stained with hematoxylin and eosin (H&E) for routine histopathological examination.

The DAT for titration of specific antibodies was performed using a standard freeze-dried *Leishmania* antigen as previously described by Schallig et al. (2002) and Cardoso et al. (2010). The cut-off was established at a serum dilution of 1:100, corresponding to a titre of 100 (Cardoso et al., 2010; Maia et al., 2015a).

Whole blood and other tissues (bone marrow, liver, lymph nodes and spleen; collected during case 1 necropsy) were used and DNA extracted according to the instructions of a commercial kit (PCR-template Preparation Kit; Roche). Polymerase chain reaction amplification was performed using specific primers for two molecular markers: the internal transcribed spacer 1 (ITS1) of the ribosomal operon (Schönian et al., 2003) and kinetoplastid minicircle DNA (kDNA) sequence specific for *Leishmania donovani* sensu lato (Cortes et al., 2004). In order to perform restriction fragment length polymorphism (RFLP), some PCR-ITS1 products were digested with *Hae*III (Schönian et al., 2003).

3. Case histories

3.1. Case 1

A 10-year-old domestic shorthaired spayed female cat, which lived in a rural environment with outdoor access, was referred to Hospital Veterinário de Trás-os-Montes (HVTM; Vila Real, Portugal) with an 1-month history of epiphora, photophobia and a corneal ulcer in the right eye (*oculus dexter*; OD) that had been treated with diclofenac (Voltaren; Novartis) and chloramphenicol drops (Clorocil; Edol). The cat was in good body condition, without any additional manifestations.

Initial ophthalmological examination revealed moderate blepharospasm OD. Menace response and dazzle test were present in both eyes. Results of Schirmer tear test (STT; Schering-Plough) were within normal limits in both eyes. Using slit lamp biomicroscopy, moderate conjunctival hyperemia and iris swelling in both eyes and an upper eyelid conjunctival nodular lesion and keratic precipitates OD were noted (Figs. 1 and 2). Both eyes had moderate aqueous flare (+2). Intraocular pressure, measured by applanation tonometry (TonoPen Vet; Medtronic Solan) was 4 and 5 OD and OS (*oculus sinister*; the left eye), respectively (reference range: 20–25 mm Hg). No funduscopic alterations were detected in either eye by direct and indirect ophthalmoscopic examination. At this point, bilateral anterior uveitis and a conjunctival mass OD were diagnosed.

A complete blood count (CBC) and urinalysis were within their normal ranges, but the biochemical profile tests revealed a marked hyperproteinemia (10.9 mg/dL; reference range: 5.7–7.8 mg/dL), hyperglobulinemia (8.2 mg/dL; reference range: 3.3–4.5 mg/dL) and low albumin:globulin ratio (0.33; reference range: 0.35–1.5 mg/dL). Serum FeLV antigen and FIV antibody were negative using a commercial rapid test (Uranotest FeLV-FIV; Uranovet).

Fig. 1. Case 1. At presentation, an upper eyelid conjunctival nodular lesion, keratic precipitates and iris swelling OD were observed.

Fig. 2. Case 1. Iris swelling OS observed at presentation.

Cytology from a fine-needle aspiration of the nodular lesion OD revealed macrophages with multiple intra- and extracellular *Leishmania* spp. amastigote forms (Fig. 3). The cat had a serum specific antibody titre of ≥102,400 (positive). Polymerase chain reaction on blood was positive for *Leishmania* spp.

Upon diagnosis of leishmaniosis, the cat was treated with subcutaneous injections of meglumine antimoniate (50 mg/kg/day, 30 days; Glucantime; Merial) and oral allopurinol (10 mg/kg/day, 6 months; Zyloric; Faes Farma). Supportive treatment comprised the topical application of ophthalmic flurbiprofen (one drop, every 8 h; Edolfene; Edol), prednisolone acetate (one drop, every 8 h; Frisolona Forte; Allergan) and cyclopentolate hydrochloride (one drop, every 8 h; Midriodavi; Dávi). One-and-a-half months after initial presentation the nodular lesion and bilateral uveitis were completely resolved (Fig. 4). The cat died 6 months after diagnosis, due to renal failure.

At necropsy, this cat was in good body condition. Macroscopic findings included: left periorbital skin crusting, pulmonary edema, mild hepatomegaly and renomegaly. Histological examination revealed bilateral blepharitis (left eyelid, granulomatous dermatitis, characterized by nodular, perivascular and adnexal moderate inflammatory infiltrate predominantly composed of plasma cells and lymphocytes, with a few macrophages, associated with follicular keratosis; right eyelid, focal intraepidermal pustular dermatitis; pulmonary edema and congestion; micro- and macrovesicular hepatic lipidosis; membranous

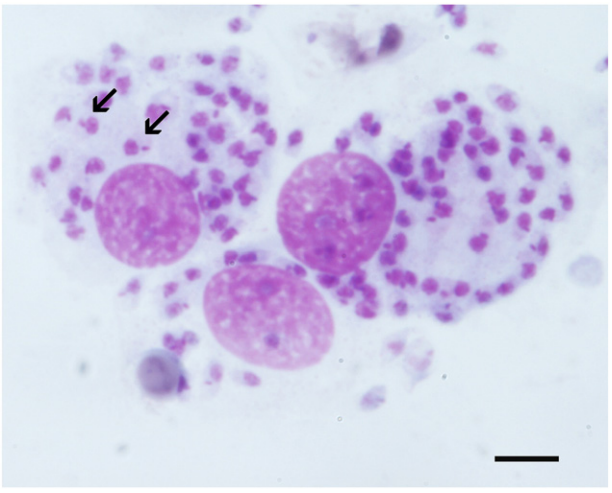

Fig. 3. Case 1. Cytologic preparation from a fine-needle aspirate of the conjunctival nodule OD. Macrophages and multiple intracellular *Leishmania* spp. amastigote forms (arrows) are present. DiffQuick, scale bar = 10 μm.