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Ocular signs, diagnosis and long-term treatment with allopurinol in a cat with leishmaniasis

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Summary

A case of leishmaniasis with predominantly ocular signs in a cat living in Switzerland and it's treatment is reported. The cat was imported from Spain 4 years earlier and was initially presented with chronic uveitis. Laboratory test results were negative for feline immunodeficiency virus (FIV), feline infectious peritonitis (FIP), feline leukaemia virus (FeLV) and *Toxoplasma gondii*, as well as for *Bartonella haenselae* and *Leishmania* spp.
Twenty-one months later the cat was presented again because of development of keratitis and granulomatous blepharitis. Blood cell count revealed severe Pancytopenia; Cytology of fine needle aspirates of granulomatous lesions on both upper eyelids and of a corneal smear revealed intracytoplasmatic microorganisms. A preliminary diagnosis of leishmaniasis was supported by positive polymerase chain reaction from bone marrow and eyelid samples for *Leishmania infantum* DNA and by a high serum antibody titer for *Leishmania* spp. Treatment with Allopurinol (10 mg/kg, BID) orally led to rapid improvement of ocular signs, general condition and blood cell count with complete remission of lid and corneal lesions within 2 months of treatment.

Keywords: cat, leishmaniasis, keratitis, uveitis, allopurinol

Augenveränderungen, Diagnostik und Langzeittherapie mit Allopurinol bei einer an Leishmaniose erkrankten Katze

Dieser Fallbericht beschreibt eine in der Schweiz lebende und an Leishmaniose erkrankte Katze mit vorwiegend Augenveränderungen und deren Behandlung. Die Katze wurde 4 Jahre zuvor aus Spanien importiert und wurde initial mit chronischer Uveitis vorgestellt. Blutuntersuchungen ergaben negative Resultate für das Feline Immunschwäche Virus (FIV), für Feline Infektiöse Peritonitis (FIP), für das Feline Leukämie Virus (FeLV), sowie für *Toxoplasma gondii*, *Bartonella haenselae* und *Leishmania* spp.
Einundzwanzig Monate später wurde die Katze erneut vorgestellt, diesmal mit Keratitis und Blepharitis. Das Differentialblutbild zeigte eine schwere Panzytopenie. Die zytologische Untersuchung der Feinnadelaspirate der granulomatösen Veränderungen an den beiden Oberlidern und des Hornhautabstriches zeigte intrazytoplasmatische Mikroorganismen. Die Verdachtsdiagnose Leishmaniose wurde durch den Nachweis von *Leishmania infantum* DNA mittels Polymerase-Kettenreaktion in Proben des Knochenmarkes und der Lider und durch hohe Serumantikörpertiter gegen *Leishmania* spp. bestätigt. Eine orale Behandlung mit Allopurinol (10 mg/kg, BID) führte zu einer raschen Verbesserung der Augensymptome, des Allgemeinzustandes und des Differentialblutbildes mit vollständiger Remission der Lid- und Hornhautveränderungen innerhalb 2 Monate nach Therapiebeginn.

Schlüsselwörter: Katze, Leishmaniose, Keratitis, Uveitis, Allopurinol

Case history and laboratory tests

A 7 year old spayed female cat had been presented for evaluation of chronic uveitis in both eyes. The cat had been imported as a stray cat from Spain 4 years earlier. According to its owners the cat had been treated by the local veterinarian for uveitis topically with gentamicin and fluorometholone as well as systemically with clindamycin for 10 days, followed by doxycycline and carprofen which had to be discontinued due to gastrointestinal side effects; laboratory examinations initiated by the local veterinarian were within normal limits regarding blood serum chemistry and were negative for FeLV and FIP.
Initial ophthalmic examination using slit lamp biomicroscopy (Kowa SL-15, Kowa Company, Tokyo, Japan) and intraocular pressure (IOP) measurements obtained

by applanation tonometry (Tonopen XL, Mentor, Norwell, MA, USA) revealed signs of chronic uveitis with iritis in both eyes (OU), aqueous humour flare + (on a scale 0–+++) OU, endothelial precipitates in the left eye (OS), perilimbal deep stromal vascularisation (ciliary flush) in the right eye (OD), pigment deposits on the anterior lens capsule OD, focal retinal scars OU and an IOP of 9 mmHg OD and 5 mmHg OS. Menace response, light perception and pupillary light reflex were positive in both eyes. Topical treatment with prednisolon acetate (Pred forte®, Allergan AG/S.A., Pfäffikon, Switzerland) TID and systemic treatment with meloxicam (Metacam®, Boehringer Ingelheim GmbH, Basel, Switzerland) SID was initiated. One week later signs of active uveitis had been cleared, and owners were instructed to slowly taper off medication over a period of 4 weeks.

The cat was presented again 12 months later. Ophthalmic examination revealed bilateral chronic active uveitis (aqueous flare, posterior synechiae, preiridial fibrovascular membranes, and diffuse cortical cataracts), superficial keratitis in the right eye and visible enlargement of the left eye (buphthalmos), which is indicative of glaucoma. The IOP was 9 mmHg in the OD and 18 mmHg in the OS. Menace response and light perception were present in the OD but were absent in the OS. Pupillary light reflex was absent in both eyes due to posterior synechiae between the iris and the anterior surface of the lens. A blood sample was taken to test for various infectious agents known to induce uveitis in cats. Serum profile was within normal limits except for mild hyperglycaemia (glucose 14.1 mmol/L, reference range 4.0–9.0 mmol/L).The following laboratory tests all yielded negative results: *Toxoplasma gondii* (IFA) and feline immunodeficiency virus (ELISA) antibodies, feline leukemia virus antigen (ELISA), PCR from blood for *Bartonella henselae* and *Leishmania infantum*. The antibody titer against feline coronavirus togethser with the results of haematology and clinical chemistry were not suggestive for feline infectious peritonitis.

Because none of the tests revealed any specific cause of infectious uveitis, anti-inflammatory treatment with topical prednisolone acetate (Pred forte®) and oral meloxicam (Metacam®) were continued. Due to an IOP within reference range (15–25 mmHg) in an eye with active uveitis (which usually induces an IOP below reference range), topical IOP lowering treatment with combination of dorzolamide and timolol maleate (Cosopt®, MSD Merck Sharp & Dohme AG, Luzern, Switzerland) was initiated in the OS to help control the intraocular pressure.

Final diagnosis and treatment

Nine months later the cat was presented again because of changes in both upper eyelids as well as opacification of the cornea OD. According to its owners the cat had been less playful and active for quite some time. The cat's condition initially improved with homeopathic therapy (nux vomica C30 and euphrasia C30, followed by euphrasia C200 and sulphur C200) prescribed by a local veterinarian, but worsened again with time despite homeopathic treatment. On general examination the cat was apathetic, the hair coat was dull, and the mucous membranes were pale. The body temperature and palpable lymph nodes were normal. Ophthalmic examination revealed nodular dermal lesions of upper eyelids OU (Fig. 1), diffuse whitish infiltrates, vascularisation and edema of the superficial corneal stroma and aqueous flare + in the OD (Fig. 2). The OS was visibly enlarged (buphthalmic) with an IOP of 8 mmHg, clear cornea, aqueous flare ++, and preiridial fibrovascular membranes covering the iris and the anterior surface of the lens, which showed cortical cataractous changes (Fig. 3). Menace response was positive in the OD and absent in the OS. Fine needle aspiration of the nodular lesions OU and a smear from the cornea OD were performed and stained with DiffQuick®. Cytology revealed multiple intracellular and extracellular protozoal organisms in both, samples from the eyelid lesions and from the cornea OD (Fig. 4). The organisms were round, measured 1–2 µm in width and 2–3 µm in length. With high magnification (1000×), a large oval nucleus and a small rod-shaped kinetoplast could be detected (Fig. 4, inset). This was considered to be most consistent with the amastigote form of the genus *Leishmania*. Due to cytological findings from ocular samples, bone marrow was sampled to determine visceral involvement and peripheral blood was taken for a blood cell profile and serology. Table 1 shows selected parameters of clinical chemistry (Konelab 30i, Thermo Fisher Corporation, Vantaa, Finland) and hematology (Sysmex XT-2000iV, Sysmex, Norderstedt, Germany). The main biochemical abnormalities were hypoalbuminemia, hyperglobulinemia, and hypergammaglobulinemia. The blood count showed a marked anemia, leucopenia and thrombocytopenia, which were confirmed by manual counting. As

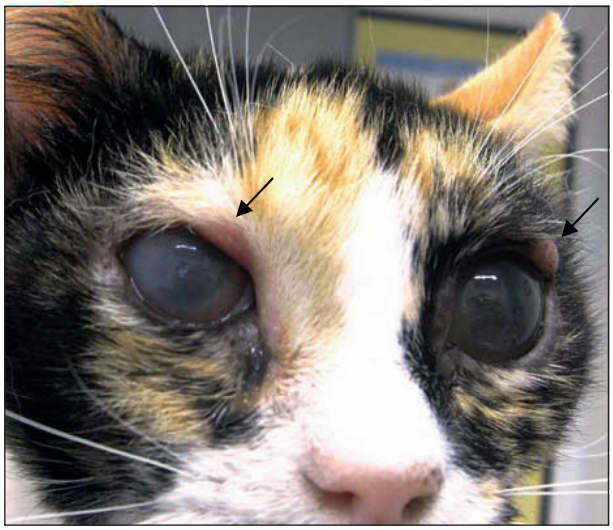

Figure 1: Nodular lesion (arrow) on upper eyelids in both eyes and keratitis (recognizable by the cloudiness of the cornea) in the right eye.

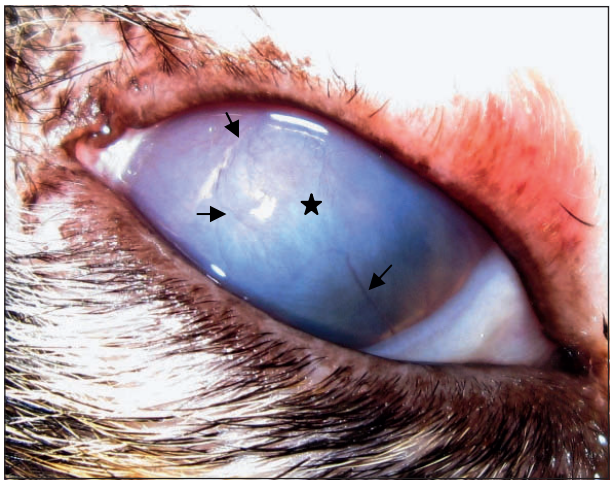

Figure 2: Stromal keratitis with vascularisation (arrows) and dense whitish infiltrates (asterisk) in the right eye.

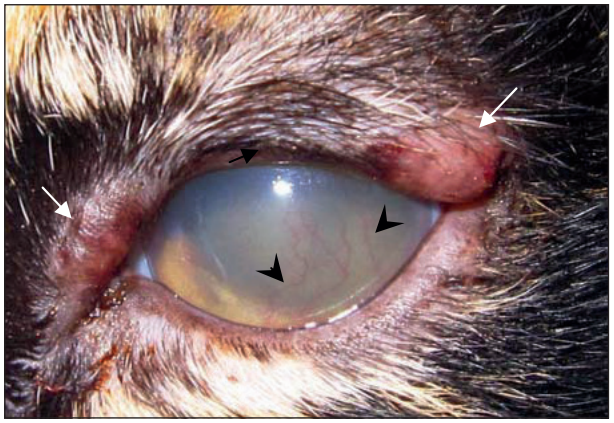

Figure 3: Nodular lesion on upper eyelid (arrow), chronic uveitis with aqueous flare (leads to cloudy appearance of the eye) and preiridal fibrovascular membranes (arrowheads) covering the iris and anterior lens surface in the left eye.

with the blood sample, the bone marrow aspirate was also deficient in all types of blood cells (empty bone marrow). No blood parasites were found in May-Grünwald Giemsa-stained blood smears and the bone marrow sample. DNA was extracted first from nodular eyelid lesions and the bone marrow aspirate. These samples tested positive by real-time PCR for *Leishmania species* DNA (Borggräfe et al., 2008). To determine the definitive species, the PCR product was directly sequenced. Comparing the nucleotide sequence to GenBank, *Leishmania infantum* was identified as the causative agent. For confirmative diagnosis, serum was investigated by indirect immunofluorescence (IFA) as described. A high IgG titer of 1:3200 was determined.

Treatment with allopurinol (Allopur®, Sandoz Pharmaceuticals AG, Cham, Switzerland) 10 mg/kg BID orally was initiated and topical treatment with prednisolon acetate (Predforte®) BID and dorzolamide and timolol maleate (Cosopt®) SID OS was continued. No topical treatment was given OD. Homeopathic treatment was discontinued. Two months following initiation of oral treatment with

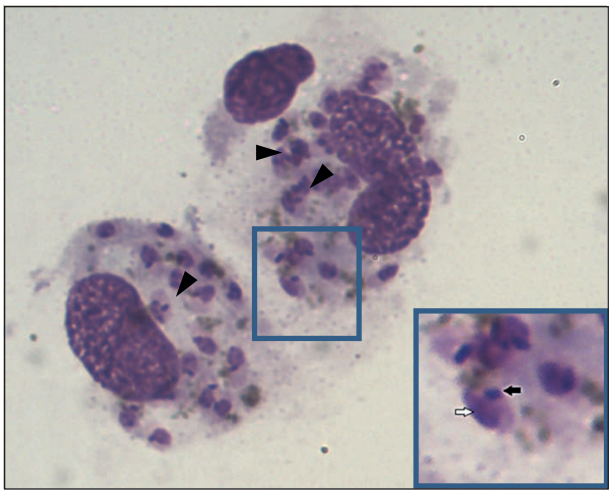

Figure 4: Smear of the cornea of the right eye (DiffQuick®, original magnification × 1000): Two macrophages with numerous intracytoplasmic protozoa (black arrowheads) consistent with the amastigote form of *Leishmania*. Inset: protozoa with a large oval light purple nucleus (white arrow) and a small rod-shaped dark purple kinetoplast (black arrow).

allopurinol, blood cell profile was markedly improved (Tab. 1), and the cat was alert and in good condition. Nodular eyelid lesions and corneal vascularisation and infiltrates had completely resolved. Although signs of active uveitis (aqueous flare, iritis) cleared OU, remnants of previous chronic uveitis (posterior synechiae OU, cortical cataract OS, preiridal fibrovascular membranes OS) were persistent. The cat was presented for follow-up examination1 year, and 2 and 3 years after initiation of allopurinol therapy. The cat, still on allopurinol 10 mg/kg BID orally without any side effects, was active and in good general condition, which is reflected by its blood cell profile with most values within reference range. The serum antibody titre for *Leishmania* spp. was reduced to 1:200 and remained stable throughout the observation period (Tab. 1). Ocular examination revealed lack of signs of active uveitis OU. However, remnants of previous uveitis, which have been present already with initiation of allopurinol treatment, remained present (Fig. 5, 6).

Discussion

Leishmaniases are infectious diseases of man and animals caused by about 20 species of *Leishmania*. Within Europe, *Leishmania infantum* (syn. *Leishmania chagasi*) is endemic in the Mediterranean region. Sand flies are the only arthropode able to transmit *Leishmania infantum*.(Solano-Gallego et al., 2009) Leishmaniasis due to *Leishmania infantum* is commonly seen in dogs in endemic regions. The disease is also diagnosed in dogs in non-endemic regions with a history of travelling to or living in endemic regions. (Shaw et al., 2003) Although the rate of infection with *Leishmania* spp. seems to be high in cats living

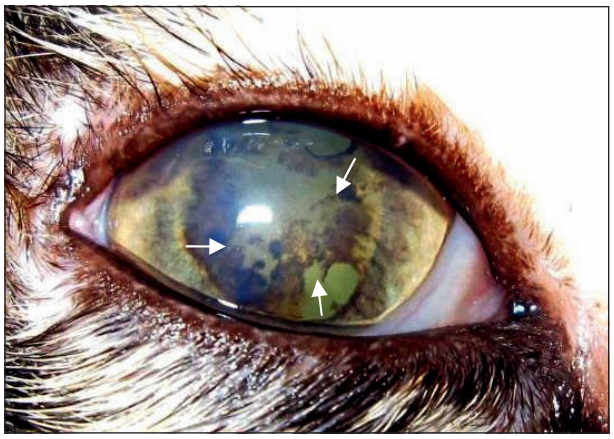

Figure 5: Right eye: Posterior synechia (arrows) 2 years after initiation of therapy.

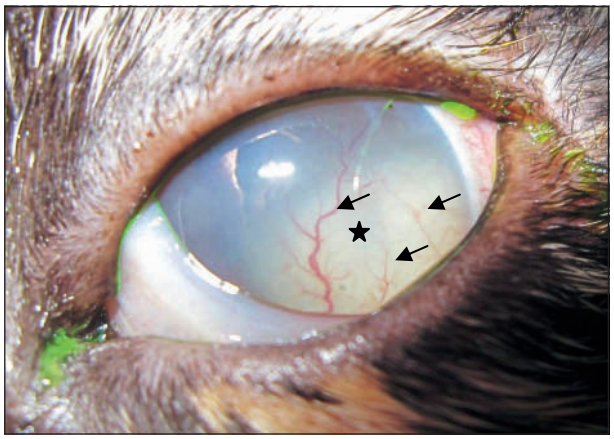

Figure 6: Left eye: Subtle corneal edema, clear aqueous humor, and remnants of chronic uveitis: diffuse cortical cataract (asterisk) with fibrovascular membranes (arrows) growing onto the anterior lens surface; 2 years after initiation of therapy.

in endemic areas (Maia et al., 2010; Martin-Sanchez et al., 2007; Pennisi, 2002), in Europe only few clinical cases of feline leishmaniasis have been described in endemic regions, like Portugal, France (Ozon et al., 1998), Spain (Hervas et al., 1999; Leiva et al., 2005) and Italy (Poli et al., 2002). The susceptibility to infection with *Leishmania* spp. and the outcome of the disease are poorly understood in cats. Experimental infection with *Leishmania* spp. resulted in spontaneous healing of lesions suggesting natural resistance of cats to leishmaniasis (Kirkpatrick et al., 1984; Simoes-Mattos et al., 2005).

Until today, feline leishmaniasis (cutaneous form) has been reported in Switzerland in a total of 3 cats originating from Spain in 1977 (Schawalder, 1977) and 2005 (Rufenacht et al., 2005).

The cat described in the present report had been most likely infected prior to its departure from Spain, because since living in Switzerland (canton Zurich) the cat was held indoors, and the sand fly *Phlebotomus perniciosus* (a potential vector) has only been observed occasionally in canton Ticino (Knechtli and Jenni, 1990). Cases of feline leishmaniasis present primarily as cutaneous disease without visceral involvement (Kirkpatrick et al., 1984; Rufenacht et al., 2005; Navarro et al., 2010). Cases of feline leishmaniasis with ocular involvement have been described in 2 cats so far (Hervas et al., 2001; Leiva et al., 2005). In dogs, ocular disease occurs in approximately 25 % of cases of leishmaniasis, generally concurrent with other systemic signs, and consists of predominantly anterior uveitis, periocular alopecia, diffuse blepharitis, conjunctivitis and keratoconjunctivitis. Solitary eyelid nodules seem to be rare in canine leishmaniasis with a prevalence of 0.09 % only (Pena et al., 2000).

Table 1: Laboratory findings before and under therapy at the indicated date. Pathological results are printed in bold.

parameter (range)	30.09.2009	19.10.2009	02.12.2009	17.09.2010	08.07.2011	29.9.2012
clinical chemistry						
albumin (30–40 g/l)	**27**	**27**	30	**29**	**29**	**29**
total protein (64–70 g/l)	**74**	**73**	70	70	65	68
Hematology						
erythrocytes (5.0–10. 10.0×10^{12}/l)	**2.8**	**3.4**	7.5	7.1	6.0	5.8
hematocrit (27–47 %)	**20**	**24**	42	37	32	34
hemoglobin (5.6–9.3 g/dl)	**3.6**	**4.6**	7.7	6.9	6.6	6.4
leukocytes ($6.0–11.0 \times 10^9$/l)	**2.4**	**2.8**	**3.4**	**3.8**	**2.5**	**4.0**
thrombocytes ($150–550 \times 10^9$/l)	**55**	249	**89**	**101**	**73**	**86**
Microbiology						
Leishmania spp. DNA	**positive** (eye and bone marrow)	n.a.	negative (eye)	n.a.	n.a	n.a.
Anti-*L. infantum* antibodies (≤ 1:50)	**1: > 1600**	n.a.	**1:800**	**1:200**	**1:100**	**1:200**

n.a. = not analysed

In the present cat, initial ocular signs consisted of bilateral chronic uveitis. Clinical signs of uveitis including aqueous flare, iris thickening, keratic precipitates and low intraocular pressure are non-pathognomonic for its aetiology. Uveitis in cats is commonly associated with infections with FeLV (feline leukemia virus), FIV (feline immunodeficiency virus), feline infectious peritonitis virus (FIP) and *Toxoplasma gondii* (Lappin, 2000). Feline herpesvirus type 1 (FHV-1) and *Bartonella* spp. have also been detected in aqueous humor of cats with uveitis (Lappin et al., 2000; Powell et al., 2010). Other reasons for uveitis are ocular trauma, corneal ulceration, cataract, and intraocular primary or metastatic neoplasia (Colitz, 2005). In many cases, the aetiology of feline uveitis remains unknown and an idiopathic uveitis is diagnosed. Similarly, the aetiology of bilateral chronic uveitis remained undetected in the present cat until solitary nodular lesions developed in both upper eyelids, and cytology revealed the presence of intracytoplasmatic microorganisms in samples from the eyelids and cornea. Specific routine diagnostic techniques to detect canine leishmaniasis include microscopy, culture, serology, and PCR (Oliva et al., 2006). Serological diagnosis is widely used in dogs because humoral response in canine leishmaniasis is usually very intense, however, detectable levels of antibodies may not be present until several months after infection by *Leishmania* (Maia and Campino, 2008). Whereas specific antibodies have been validated for the diagnosis of canine leishmaniasis, this is not the case with leishmaniasis in cats (Martin-Sanchez et al., 2007; Maia et al., 2010). In fact, *Leishmania*-infected cats often develop a low anti-*Leishmania* antibody response or remain seronegative and serum protein changes are found to be much less severe than in canine leishmaniasis (Pennisi, 2002; Martin-Sanchez et al., 2007; Maia et al., 2010). In contrast demonstration and identification of the parasite by cytology or biopsy and PCR allows confirmation of leishmaniasis in cats. (Maia et al., 2010; Navarro et al., 2010) In the present case, a PCR test for *Leishmania* spp was included in the work up for infectious diseases. The PCR test was negative for *Leishmania* spp. in the blood sample. With the appearance of nodular lid lesions and keratitis in one eye, samples taken from the affected tissues revealed high numbers of amastigotes (using cytology) and tested positive by real-time PCR for *Leishmania* spp. DNA. Systemic infection was proven by the presence of organisms (using PCR) in bone marrow aspirates. Non-invasive sampling of the ocular surface may serve as an additional diagnostic tool when invasive sampling such as spleen or bone narrow aspiration is not possible. Further studies of *Leishmania*-infected cats and dogs with ocular lesions are necessary to evaluate if this method could be a valuable and powerful diagnostic method.

With initiation of antileishmanial treatment, the cat's ocular and general condition improved rapidly and markedly. Treatment with allopurinol often leads to clinical cure in dogs. However, clinical relapse may be seen in dogs during or after cessation of treatment, indicating persistent infection (Solano-Gallego et al., 2009). To the best of the author's knowledge, no studies evaluating allopurinol treatment in cats have been performed so far. Therefore it is unknown, whether clinical relapse may happen with cessation of treatment in feline leishmaniasis. Since allopurinol has been well tolerated by the present cat for 3 years and stopped its active uveitis, the treatment was continued to prevent a possible relapse which could lead to blindness.

References

Borggräfe A., Schaarschmidt D., Müller W., Walla L.: Reaktive Arthritis beim Hund nach Leishmanien-Infektion? – Nachweis von Leishmania-infantum-DNA mittels PCR in einem arthritischen Gelenk. Tierärztl. Prax. Kleintiere. 2008, 36: 35–41.

Colitz C. M.: Feline uveitis: Diagnosis and treatment. Clin. Tech. Small Anim. Pract. 2005, 20: 117–120.

Hervas J., Chacon-Manrique de Lara F., Lopez J., Gomez-Villamandos J. C., Guerrero M. J., Moreno A.: Granulomatous (pseudotumoral) iridociclitis associated with leishmaniasis in a cat. Vet. Rec. 2001, 149: 624625.

Hervas J., Chacon M., De Lara F., Sanchez-Isarria M. A., Pellicer S., Carrasco L., Castillo J. A., Gomez-Villamandos J. C.: Two cases of feline visceral and cutaneous leishmaniosis in Spain. J. Feline Med. Surg. 1999, 1: 101–105.

Kirkpatrick C. E., Farrell J. P. and Goldschmidt M. H.: Leishmania chagasi and L. donovani: Experimental infections in domestic cats. Exp. Parasitol. 1984, 58: 125–131.

Knechtli R., Jenni L.: Experimental transmission of leishmania infantum by the bite of phlebotomus perniciosus from Switzerland. Acta Trop. 1990, 47: 213–216.

Lappin M. R.: Feline infectious uveitis. J. Feline Med. Surg. 2000, 2 (3): 159–163.

Lappin M. R., Kordick D. L., Breitschwerdt E. B.: Bartonella spp antibodies and DNA in aqueous humour of cats. J. Feline Med. Surg. 2000, 2 (1): 61–68.

Leiva M., Lloret A., Pena T., and Roura X.: Therapy of ocular and visceral leishmaniasis in a cat. Vet. Ophthalmol. 2005, 8: 71–75.

Maia C., Campino L.: Methods for diagnosis of canine leishmaniasis and immune response to infection. Vet. Parasitol. 2008, 158: 274–287.

Maia C., Gomes J., Cristovao J., Nunes M., Martins A., Rebelo E., Campino L.: Feline leishmania infection in a canine leish-

maniasis endemic region, Portugal. Vet. Parasitol. 2010, 174: 336–340.

Martin-Sanchez J., Acedo C., Munoz-Perez M., Pesson B., Marchal O., Morillas-Marquez F.: Infection by leishmania infantum in cats: Epidemiological study in Spain. Vet. Parasitol. 2007, 145: 267–273.

Navarro J. A., Sanchez J., Penafiel-Verdu C., Buendia A. J., Altimira J., Vilafranca M.: Histopathological lesions in 15 cats with leishmaniosis. J. Comp. Pathol. 2010, 143: 297–302.

Oliva G., Scalone A., Foglia Manzillo V., Gramiccia M., Pagano A., Di Muccio T., Gradoni L.: Incidence and time course of leishmania infantum infections examined by parasitological, serologic, and nested-pcr techniques in a cohort of naive dogs exposed to three consecutive transmission seasons. J. Clin. Microbiol. 2006, 44: 1318–1322.

Ozon C., Marty P., Pratlong F., Breton C., Blein M., Lelievre A., Haas P.: Disseminated feline leishmaniosis due to leishmania infantum in southern france. Vet. Parasitol. 1998, 75: 273–277.

Pena M. T., Roura X, Davidson M. G.: Ocular and periocular manifestations of leishmaniasis in dogs: 105 cases (1993–1998). Vet. Ophthalmol. 2000, 3: 35–41.

Pennisi M. G.: A high prevalence of feline leishmaniasis in southern Italy. Proceedings of the International Canine Leishmaniasis Forum, Sevilla, Spain, 2002, 39–48.

Poli A., Abramo F., Barsotti P., Leva S., Gramiccia M., Ludovisi A., Mancianti F.: Feline leishmaniosis due to leishmania infantum in Italy. Vet. Parasitol. 2002, 106: 181–191.

Powell C. C., McInnis C. L., Fontenelle J. P., Lappin M. R.: Bartonella species, feline herpesvirus-1, and toxoplasma gondii pcr assay results from blood and aqueous humor samples from 104 cats with naturally occurring endogenous uveitis. J. Feline Med. Surg. 2010, 12: 923–928.

Rufenacht S., Sager H., Muller N., Schaerer V., Heier A., Welle M. M., Roosje P. J.: Two cases of feline leishmaniosis in Switzerland. Vet. Rec. 2005, 156: 542–545.

Schawalder P.: Leishmaniasis in dogs and cats. Kleintierpraxis. 1977, 237–246.

Shaw S. E., Lerga A. I., Williams S., Beugnet F., Birtles R. J., Day M. J., Kenny M. J.: Review of exotic infectious diseases in small animals entering the united kingdom from abroad diagnosed by pcr. Vet. Rec. 2003, 152: 176–177.

Simoes-Mattos L., Mattos M. R., Teixeira M. J., Oliveira-Lima J. W., Bevilaqua C. M., Prata-Junior R. C., Holanda C. M., Rondon F. C., Bastos K. M., Coelho Z. C., Coelho I. C., Barral A., Pompeu M. M.: The susceptibility of domestic cats (felis catus) to experimental infection with leishmania braziliensis. Vet. Parasitol. 2005, 127: 199–208.

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

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