Intramedullary spinal cord mass presumptively associated with leishmaniasis in a dog

Roberto José-López, DVM; Cristian de la Fuente, DVM, MSc; Martí Pumarola, DVM, PhD; Sonia Añor, DVM, PhD

Case Description—A 9-year-old male Miniature Poodle was evaluated because of progressive severe right hemiparesis, right forelimb lameness, and signs of cervical pain.

Clinical Findings—A low body condition score (2/9) and popliteal lymphadenopathy were detected. Results of a CBC, serum biochemical analyses, urinalysis, cytologic examination of bone marrow and popliteal lymph node aspirates, and serum ELISA were consistent with systemic leishmaniasis. Magnetic resonance imaging of the cervical spinal cord revealed an intramedullary mass extending from the caudal aspect of the C5 vertebral body to the C5-6 intervertebral disk space with a contrast medium–enhanced pattern that had 3 zones (central contrast medium–enhanced core, intermediate isointense zone, and peripheral contrast medium–enhanced ring). Surgical biopsy of the mass was performed by means of a right C5-6 dorsal hemilaminectomy. Results of PCR assays for detection of *Leishmania* DNA in CSF and tissue biopsy samples were positive.

Treatment and Outcome—Treatment for systemic leishmaniasis was initiated. Two months later, body condition, neurologic signs, and gait of the dog had substantially improved; the dog had mild right forelimb paresis at that time. Results of follow-up MRI indicated resolution of the cervical spinal cord lesion. Four months after diagnosis, the dog's neurologic condition was stable.

Clinical Relevance—To the authors' knowledge, this report is the first in which clinical findings, clinicopathologic data, and MRI characteristics of an intramedullary inflammatory spinal cord lesion presumptively attributable to leishmaniasis in a dog have been reported, and the first report of CNS leishmaniasis in a dog with MRI resolution and a successful clinical response to treatment. (*J Am Vet Med Assoc* 2014;244:200–204)

A 9-year-old male Miniature Poodle was examined by personnel of the Neurology and Neurosurgery Service of the Veterinary Teaching Hospital at the Universitat Autònoma de Barcelona because of progressive right hemiparesis, right forelimb lameness, and signs of cervical pain of 10 days' duration. In addition to and beginning at the same time as the neurologic signs, the dog had hyporexia and progressive weight loss. Results of physical examination revealed a body condition score of 2 of 9 and popliteal lymphadenopathy. Results of neurologic examination indicated the dog had severe right forelimb and hind limb ambulatory hemiparesis and postural reaction deficits in those same limbs. Low muscle tone and muscle atrophy, most obvious in the supraspinatus and infraspinatus muscles, were detected in the right forelimb. The flexor withdrawal reflex was weak in this limb, and the animal had severe signs of pain during palpation and manipulation of the cervical vertebrae. Neuroanatomic localization of clinical signs was consistent with a right-sided lesion in the C6-T2 spinal cord segments. The primary differential diagnoses included inflammation (immune mediated or infectious), neoplasia, or lateralized intervertebral disk extrusion.

Results of hematologic and serum biochemical analyses indicated mild, nonregenerative (normocytic and normochromic) anemia (PCV, 33% [reference range, 37% to 55%]; RBC count, 4.72 × 10^6 RBCs/µL [reference range, 5.5 × 10^6 RBCs/µL to 8.5 × 10^6 RBCs/µL]) and marked hyperproteinemia (9.79 g/dL; reference range, 5.4 to 7.1 g/dL) attributable to severe polyclonal hypergammaglobulinemia (4.67 g/dL; reference range, 0.3 to 0.8 g/dL). Results of thoracic radiography and abdominal ultrasonography were unremarkable. Results of urinalysis indicated mild proteinuria with a normal specific gravity (1.036; reference range, > 1.030) and unremarkable urine sediment. The urine protein-to-creatinine concentration ratio was 1.4 (reference range, < 0.5).

Results of cytologic examination of a bone marrow fine-needle aspirate obtained from a costochondral junction indicated abundant *Leishmania* amastigotes in macrophages. Results of cytologic examination of a left popliteal lymph node fine-needle aspirate indicated a predominance of small lymphocytes with a high number of plasma cells and intermediate-sized lymphocytes, but no *Leishmania* amastigotes were detected. Results of a serum ELISA for detection of *Leishmania infantum* infection were positive (optical density, > 300% of the

From the Departament de Medicina i Cirurgia Animals (José-López, de la Fuente, Pumarola, Añor) and the Fundació Hospital Clínic Veterinari (José-López, de la Fuente, Añor), Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain. Dr. José-López's present address is Willows Referral Service, Highlands Rd, Solihull, West Midlands, B90 4NH, England.

This work was performed at the Departament de Medicina i Cirurgia Animals and the Fundació Hospital Clínic Veterinari, Facultat de Veterinària, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.

Address correspondence to Dr. Añor (sonia.anor@uab.es).

value for a positive control serum sample; cutoff value for a positive result, 24% of the optical density for a positive control serum sample).[1]

One day after admission, MRI of the cervical spinal cord was performed with a 0.2-T permanent open magnet.[a] Transverse, sagittal, and dorsal plane images were obtained with T2-weighted spin echo sequences (repetition time, 2,800 milliseconds; echo time, 80 milliseconds) and T1-weighted spin echo sequences (repetition time, 690 milliseconds; echo time, 26 milliseconds) before and after IV administration of gadopentate dimeglumine[b] (0.1 mmol/kg [0.045 mmol/lb]). In the T2-weighted sequence images, there was a poorly defined, markedly hyperintense intramedullary lesion in the right side of the spinal cord that extended over the caudal aspect of the C3 through C6 vertebrae (**Figure 1**). At this level, the spinal cord diameter was slightly enlarged, and the signal intensity of the subarachnoid fluid and epidural fat was attenuated. Evaluation of precontrast T1-weighted sequence images indicated a slightly hyperintense, round focal intramedullary mass that was in contact with the meninges on the right side. The lesion extended from the caudal half of the C5 vertebral body to the C5-6 intervertebral disk space; it was well defined and, in postcontrast T1-weighted sequence images, had a central contrast medium–enhanced core surrounded by an intermediate isointense zone and a peripheral contrast medium–enhanced rim. The MRI findings were considered consistent with an immune-mediated or infectious granuloma or intramedullary neoplasia.

Results of analysis of a CSF sample collected from the cerebellomedullary cistern indicated a total nucleated cell count in the reference range (2 cells/μL; reference range, < 5 cells/μL) and a mildly high protein concentration (29.8 mg/dL; reference range, < 25 mg/dL). Results of cytologic examination of the CSF sample indicated 30% of the cells were degenerated neutrophils (reference range, < 2% nondegenerated neutrophils).[2] The high percentage of degenerated neutrophils and the mildly high protein concentration were considered consistent with mild or early inflammation that was suspected to be associated with infection (eg, bacterial [including rickettsial], fungal, or protozoal). Results of real-time PCR assays performed in the CSF sample were negative for *Toxoplasma gondii*, *Neospora caninum*, and *Ehrlichia canis* but positive for *Leishmania donovani* complex (*L donovani* and *L infantum* [including synonymous *Leishmania chagasi*]).

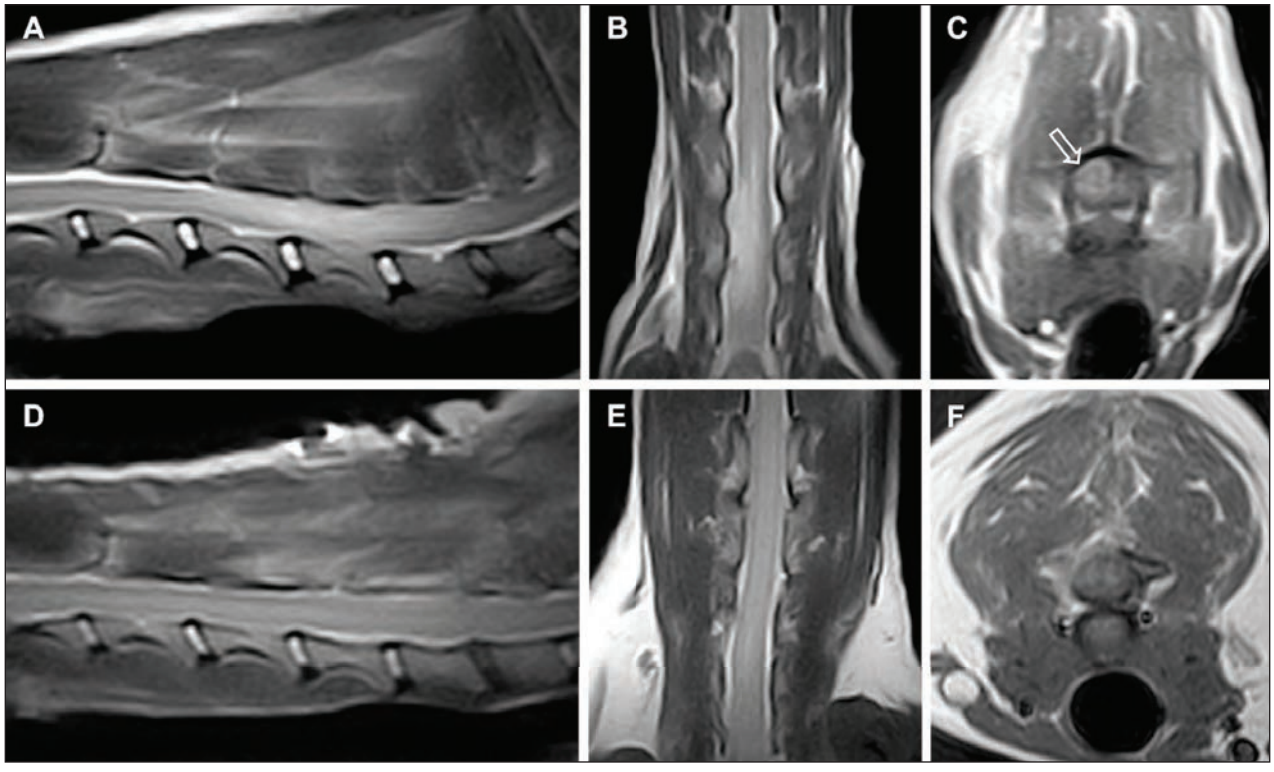

Figure 1—Magnetic resonance images of the cervical spinal cord of a 9-year-old male Miniature Poodle that was evaluated for signs of progressive severe ambulatory right hemiparesis, right forelimb lameness, and signs of cervical pain. Midsagittal (A) and dorsal (B) plane T2-weighted sequence MRI images indicate a hyperintense, right-sided intramedullary lesion that extends from the caudal aspect of the C3 through C6 vertebra. Notice there is a slight enlargement of the spinal cord and loss of the T2-weighted subarachnoid fluid and epidural fat signal intensities from the C4 through C6 vertebrae. A transverse postcontrast T1-weighted sequence image (C) at the level of the caudal aspect of the C5 vertebral body indicates a well-defined, right-sided intramedullary mass with a contrast medium–enhanced peripheral rim, an intermediate isointense zone, and a central contrast medium–enhanced core, resembling a target sign (arrow). Follow-up MRI was performed 2 months after diagnosis and treatment for systemic leishmaniasis (D, E, and F). Midsagittal (D) and dorsal (E) T2-weighted sequence images obtained at the same level as the images in panels A and B indicate substantial improvement of the lesion with mild, residual poorly defined intramedullary T2-weighted hyperintensity in the spinal cord from the caudal aspect of the C3 through C5 vertebrae. A transverse postcontrast T1-weighted sequence image obtained at the level of the caudal aspect of the C5 vertebral body (F) indicates resolution of the intramedullary contrast medium–enhanced mass and changes secondary to the right C5-C6 dorsal hemilaminectomy performed for collection of a biopsy sample.

Because the intramedullary mass appeared to be in contact with the spinal cord surface and the meninges, surgical biopsy was attempted by means of a right C5-C6 hemilaminectomy by use of a dorsal approach. Durectomy was performed, and an intramedullary mass was identified that was bulging from the spinal cord. The mass was solid, had gray discoloration, and appeared to be infiltrating the spinal cord parenchyma. A small amount of the abnormally discolored tissue and the overlying dura mater were resected and fixed in neutral-buffered 10% formalin for histologic examination.

After surgery, administration of enrofloxacin[c] (5 mg/kg [2.3 mg/lb], PO, q 12 h for 14 days) and prednisolone[d] (0.5 mg/kg [0.23 mg/lb], PO, q 24 h for 14 days) was started. Treatment for systemic leishmaniasis with allopurinol[e] (10 mg/kg [4.5 mg/lb], PO, q 12 h) and meglumine antimoniate[f] (100 mg/kg [45 mg/lb], SC, q 24 h) was also initiated.

Results of histologic examination of the surgically obtained, paraffin-embedded biopsy sample revealed a fusiform cell population (fibroblasts and fibrocytes) with some lymphocytes in an eosinophilic matrix covered by a thin layer of collagen fibers. No cellular pleomorphism, anisokaryosis, or neovascularization was detected. The dura mater was histologically normal. Histopathologic findings for the resected tissue were consistent with the periphery of a chronic inflammatory process, most likely a granuloma. However, a tumor could not be ruled out because of the small size of the tissue sample. Results of real-time quantitative PCR analysis performed on the paraffin-embedded tissue were positive for *L infantum*.[3]

The presumptive diagnosis determined for the dog was myelopathy caused by an intramedullary *L infantum* inflammatory lesion. One week after the diagnostic procedures were performed, the dog was discharged from the hospital with greatly improved motor function and postural reactions in the right limbs. Treatment with meglumine antimoniate[f] (100 mg/kg, SC, q 24 h) was stopped after 1 month, and treatment with allopurinol[e] (10 mg/kg, PO, q 12 h) was continued. At the time of reevaluation 2 months after determination of the diagnosis, the dog's body condition score was 4 of 9 and the neurologic condition had substantially improved. The dog's gait was almost normal, with very mild right forelimb paresis. Postural reaction deficits were marked in the right hind limb but mild in the right forelimb. Segmental spinal reflexes were normal in all 4 limbs, and mild to moderate signs of cervical pain were detected during right lateral flexion of the neck, although the dog was able to move its head freely without signs of discomfort.

Results of hematologic and serum biochemical analyses performed at this time indicated persistent, mild nonregenerative anemia (PCV, 32%; RBC count, 4.44×10^6 RBCs/µL) and mild polyclonal hypergammaglobulinemia (1.48 g/dL). The urine protein-to-creatinine concentration ratio had decreased to 0.5. Results of follow-up MRI indicated almost complete resolution of the cervical spinal cord lesion. Very mild, poorly defined intramedullary T2-weighted hyperintensity in the spinal cord over the caudal aspect of the C3 through C5 vertebrae persisted (Figure 1). However, the intramedullary mass lesion that had been observed in pre- and postcontrast T1-weighted sequence images was not detected. Results of CSF sample analysis were unremarkable, and results of a CSF PCR assay for detection of *Leishmania* spp were negative. Four months after determination of the diagnosis, the dog's neurologic condition remained stable.

Discussion

Leishmaniasis in dogs is a severe systemic disease caused by the protozoan parasite *L infantum*. It is transmitted by sandflies of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World. In many endemic countries and geographic areas, visceral leishmaniasis in dogs is an important cause of zoonotic disease.[4] Determination of a definitive diagnosis of leishmaniasis is typically made on the basis of cytologic or histologic identification of amastigotes (either inside or outside macrophages) in routinely stained smears from lymph node or splenic aspirates, skin touch impressions, or bone marrow samples. Additionally, the diagnosis can be confirmed by means of serologic testing, culture of the organism in appropriate medium, or detection of DNA of the parasite with molecular methods.[4]

Neurologic signs in animals with *Leishmania* spp infection are rare but have been detected in affected humans and dogs.[5–12,g] Although neurologic signs associated with visceral leishmaniasis may be obvious in some dogs, few data are available regarding the relationship between such signs and nervous system lesions.[13]

To the authors' knowledge, the present report is the first in which the clinical findings, clinicopathologic data, and MRI features of an intramedullary inflammatory mass presumptively caused by *L infantum* infection in a dog have been reported. The markedly positive PCR assay results for biopsy samples of this dog indicated it had *L infantum* in the CNS. Therefore, this is the first report of CNS leishmaniasis with MRI resolution of the lesion and successful clinical response to medical treatment in a dog. Regarding treatments for this dog, it is important to note that meglumine antimoniate[f] is not commercially available in the United States.

A vertebral canal extradural granuloma attributable to leishmaniasis causing spinal cord compression has been described in a dog of another report,[5] but spinal cord infiltration was not detected in MRI images of that dog. A lumbar extradural mass was surgically removed from that dog, and histopathologic analysis indicated epidural fat extensively infiltrated with inflammatory cells and *Leishmania* amastigotes in and outside macrophages. Complete resolution of clinical signs was detected in that dog during recheck examinations 8 and 12 months after initiation of treatment for systemic leishmaniasis. Information in another report[g] indicates cervical spinal cord ischemic infarctions were suspected in a dog associated with meningomyelitis that was caused by *L infantum* infection as determined on the basis of results of MRI and cytologic examination of bone marrow samples and a positive CSF PCR assay result for *Leismania* spp. Two weeks after initiation of treatment with allopurinol and meglumine antimoniate, that dog was euthanized because of lack of response. Postmortem histologic examination of the spinal cord of that dog indicated vasculitis and malacia

secondary to ischemia, but parasites were not found in nervous system tissue and results of a PCR assay for *Leishmania* spp performed for nervous system tissue samples were negative.

In 2 other studies,[13,14] *Leishmania* amastigotes were detected in the meninges and choroid plexuses of dogs naturally infected with *L infantum*. Although dogs in those studies[13,14] had typical features of granulomatous meningitis and choroiditis, well-defined granulomas or giant multinucleated cells were not detected. In a recent report[12] of a tetraplegic Labrador Retriever that died a short time after admission to a hospital, microscopic examination of nervous system tissue samples indicated *Leishmania* amastigotes in the spinal nerves, spinal cord, brain parenchyma, and choroid plexuses, which induced radiculoneuritis, myelitis, and meningoencephalitis in the dog. Results of other studies[15–17] suggest that failure of the blood-CSF barrier may cause the transfer of *Leishmania* antibodies and antigens from blood to the CSF of dogs naturally infected with *L infantum* or *L chagasi*. That same mechanism could cause transfer of *Leishmania* parasites to the subarachnoid space and, subsequently, the CNS of a dog.

For the dog of the present report, antemortem diagnosis of CNS leishmaniasis was determined on the basis of detection of systemic infection, MRI findings, and positive results of PCR assays for *Leishmania* DNA in CSF and intramedullary tissue biopsy samples. The MRI findings for the dog were similar to those for cats with segmental protozoal myelitis and humans with toxoplasmosis (T2-weighted sequence hyperintense and T1-weighted sequence contrast medium–enhanced, focal intramedullary mass), although in such cats and humans, contrast medium enhancement is typically stronger.[18,19] In addition, the contrast medium–enhanced MRI pattern for the dog of this report indicated 3 alternating zones (a central contrast medium–enhanced core, an intermediate isointense zone, and a peripheral contrast medium–enhanced ring), which was similar to an MRI target sign detected in humans with cerebral toxoplasmosis.[20] Therefore, on the basis of the MRI findings and positive PCR assay results for a CSF sample of the dog, visceral leishmaniasis–induced myelitis was considered a primary differential diagnosis. However, focal intramedullary space-occupying lesions in dogs are commonly neoplastic (either primary spinal parenchymal tumors [eg, astrocytoma, oligodendroglioma, or ependymoma] or intramedullary metastases [hemangiosarcoma or lymphosarcoma]).

Because the dog of the present report lived in a geographic area endemic for visceral leishmaniasis and because the positive PCR assay results for detection of *Leishmania* DNA in a CSF sample could have been attributable to severe systemic leishmaniasis and failure of the blood-CSF barrier, other diseases were ruled out. Results of recent studies[21,22] of dogs with histologically confirmed brain lesions indicate that MRI is sensitive and specific for classification of brain lesions attributable to such processes as inflammatory or neoplastic, but results also suggested limitations of MRI and the need to obtain tissue biopsy samples for determination of a definitive diagnosis. Surgical exploration of intramedullary spinal cord lesions in dogs can be associated with a good outcome.[23,24] Because the mass in the dog of this report appeared to be in contact with the spinal cord surface in MRI images, surgical biopsy was attempted to determine a definitive diagnosis.

The surgically obtained biopsy sample was insufficient for determination of a histopathologic diagnosis. A quantitative PCR assay to detect *Leishmania* DNA in paraffin-embedded skin biopsy samples of dogs is as sensitive as immunohistochemical analysis for determination of a diagnosis.[25] Therefore, real-time quantitative PCR assay was performed with paraffin-embedded tissue samples of the dog, and the markedly positive results for *L infantum* DNA suggested a presumptive diagnosis of an intramedullary inflammatory mass attributable to leishmaniasis. However, determination of a diagnosis of visceral leishmaniasis in a dog is challenging in endemic areas and, despite the high sensitivity of PCR assays for determination of a diagnosis of leishmaniasis in such animals, false-positive results are a limitation of that technique.[26] Although the gold-standard methods for determination of a definitive diagnosis of visceral leishmaniasis in dogs are cytologic or histologic observation of amastigotes (either inside or outside macrophages), the positive results of real-time quantitative PCR assay for an intramedullary spinal cord mass sample of the dog of the present report suggested it had a high parasite burden and the intramedullary mass was an inflammatory lesion caused by *L infantum*.[3]

The long-term clinical response to treatment for systemic leishmaniasis and the resolution of the intramedullary lesion (as indicated by means of evaluation of follow-up MRI images) were supportive of a diagnosis of an inflammatory mass attributable to leishmaniasis in the dog of this report. To our knowledge, a spinal cord intramedullary inflammatory mass presumptively attributable to leishmaniasis in a dog has not been previously reported, and this diagnosis should be considered a differential for dogs with intramedullary spinal cord masses in geographic areas endemic for leishmaniasis.

a. Vet-MR, Esaote, Genova, Italy.
b. Magnevist, Bayer Schering Pharma AG, Berlin, Germany.
c. Baytril, Bayer Healthcare, Kiel, Germany.
d. Dacortin, Merck, Mollet del Vallés, Spain.
e. Zyloric, GlaxoWellcome, Bad Oldesloe, Germany.
f. Glucantime, Merial, Lyon, France.
g. Sanchez D, Navalon I, Font A, et al. Central nervous system involvement induced by *Leishmania infantum* in a dog (abstr), in *Proceedings*. 20th Annu Symp Eur Soc Vet Neurol 2007;(A15)9.

References

1. Riera C, Valladares JE, Gallego M, et al. Serological and parasitological follow-up in dogs experimentally infected with *Leishmania infantum* and treated with meglumine antimoniate. *Vet Parasitol* 1999;84:33–47.
2. Wood A, Garosi L, Platt S. Cerebrospinal fluid analysis. In: Platt S, Garosi L, eds. *Small animal neurological emergencies*. London: Manson Publishing, 2012;121–136.
3. Francino O, Altet L, Sánchez-Robert E, et al. Advantatges of real-time PCR assay for diagnosis and monitoring of canine leishmaniosis. *Vet Parasitol* 2006;137:214–221.
4. Baneth G. Leishmaniasis. In: Greene CE, ed. *Infectious diseases in the dog and cat*. 3rd ed. St Louis: Saunders Elsevier, 2006;685–695.
5. Cauduro A, Favole P, Lorenzo V, et al. Paraparesis caused by vertebral canal leishmaniotic granuloma in a dog. *J Vet Intern Med* 2011;25:398–399.

6. Jackson JE, Iwu MM, Okunji CO, et al. Chronic canine visceral leishmaniasis (VL): prepatent VL neurologic manifestations. *Am J Trop Med Hyg* 2002;67:222–223.
7. Font A, Mascort J, Altimira J, et al. Acute paraplegia associated with vasculitis in a dog with leishmaniasis. *J Small Anim Pract* 2004;45:199–201.
8. José-López R, de la Fuente C, Añor S. Presumed brain infarctions in two dogs with systemic leishmaniasis. *J Small Anim Pract* 2012;53:554–557.
9. Petersen CA, Greenlee MHW. Neurologic manifestations of *Leishmania* spp infection. *J Neuroparasitol* 2011;2:N110401.
10. Walker M, Kublin JG, Zunt JR. Parasitic central nervous infections in immunocompromised hosts: malaria, microsporidiosis, leishmaniasis, and African trypanosomiasis. *Clin Infect Dis* 2006;42:115–125.
11. Prasad LS, Sen S. Migration of *Leishmania donovani* amastigotes in cerebrospinal fluid. *Lancet* 1995;346:183–184.
12. Márquez M, Pedregosa JR, López J, et al. *Leishmania* amastigotes in the central nervous system of a naturally infected dog. *J Vet Diagn Invest* 2013;25:142–146.
13. Viñuelas J, García-Alonso M, Ferrando L, et al. Meningeal leishmaniosis induced by *Leishmania infantum* in naturally infected dogs. *Vet Parasitol* 2001;101:23–27.
14. Nieto CG, Viñuelas J, Blanco A, et al. Detection of *Leishmania infantum* amastigotes in canine choroid plexus. *Vet Rec* 1996;139:346–347.
15. García-Alonso M, Nieto CG, Blanco A, et al. Presence of antibodies in the aqueous humour and cerebrospinal fluid during *Leishmania* infections in dogs. Pathological features at the central nervous system. *Parasite Immunol* 1996;18:539–546.
16. Lima VMF, Gonçalves ME, Ikeda FA, et al. Anti-*Leishmania* antibodies in cerebrospinal fluid from dogs with visceral leishmaniasis. *Braz J Med Biol Res* 2003;36:485–489.
17. Melo GD, Marcondes M, Vasconcelos RO, et al. Leucocyte entry into the CNS of *Leishmania chagasi* naturally infected dogs. *Vet Parasitol* 2009;162:248–256.
18. Alves L, Gorgas D, Vandevelde M, et al. Segmental meningomyelitis in 2 cats caused by *Toxoplasma gondii*. *J Vet Intern Med* 2011;25:148–152.
19. Taber KH, Hayman LA, Shandera WX, et al. Spinal disease in neurologically symptomatic HIV-positive patients. *Neuroradiology* 1999;41:360–368.
20. Masamed R, Meleis A, Lee EW, et al. Cerebral toxoplasmosis: case review and description of a new imaging sign. *Clin Radiol* 2009;64:560–563.
21. Wolff CA, Holmes SP, Young BD, et al. Magnetic resonance imaging for the differentiation of neoplastic, inflammatory, and cerebrovascular brain disease in dogs. *J Vet Intern Med* 2012;26:589–597.
22. Ródenas S, Pumarola M, Gaitero L, et al. Magnetic resonance imaging findings in 40 dogs with histologically confirmed intracranial tumours. *Vet J* 2011;187:85–91.
23. Jeffery ND, Phillips SM. Surgical treatment of intramedullary spinal cord neoplasia in two dogs. *J Small Anim Pract* 1995;36:553–557.
24. Leskovar A, Coates JR, Russell KE, et al. Surgical removal of an intramedullary spinal cord foreign body granuloma in a dog. *J Am Anim Hosp Assoc* 2003;39:306–310.
25. Roura X, Fondevila D, Sánchez A, et al. Detection of *Leishmania* infection in paraffin-embedded skin biopsies of dogs using polymerase chain reaction. *J Vet Diagn Invest* 1999;11:385–387.
26. Moreira MA, Luvizotto MC, García JF, et al. Comparison of parasitological, immunological and molecular methods for the diagnosis of leishmaniasis in dogs with different clinical signs. *Vet Parasitol* 2007;145:245–252.