

A case of canine neurological leishmaniasis

Rosanna Zobba^{1*}, Maria Antonietta Evangelisti¹, Maria Lucia Manunta¹, Alberto Alberti¹, Deborah Zucca² and Maria Luisa Pinna Parpaglia¹

¹ Dipartimento di Medicina Veterinaria, Università di Sassari, via Vienna 2, 07100 Sassari, Italy.

² Private Practice, Clinica Veterinaria Nemo, Via Budapest 26, 07100 Sassari, Italy.

*Corresponding author at: Dipartimento di Medicina Veterinaria, Università di Sassari, via Vienna 2, 07100 Sassari, Italy.
Tel.: +39 079 229523, e-mail: zobba@uniss.it.

Veterinaria Italiana 2017, **53** (4), 321-326. doi: 10.12834/VetIt.307.1193.4

Accepted: 09.10.2016 | Available on line: 29.12.2017

Keywords

Canine leishmaniasis,
Cerebellum,
Cerebrospinal fluid,
Magnetic resonance
images,
PCR assay,
Vestibular system.

Summary

In this study we describe a case of neurological leishmaniasis in a dog, reported in an endemic area, with signs of multifocal intracranial lesions involving the vestibular system and the cerebellum. Serum biochemistry revealed a decrease of albumin-globulin ratio caused by an increase of alfa2-, beta-, and gamma- globulin, while antibody titers were highly positive for *Leishmania* spp. Magnetic resonance images of the brain were consistent with a granulomatous inflammation/infection. Cerebrospinal fluid revealed a marked mononuclear pleocytosis and was positive to the Pandy Test, as well as to a *Leishmania* spp.-specific polymerase chain reaction (PCR) assay. Sequencing of the PCR products revealed the highest similarity with several strains belonging to the *Leishmania infantum* / *Leishmania chagasi* group. Clinical response to treatment for systemic leishmaniasis was supportive of diagnosis. This report focuses on an atypical form of canine leishmaniasis and suggests that in endemic geographic areas leishmaniasis has to be considered for differential diagnosis in neurological manifestations. Also, cerebrospinal liquor should always be tested when neurological symptoms are present in order to differentiate neurological leishmaniasis from unrelated neurological signs in *Leishmania* positive patients.

Un caso di leishmaniosi neurologica canina

Parole chiave

Leishmaniosi canina,
Cervelletto,
Liquido cerebrospinale,
Risonanza magnetica,
PCR,
Sistema vestibolare.

Riassunto

In questo articolo si descrive un caso di leishmaniosi neurologica canina, accertato in un'area endemica, che ha interessato il sistema vestibolare e il cervelletto dell'animale con lesioni intracraniche multifocali. Gli esami ematochimici hanno rivelato una riduzione del rapporto albumine-globuline causata da un aumento delle alfa2-, delle beta- e delle gamma-globuline; il titolo anticorpale nei confronti di *Leishmania* spp. è risultato altamente positivo. La risonanza magnetica del cervello ha evidenziato la compatibilità con un'infezione/infezione granulomatosa. L'esame del liquido cerebrospinale ha indicato una grave pleocitosi mononucleare e una positività, sia con il test di Pandy e sia in PCR specifica, per la *Leishmania* spp. Il sequenziamento del prodotto di PCR ha rivelato un'alta similarità con vari ceppi appartenenti al gruppo *Leishmania infantum* / *Leishmania chagasi*. La risposta clinica al trattamento specifico ha avvalorato la diagnosi di leishmaniosi sistemica. Questo caso atipico di leishmaniosi canina suggerisce la necessità, nelle aree endemiche, di considerare la leishmaniosi tra le diagnosi differenziali nelle manifestazioni neurologiche. Inoltre, l'esame del liquido cerebrospinale in caso di manifestazioni neurologiche potrebbe essere utile allo scopo di differenziare forme di leishmaniosi neurologica da sintomi neurologici non correlabili a soggetti positivi per *Leishmania*.

Introduction

Canine leishmaniasis (CL) is a cutaneous, mucocutaneous, and visceral disease caused by species of flagellate protozoa belonging to the genus *Leishmania*, which are transmitted through the bite of female sand flies. In Mediterranean Countries, the disease is endemic, and it is caused by *Leishmania infantum* (Font et al. 2004, Ordeix et al. 2005).

Leishmania spp. produce lesions throughout the body of the host, especially in the lymph nodes, liver, spleen, and skin, where the proliferative inflammatory infiltrate is dominated by macrophages. *Leishmania* spp. also induce immune-complexes deposits in some tissues (Alvar et al. 2004, Baneth et al. 2008). Clinical manifestations are highly variable, ranging from subclinical infections to generalised disorders characterised by fever, weight loss, generalised lymphadenopathy, splenomegaly, ulcerative, exfoliative or nodular skin lesions, onychogryphosis, ocular lesions, epistaxis, lameness and polyuria, and polydipsia. Typical laboratory abnormalities are polyclonal hyperglobulinaemia and hypoalbuminaemia, non-regenerative anemia, leukocytosis or leucopenia, thrombocytopenia or thrombocytopathy, and abnormality of hepatic or renal profile (Solano-Gallego et al. 2011). Clinical diagnosis is relatively easy in patients showing multiple typical signs, especially when the animal lives in or has spent time in an endemic area. However, if the dog presents only 1 symptom or lesion, diagnosis may become more difficult. Diagnosis may be tricky when CL appears in unusual clinical forms, for instance associated to specific skin lesions, oral lesions, monoclonal gammopathy, chronic colitis, haemostatic problems and/or disorders of the cardiovascular, respiratory and musculo-skeletal system (Blavier et al. 2001, Pinna Pargaglia et al. 2007).

Neurologic manifestations of leishmaniasis involving cerebral tissue or spinal cord have also been reported in veterinary medicine, worldwide (Viñuelas et al. 2001, Lima et al. 2003, Font et al. 2004, Ikeda et al. 2007, Cauduro et al. 2011, José-López et al. 2012, Márquez et al. 2013, José-López et al. 2014). In endemic areas, where dogs with leishmaniasis may be co-infected with other vector-borne pathogens or might suffer from other concomitant infectious or noninfectious diseases, specific diagnostic approach should be designed for each patient in order to differentiate leishmaniasis infection from other disease. According to guidelines proposed by Paltrinieri and colleagues (Paltrinieri et al. 2010), diagnosis of clinical leishmaniasis should be based on clinicopathological manifestations and must be confirmed by serological and molecular techniques. In this report, we describe clinical findings, magnetic resonance images (MRI) features, cerebrospinal fluid

(CSF) alterations, and successful clinical response to medical treatment in a dog with neurological leishmaniasis.

Case report

A 6-year-old, male toy mixed breed dog, living in Sardinia, was presented to the Veterinary Teaching Hospital of the University of Sassari with a 20 days history of ataxia, blepharitis, head tilt, and tremors. Few days before clinical evaluation, keratoconjunctivitis, nasal dermatitis, epidermal scaling, depression, and anorexia also appeared. On physical examination, dermatological signs, ocular lesions, and head tilt were confirmed. In addition, tendency to recumbency, vestibular ataxia, circling, ventral strabismus, proprioceptive deficit, menace deficit, tremors, and hypermetria were observed. These neurological signs were consistent with multifocal intracranial lesions involving vestibular system and cerebellum.

Results of cell blood count and serum biochemical profile displayed trombocytosis [$547 \times 10^9/L$, reference interval (RI) $92.2 - 370.0 \times 10^3/\mu l$] and a decrease of albumin-globulin ratio [A/G 0.45, (RI) 0.79-1.02] caused by an increase of alfa2-, beta- and gamma- globulin. Indirect fluorescent antibody test (IFAT) for *Ehrlichia canis* (IgG), *Toxoplasma gondii* (IgM, IgG), and *Neospora caninum* (IgG) were negative, while IFAT for *Rickettsia* spp. (IgG 1:512) and *Leishmania* spp. (IgG 1:1280) were positive.

Based on the anamnestic and clinical presentation, the primary differential diagnoses included inflammatory-infectious diseases and neoplasia. Considering the onset and course of illness, vascular alterations were ruled out from differential diagnosis. Similarly, degenerative forms were excluded considering the age of the dog. A MRI scan of the brain was performed using a 0.23 Tesla machine (Paramed, Genova, Italy) and a designed human knee coil with the dog positioned on sternal recumbence. The animal was anaesthetized using intravenous fentanyl ($5 \mu g/kg$) and medetomidin ($0.01 mg/kg$) for premedication, ketamin ($3 mg/kg$), and diazepam ($0.3 mg/kg$) for induction and maintained with sevoflurane in oxygen delivered via a circle system. MRI images were acquired in 3 planes (sagittal, transverse, and dorsal) and included the head from the forth-cervical vertebra to the nasal cavity. The study included SE T1W pre- and post-contrast [following intravenous administration of $0.1 mmol/kg$ gadopentetate dimeglumine (Gadovist $0.05 mmol/kg$, IV)], and FSE T2W sequences (Figure 1). The T2W sequence detected an oval shaped hypointense lesion ($0.5 cm$ diameter) located intra-axially within the vermis of the cerebellum without mass effect. The lesion was

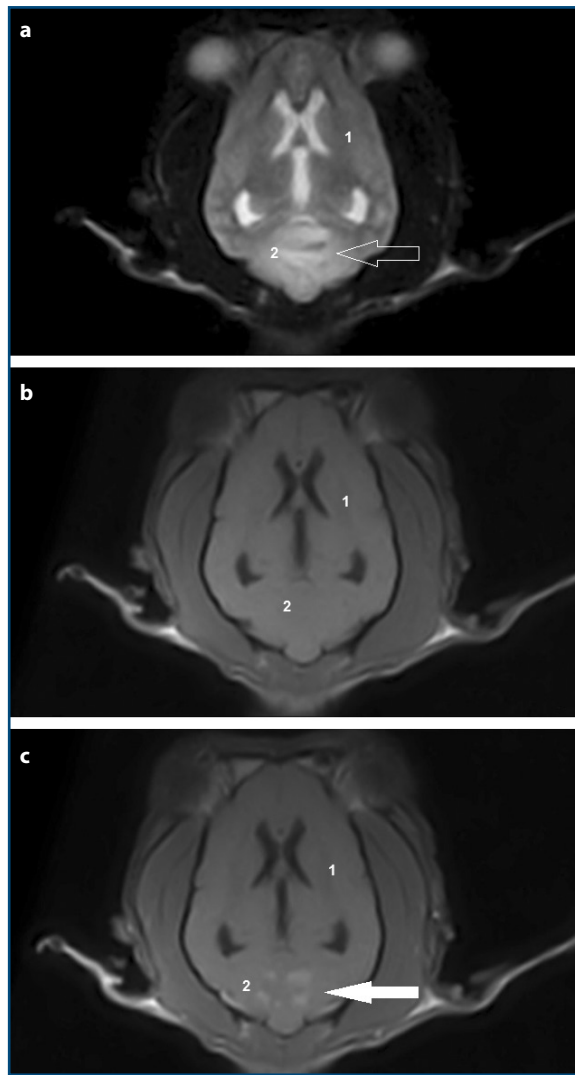


Figure 1. Magnetic resonance images of the brain in dorsal plane in FSE T2W (a), Se T1W pre (b) and post contrast injection (c): 1 forebrain, 2 cerebellum. a) oval shape hypointense lesion (0.5 cm diameter) intra-axially within the vermis of the cerebellum and diffuse perilesional edema (arrow); b) isointense cerebellar parenchyma; c) several smaller hyperintense, well-defined areas, egg-shaped with sharp edges within the vermis of the cerebellum (arrow white).

surrounded by a diffuse hyperintensity. On T1W sequences the area was isointense. After injection of contrast medium it appeared with a mild ring enhancement and several smaller hyperintense, well-defined areas, egg-shaped with sharp edges within the vermis of the cerebellum were observed. A mild dilatation of the ventricular system, particularly on the mesencephalic aqueduct was noted. The MRI images were compatible with granulomatous inflammatory/infectious, and primary or metastatic neoplastic lesions.

Cerebrospinal fluid was collected via atlanto-occipital cisternal puncture. The macroscopic appearance was clear and transparent and fluid analysis revealed a Pandy Test positive reaction (1+) and a marked

pleocytosis with a total nucleated cell count of 134/ μ L (reference interval < 5 cells/ μ L) (Lorenz et al. 2011). On a cytocentrifuged specimen stained with Diff Quick (Dade Behring AG, Düringen, Switzerland), mononuclear pleocytosis (~85.5%) was evident with a prevalence of monocytoïd/macrophage cells (~60%) vs lymphocytes (~25.5%). Reactive lymphocytes with basophilic cytoplasm and/or plasmocytoid cells with more abundant basophilic cytoplasm and perinuclear clear zone were occasionally observed. Moderate number of non-degenerate neutrophils (14.1%) and occasional eosinophils (0.4%) were also observed (Figure 2). The CSF appeared slightly contaminated by blood (the absence of erythrophagocytosis or hemosiderin crystals excluded a pathological subarachnoid haemorrhage). However, the contamination appeared minimal (red blood cells in CSF were 125/ μ L) and it was not so high to affect significantly the total nucleated cell count or the differential count.

On the basis of serologic results, MRI images, and CSF cytological aspect, a *Leishmania* spp. specific heminested polymerase chain reaction (PCR) was performed in our laboratory from blood and CSF, whereas a specific *Rickettsia* spp. and *Leishmania infantum* real-time PCRs were performed in private laboratories from CSF, for *Leishmania*, and *Rickettsia*, as well as from blood for *Rickettsia*. The DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Milano, Italy).

The *Leishmania* spp. specific PCR used in this study target the 5.8S rRNA gene and the flanking internal transcribed spacer regions (ITS1 and ITS2). Two already published primers (LITSR: 5'- CTG GAT CAT TTT CCG ATG - 3' and LITSV: 5' - ACA CTC AGG TCT GTA AAC - 3') were used in the first PCR round

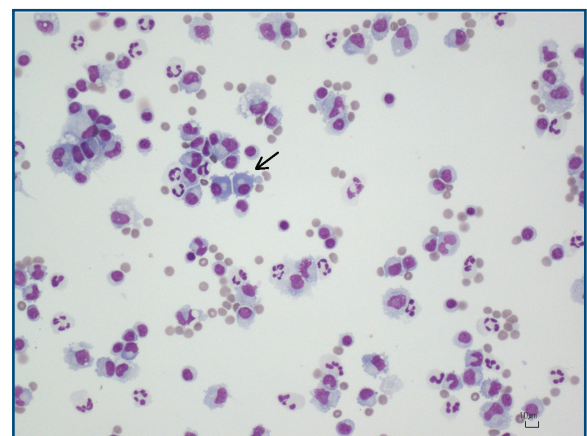


Figure 2. Cytocentrifuged preparation of cerebrospinal fluid (CSF). Mononuclear pleocytosis with a prevalence of monocytoïd/macrophage cells vs lymphocytes. Moderate number of non-degenerated neutrophils are present. Two plasma cells are observed (arrow). The CSF appeared slightly contaminated by blood. Diff Quick, x 20 objective.

to amplify a fragment of 1034 bp (Mahdy *et al.* 2010). The PCR product obtained in the first round was used as a DNA target in the heminested PCR designed by combining the new primer LeishS: 5' – CAT TTT CCG ATG ATT ACA CCC – 3' with the primer LITSV, to obtain a fragment of 1028 bp. Briefly, 150 ng of DNA extraction were used in a 50 µl PCR reaction containing 200 µM dNTPs, 0.3 µM of each of the 2 primers, and 1.25 U of Taq DNA polymerase (Qiagen, Milano, Italy). Both first and heminested PCR amplification were performed with an initial denaturation at 94 °C for 3 minutes; 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 56 °C for 30 seconds and extension at 72 °C for 1 minute; final extension at 72 °C for 10 minutes. Agarose gel electrophoresis revealed a band of predicted size for both the primary and second PCR performed on CSF (Figure 3). A 1028 bp amplicon was cloned into the pCR4-TOPO vector (Invitrogen SRL, Milan, Italy) and sequenced by using an automatic sequencer (BMR Genomics, Padova, Italy). Upon BLAST analyses, the sequence revealed the highest similarity with sequences obtained from strains belonging to the *L. infantum* / *L. chagasi* group (98% identity with 100% coverage). Nucleotide sequence of the 5.8S rRNA gene and the flanking internal transcribed spacer regions (ITS1 and ITS2) obtained in this study was deposited in GenBank under accession number KJ573795. Real time PCR performed on CSF confirmed the presence of *L. infantum* with a parasite load of 115,000 copies of kinetoplast DNA / ml. Results of *Leishmania* spp. specific heminested PCR performed on blood sample and specific *Rickettsia* spp. real-time PCR performed on CSF and blood sample were negative.

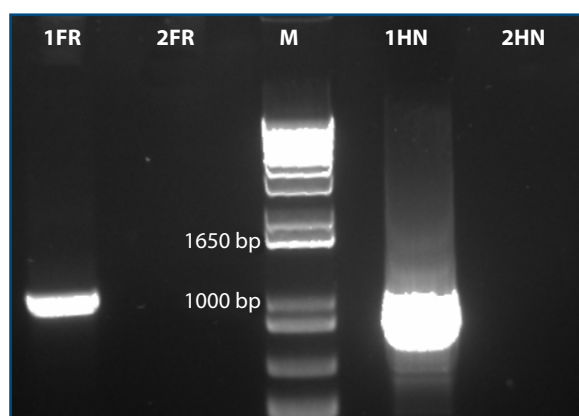


Figure 3. Gel electrophoresis result of PCR specific for *Leishmania* spp. amplifying a fragment of 1034 bp in the first round (FR) and a fragment of 1028 bp in the heminested (HN) of the ITS1-5.8SrRNA-ITS2 region. M = 1 kb plus DNA ladder (Invitrogen, Monza, Italy). FR = first round PCR results, 1FR = amplicon obtained from DNA extracted from the cerebral-spinal fluid of our patient, 2FR = negative control. HN = Heminested PCR results, 1HN = amplicon obtained from DNA extracted from the cerebral-spinal fluid of our patient, 2HN = negative control.

Treatment for systemic leishmaniasis with allopurinol at 10 mg/kg every 12 hours for 12 months, meglumine antimoniate at 100 mg/kg every 24 hours for 45 days, and prednisolone at 1 mg/kg every 24 hours for a week was prescribed. During the first days the dog showed a rapid improvement of general conditions with an increase of appetite, while at the end of therapy the dog showed an important decrease of neurological signs with complete remission of vestibular ataxia, significant decrease of head tilt, reduction of menace deficit present only on the left eye, mild deficit of postural reactions, and total disappearance of dermatological and ocular signs. At the 3-month follow up appointment, the normalization of serum electrophoresis pattern was observed.

Discussion

Canine leishmaniasis is an endemic pathology in the Mediterranean countries, where it is characterised by progressive wasting due to the involvement of multiple organs (Alvar *et al.* 2004, Baneth *et al.* 2008). Clinical presentation varies widely depending on the affected organ(s). Occasionally, neurological signs and/or histopathological lesions affecting the central or peripheral nervous system have been observed (Garcia-Alonso *et al.* 1996, Nieto *et al.* 1996, Viñuelas *et al.* 2001, Lima *et al.* 2003, Font *et al.* 2004, Ikeda *et al.* 2007, Cauduro *et al.* 2011, José-López *et al.* 2012, Márquez *et al.* 2013, José-López *et al.* 2014) and named as “cerebral leishmaniasis” (Garcia-Alonso *et al.* 1996).

Previous investigations of canine symptomatic neurologic leishmaniasis reported granulomatous inflammations composed of macrophages, lymphocytes, plasma cells, neutrophils and, sometimes, eosinophils with occasional presence of amastigotes free or inside the macrophages. These proliferative reactions were localised in the cervical spinal nerves or in the spinal cord (Márquez *et al.* 2013), in the vertebral canal extradural masses (Cauduro *et al.* 2011), or in the meninges (Viñuelas *et al.* 2001). Other histopathological findings were characterised by malacia and haemorrhage associated with multiple foci of vasculitis, rupture or thrombosis of inflamed vessels, and consequent ischemia in the spinal cord (Font *et al.* 2004), perivascular mononuclear cuffs in the spinal cord, in the brain and the leptomeningeal vessels (Márquez *et al.* 2013), neuronophagia, gliosis, leptomeningitis, vascular congestion, presence of perivascular lymphoplasmacytic infiltrate, and areas of focal micro hemorrhage of the encephalon (Ikeda *et al.* 2007). The involvement of *Leishmania* in the vascular injury was demonstrated with the presence of amastigotes or antigens inside the lesions (Ikeda *et al.* 2007, Márquez *et al.* 2013), or

it was suspected because of the finding of the parasite in the bone marrow (Font *et al.* 2004). Some authors (Jose-Lopez *et al.* 2012) described 2 other cases of symptomatic neurological canine leishmaniasis, and presented MRI of the brain consistent with multifocal, non-haemorrhagic, and ischaemic lesions. Histopathological lesions were not described, and amastigotes were found in bone marrow but not in the CNS. In 2 reports published in 1996, *Leishmania amastigotes* were identified in the choroid plexus of dogs with systemic leishmaniasis without neurologic signs (Garcia-Alonso *et al.* 1996, Nieto *et al.* 1996). Garcia Alonso and colleagues (Garcia Alonso *et al.* 1996) described the presence of inflammatory processes in the choroid plexus with hypercellularity and deposition of amyloid substance accompanied with pathological reactions in the encephalon and cerebellum, while Nieto and colleagues (Nieto *et al.* 1996) observed capillary congestion and infiltration with lymphocytes and polymorphonuclear cells associated with thrombosis and epithelial metaplasia in the choroid plexus. Another report in 2003 described 3 cases of neurological manifestations in dogs with systemic leishmaniasis and reported the presence of Anti-*Leishmania* antibody in CSF, but without any confirmation of the presence of amastigotes, antigens or DNA of the parasite, and of histological lesions in the nervous tissue (Lima *et al.* 2003).

In this article, we described a case of neurological leishmaniasis in a dog characterised by multifocal intracranial granulomatous lesions involving the vestibular system and cerebellum. *Ante mortem* diagnosis of CNS leishmaniasis has been achieved on the basis of detection of systemic infection, MRI findings, and a specific PCR assay performed on DNA extracted from CSF. Clinical response to treatment for systemic leishmaniasis was also supportive of diagnosis. In endemic areas, where dogs with leishmaniasis might be co-infected with other vector borne pathogens or might suffer from other concomitant infectious or noninfectious diseases, specific diagnostic approach should be conducted for each patient in order to differentiate leishmaniasis infection from other diseases. According to guidelines proposed by Paltrinieri and colleagues (Paltrinieri *et al.* 2010), diagnosis of clinical leishmaniasis has to be suspected on the basis of clinicopathological manifestations and must be confirmed by serological and molecular

techniques. Previous studies about MRI images in canine neurologic leishmaniasis are scarce and describe images consistent with cerebral ischemic infarction induced by vasculitis (José-López *et al.* 2012) and extradural spinal cord mass referred to granulomatous lesion (Cauduro *et al.* 2011). Recent studies indicate that MRI is a sensitive and specific tool for classification of brain lesions attributable to such processes as inflammatory and neoplastic. However, the limits of MRI and the need to obtain tissue biopsy samples for determination of a definitive diagnosis have also been stressed more recently (Wolff *et al.* 2012). Alterations of CSF have low specificity for the detection of neurological diseases because the possible abnormalities are relatively limited. Four previous reports described alterations of CSF in dogs with neurologic leishmaniasis (Lima *et al.* 2003, José-López *et al.* 2012, Márquez *et al.* 2013, José-López *et al.* 2014) and the cytological findings were ascribed to a nonspecific inflammatory reaction characterised by mixed pleocytosis or cells into normal range with an increase of percentage of neutrophil granulocytes (Márquez *et al.* 2013, José-López *et al.* 2014).

In our experience, MRI images associated with the result of CSF examination, characterised by a marked mononuclear pleocytosis and positivity for Pandy test, confirmed the suspect of an intracranial inflammation without specificity for a particular etiological agent, but it was not possible to exclude a neoplasia. When routine analyses of CSF are associated with more specific investigations, such as specific antibodies titer and index or PCR, analysis of CSF becomes a more reliable diagnostic tool. The presence of *Leishmania* DNA in CSF supports the diagnosis of neurological leishmaniasis and suggests a breakdown of the filtration barrier with the consequent transfer of antigens from blood to the CNS compartment. According to other reports (Márquez *et al.* 2013, José-López *et al.* 2014), PCR positivity for *Leishmania* DNA in CSF has confirmed a definitive diagnosis of inflammatory masses caused by neurological leishmaniasis.

Concluding, data suggest the need of considering the existence of different neurologic presentations or of other uncommon forms of canine Leishmaniasis in endemic geographic regions, such as Sardinia (Italy), where another atypical form has already been described (Pinna Parpaglia *et al.* 2007).

References

- Alvar J., Cañavate C., Molina R., Moreno J. & Nieto J. 2004. Canine leishmaniasis. *Adv Parasitol*, **57**, 1-88.
- 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**, 324-330.
- Blavier A., Keroack S., Denerolle P., Goy-Thollot I., Chabanne L., Cadore J.L. & Bourdoiseau G. 2001. Atypical forms of canine leishmaniosis. *Vet J*, **162**, 108-120.
- Cauduro A., Favole P., Lorenzo V., Simonetto L., Barda B., Cantile C. & Asperio R.M. 2011. Paraparesis caused by vertebral canal leishmaniotic granuloma in a dog. *J Vet Intern Med*, **25**, 398-399.
- Font A., Mascort J., Altimira J., Closa J.M. & Vilafranca M. 2004. Acute paraplegia associated with vasculitis in a dog with leishmaniasis. *J Small Anim Pract*, **45** (4), 199-201.
- García-Alonso M., Nieto C.G., Blanco A., Requenzna J.M., Alonso C. & Navarrete I. 1996. Presence of antibodies in the aqueous humour and cerebrospinal fluid during *Leishmania* infections in dogs. Pathological features at the central nervous system. *Parasite Immunol*, **18**, 539-546.
- Ikeda F.A., Laurenti M.D., Corbett C.E., Feitosa M.M., Machado G.F. & Perri S.H.V. 2007. Histological and immunohistochemical study of the central nervous system of dogs naturally infected by *Leishmania (Leishmania) chagasi*. *Braz J Vet Res Anim Sci*, **44**, 5-11.
- José-López R., De la Fuente C. & Añor S. 2012. Presumed brain infarctions in two dogs with systemic leishmaniasis. *J Small Anim Pract*, **53** (9), 554-557.
- José-López R., De la Fuente C., Pumarola M. & Añor S. 2014. Intramedullary spinal cord mass presumptively associated with leishmaniasis in a dog. *J Am Vet Med Assoc*, **244** (2), 200-204.
- Lima V.M., Gonçalves M.E., Ikeda F.A., Luvizotto M.C. & Feitosa M.M. 2003. Anti-leishmania antibodies in cerebrospinal fluid from dogs with visceral leishmaniasis. *Braz J Med Biol Res*, **36**, 485-489.
- Lorenz M.D., Coates J. & Kent M. 2011. Confirming a diagnosis. In *Handbook of Veterinary Neurology*, 5th Ed. (Lorenz M.D., Coates J.R., Kent M., eds). Elsevier Saunders, St. Louis, 75-93.
- Mahdy M.A., Al-Mekhlafi H.M., Al-Mekhlafi A.M., Lim Y.A., Bin Shuaib N.O., Azazy A.A. & Mahmud R. 2010. Molecular characterization of *Leishmania* species isolated from cutaneous leishmaniasis in Yemen. *PLoS One*, **20**, 5 (9).
- Márquez M., Pedregosa J.R., López J., Marco-Salazar P., Fondevila D. & Pumarola M. 2013. *Leishmania amastigotes* in the central nervous system of a naturally infected dog. *J Vet Diagn Invest*, **25**, 142-146.
- Nieto C.G., Vinuelas J., Blanco A., García-Alonso M., Verdugo S.G. & Navarrete I. 1996. Detection of *Leishmania infantum amastigotes* in canine choroid plexus. *Vet Rec*, **139**, 346-347.
- Ordeix L., Solano-Gallego L., Fondevila D., Ferrer L. & Fondati A. 2005. Papular dermatitis due to *Leishmania* spp. infection in dogs with parasite-specific cellular immune responses. *Vet Dermatol*, **16**, 187-191.
- Paltrinieri S., Solano-Gallego L., Fondati A., Lubas G., Gradoni L., Castagnaro M., Crotti A., Maroli M., Oliva G., Roura X., Zatelli A. & Zini E. 2010. Canine Leishmaniasis Working Group, Italian Society of Veterinarians of Companion Animals. Guidelines for diagnosis and clinical classification of leishmaniasis in dogs. *J Am Vet Med Assoc*, **236** (11), 1184-1191.
- Pinna Parpaglia M.L., Vercelli A., Cocco R., Zobba R. & Manunta M.L. 2007. Nodular lesions of the tongue in canine leishmaniosis. *J Vet Med A Physiol Pathol Clin Med*, **54** (8), 414-417.
- Solano-Gallego L., Miró G., Koutinas A., Cardoso L., Pennisi M.G., Ferrer L., Bourdeau P., Oliva G. & Baneth G. The LeishVet Group. 2011. LeishVet guidelines for the practical management of canine leishmaniosis. *Parasit Vectors*, **4**, 86.
- Viñuelas J., García-Alonso M., Ferrando L., Navarrete I., Molano I., Mirón C., Carcelén J., Alonso C. & Nieto C.G. 2001. Meningeal leishmaniosis induced by *Leishmania infantum* in naturally infected dogs. *Vet Parasitol*, **101**(1), 23-27.
- Wolff C.A., Holmes S.P., Young B.D., Chen A.V., Kent M., Platt S.R., Savage M.Y., Schatzberg S.J., Fosgate G.T. & Levine J.M. 2012. Magnetic resonance imaging for the differentiation of neoplastic, inflammatory, and cerebrovascular brain disease in dogs. *J Vet Intern Med*, **26** (3), 589-597.