J Am Anim Hosp Assoc 2016; 52:119–123. DOI 10.5326/JAAHA-MS-6240

CASE REPORTS

Seizures as a Consequence of Hyperviscosity Syndrome in Two Dogs Naturally Infected with Leishmania infantum

Daniela Proverbio, DVM, PhD, Eva Spada, DVM, PhD, Roberta Perego, DVM, PhD, Giada Bagnagatti de Giorgi, DVM, PhD

From Department of Health, Animal Science and Food Safety, University of Milan, Milan, Italy.

Correspondence: daniela.proverbio@unimi.it (D.P.)

CanL, canine leishmaniasis; cps, counts per s; HVS, hyperviscosity syndrome; IFAT, indirect immunofluorescence assay; PCR, polymerase chain reaction

ABSTRACT

Serum hyperviscosity syndrome (HVS) was documented in two dogs with canine leishmaniasis (CanL) and seizures as the major clinical complaint. In both cases, laboratory abnormalities included mild non-regenerative anemia, thrombocytopenia, hypoalbuminemia, hyperproteinemia with monoclonal gammopathy, and marked serum hyperviscosity. CanL was diagnosed using cytology in one case and indirect immunofluorescence assay and conventional polymerase chain reaction in the second. Specific therapy with meglumine antimoniate and allopurinol[c] led to short- term remission in both dogs and normalization of serum viscosity. Although dogs rarely develop HVS, it should be suspected if hyperproteinemia and monoclonal gammopathy are present. Since CanL manifests with a variety of clinical presentations, including seizures resulting from HVS-induced central nervous system hypoxia, it should also be considered as a differential diagnosis in animals with seizures as a primary presenting sign.

Introduction

Canine leishmaniasis (CanL) is caused by the protozoan Leishmania infantum and is prevalent in the countries around the Mediterranean basin. The development of disease in infected dogs depends on the host–immune response, which is, in turn, influenced by the genetic makeup of the host. Not only do typical forms of systemic chronic CanL present with nonspecific symptoms (weight loss, lethargy, enlarged lymph nodes, splenomegaly) and dermatological, renal, or ocular signs but many atypical forms of CanL also exist.[1] Neurological signs are an uncommon primary presentation of CanL.[2–5]

Hyperviscosity syndrome (HVS) manifests with a variety of clinical signs due to increased blood viscosity. Whole-blood viscosity is affected by a number of factors, of which plasma protein concentrations are a major component. Clinical manifestations are a result of decreased blood flow to the tissues and sludging of blood, often associated with ocular (e.g., tortuosity of vessels, retinal detachment), neurological (vertigo, paraesthesia, ataxia, seizures), cardiac (congestive heart failure, lower extremity edema), or hemostatic (spontaneous gum bleeding, epistaxis) abnormalities.[6]

Therapeutic approaches are aimed at treating the primary cause.[7] This syndrome is rarely reported in veterinary medicine and, to the authors' knowledge, there are no reports of CanL where the primary manifestations are neurological signs as a consequence of HVS. This report describes two cases with an uncommon presentation of CanL in which seizures, secondary to HVS, were the major clinical complaint, and that were successfully treated with antimonial drugs.

Case Report

Case 1 was a 6 yr old male boxer weighing 30 kg who was admitted to the Department of Health, Animal Science and Food Safety, University of Milan, Italy, with a 2 mo history of anorexia, weight loss, polyuria and polydipsia, and recurrent seizures after exercise. The seizure episodes had intensified to three episodes in the preceding wk and were characterized by loss of consciousness, coordinated paddling movements of all limbs, and drooling. Physical examination revealed a moderate, generalized lymph node enlargement. Complete neurological examination was normal and cardiological examination, with electrocardiogram and transthoracic echocardiography, showed no abnormalities. Ophthalmic examination revealed marked venous distension and tortuous retinal vessels. A complete blood count showed non-regenerative anemia (Reticulocyte Production Index of 0.7), leukopenia, and severe thrombocytopenia. Biochemical abnormalities included hyperproteinemia, hypoalbuminemia, hypergammaglobulinemia, and decreased albumin to globulin ratio (Table 1). Serum protein electrophoresis revealed a narrow spike in the c-globulin region attributable to a monoclonal gammopathy (Figure 1). Serum viscosity relative to water, calculated using an glass capillary viscosimeter, was markedly elevated (6.4 counts per s [cps], reference range 1.4–1.8 cps).[8] Due to the serum hyperviscosity, two coagulation tests (prothrombin time and partial thromboplastin time) were performed and were both within reference intervals. The main differential diagnoses included multiple myeloma, CanL, and canine ehrlichiosis.[9,10] Indirect immunofluorescence assays (IFATs) for Leishmania infantum and Ehrlichia canis were negative. Conventional polymerase chain reaction (PCR) analyses of blood and fine-needle aspirates from popliteal lymph nodes were also negative for L. infantum and E. canis. Bence-Jones proteinuria, assessed by SDS-Page[a] urinary electrophoresis test, was negative. No osteolytic lesions were identified on radiographic evaluation of the long bones. Hepatomegaly and splenomegaly were identified on ultrasonographic examination, during which fine-needle aspirates of liver and spleen were collected. Cytological examination of the latter showed many macrophages containing numerous Leishmania protozoa. The clinical and laboratory findings were diagnostic for HVS (due to monoclonal gammopathy) associated with L. infantum infection. Specific therapy with meglumine antimoniate[b] at 50 mg/kg SC q 12 hr and allopurinol[c] at 15 mg/kg q 12 hr for 8 wk was instituted.[1] Therapeutic progress was monitored twice a wk by physical examination, complete blood count, and serum protein electrophoresis. Anorexia, polyuria, and polydipsia rapidly decreased after treatment. At follow-up examination, 2 mo after antimonial therapy initiation, clinical signs had dramatically improved and seizure episodes had ceased. Haematological and biochemical analyses were within reference intervals. Total serum protein was 7.8 g/dL with normal albumin and globulin fractions. The serum viscosity had also returned to within normal limits (1.5 cps). Over the following 4 yr, no further seizure episodes were reported. The dog was treated with four more cycles of meglumine antimoniate[b] at 50 mg/kg SC q 24 hr and allopurinol[c] at 15 mg/kg q 12 hr to control recurrent hypergammaglobulinemia and uveitis associated with leishmaniasis. The dog was euthanized 4 yr after initial presentation due to chronic renal failure.

FIGURE 1 *Serum protein electrophoretogram on agarose gel was consistent with monoclonal gammopathy in a boxer with collapse and hyperviscosity syndrome due to* Leishmania infantum *infection.*

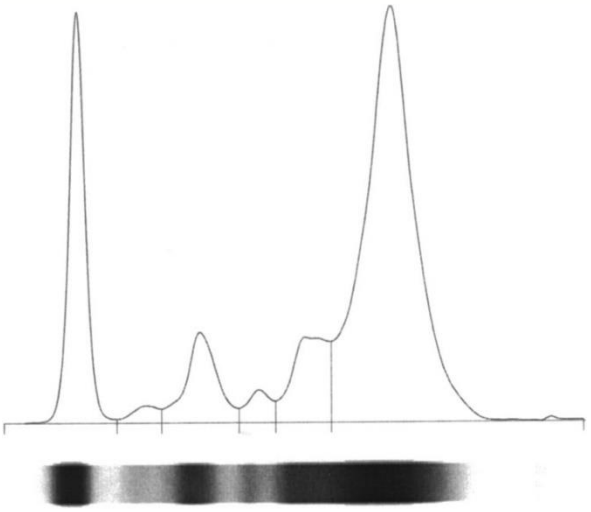

TABLE 1

Haematological and Biochemical Alterations in a Boxer (Case 1) and a Mixed-Breed Dog (Case 2) with Hyperviscosity Syndrome Due to *Leishmania infantum* Infection

Case 1	Case 1	Case 2	Reference Range
RBC	4.87 × 10^6/μL	4.91 × 10^6/μL	5-8 × 10^6/μL
Hb	11.2 g/dL	10.9 g/dL	11.5-18.0 g/dL
PCV	31.5%	30.4%	35-55%
MCV	74.0 fL	75.0 fL	62.0-73.0 fL
MCHC	32.0 g/dL	31.0 g/dL	33.0-36.0 g/dL
WBC	5.93 × 10^3/mL	6.45 × 10^3/mL	6-12 × 10^3/mL
PLT	72 × 10^3/L	105 × 10^3/L	200-500 × 10^3/L
Total protein	11.9 g/dL	10.6 g/dL	6-8 g/dL
Albumin	2.30 g/dL	2.20 g/dL	3.0-4.5 g/dL
Alpha1 globulins	0.15 g/dL	0.17 g/dL	0.2-0.5 g/dL
Alpha2 globulins	0.95 g/dL	1.43 g/dL	0.3-1.1 g/dL
Beta1 globulins	0.26 g/dL	0.32 g/dL	0.7-1.3 g/dL
Beta2 globulins	1.07 g/dL	1.26 g/dL	0.6-1.4 g/dL
Gamma globulins	7.16 g/dL	5.22 g/dL	0.9-2.3 g/dL
Albumin-to-globulin ratio	0.24	0.26	0.8-1.7
Serum viscosity	6.4	5.8	1.4-1.8 cps

cps, counts per s; Hb, haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; PLT, platelets; RBC, red blood cells.

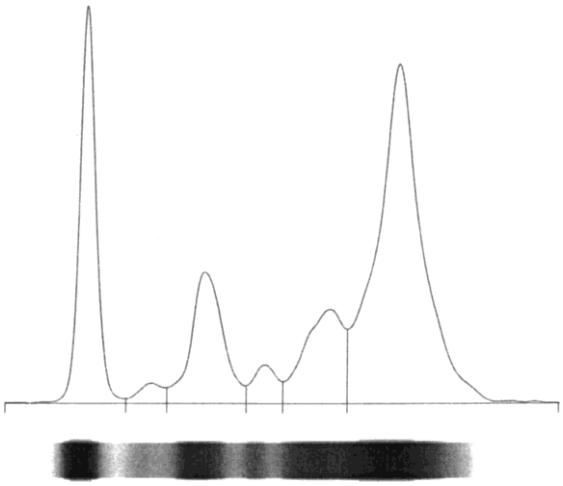

FIGURE 2 *Results of serum protein agarose gel electrophoresis were consistent with monoclonal gammopathy in a mixed-breed dog with collapse and hyperviscosity syndrome due to* Leishmania infantum *infection.*

Case 2 was a 10 yr old male mixed-breed dog weighing 15 kg who was referred to the clinic for evaluation following two seizures in the previous 24 hr. The two episodes appeared to have been associated with excitement. The dog was unresponsive for no longer than 2 min on each occasion with extensor spasm of the forelimbs. There was no prior history of such an episode, but over the previous 2 wk, the dog had appeared lethargic and anorexic, with polyuria and polydipsia. Physical examination revealed pale mucous membranes. Dermatological examination revealed gener- alized hypotrichosis and dry coat. Neurological examination was normal. A complete cardiologic assessment, including electrocardiogram and echocardiogram, did not find any cardiac abnormalities. Hematological analysis, including blood smear evaluation, revealed mild non-regenerative normocytic normochromic anemia and marked thrombocytopenia. Hyperproteinemia, hypoalbuminemia, hypergammaglobulinemia, and decreased albumin to glob- ulin ratio were evident on biochemical analysis. The electrophoretic profile showed sharp peaks, highly suggestive of a monoclonal gammopathy (Figure 2). Serum viscosity relative to water, measured using an glass capillary viscosimeter, was markedly increased (5.8 cps, reference range 1.4–1.8 cps).[8] Coagulation tests (prothrombin time and partial thromboplastin time) were both within reference intervals. The main differential diagnoses included multiple myeloma, CanL, and canine E. canis infection.[9,10] SDS- Page was negative for Bence-Jones protein[a]. No osteolytic lesions were seen in the extremities, pelvis, or spine on radiographic examination. The IFAT assay was positive for L. infantum (1:320, cut-off .1:80), but it was negative for E. canis. Conventional PCR of the blood was also positive for L. infantum, but it was negative for E. canis. The clinical and laboratory findings were diagnostic for HVS due to monoclonal hypergammaglobulinemia associated with L. infantum infection. The dog was treated with meglumine antimoniate[b] at 100 mg/kg SC q 24 hr and allopurinol[c] at 15 mg/kg q 12 hr for 6 wk.[1] An assessment every wk revealed improvement in both the clinical sings and the biochemical abnormalities. This included a cessation of the seizures and reduction of the lethargy, anorexia, polyuria, and polydipsia, as well as decrease in the hyperproteinemia and hypergammaglobulinemia. At the initial follow-up, 40 days after cessation of the first round of meglumine[b] and allopurinol[c] therapy, total protein and serum viscosity had returned to normal (7.8 g/dl and 1.7 cps, respectively). Six mo after the first presentation, and approximately every 8 mo,

the dog received three further 4 wk cycles of meglumine antimoniate[b] at 50 mg/kg SC q 12 hr and allopurinol[c] at 15 mg/kg q 12 hr to control relapses of hypergammaglobulinemia and dermatologic signs. No further seizure episodes were reported and the dog was euthanized approximately 3 yr following the initial presentation due to anemia and poor condition.

Discussion

This report describes two cases of monoclonal gammopathy with HVS caused by Leishmania infantum infection. The diagnosis of CanL associated with HVS in the two clinical cases was based on the clinico-pathological findings, the presence of monoclonal hypergammaglobulinemia, a positive IFAT test, and identification of parasites by PCR or cytological examination and on increased serum viscosity. In one of the two clinical cases presented in this report, the IFAT for the detection of antibodies against Leishmania was negative. In this case, the diagnosis of CanL was performed on the detection of amastigotes in cytological smears of aspirates from the spleen. IFAT is considered the ''gold standard'' of serologic diagnosis, but it has been demonstrated that a number of dogs remains seronegative for variable periods after being infected with Leishmania.[1,11]

IFAT test is a quantitative serological test that detects specific serum antibodies (IgG). Leishmania antibodies include various classes among which the main ones are IgG1 and IgG2, but, in most cases, IgM, IgE, and IgA are also produced at a lower frequency and concentration. In the course of CanL we also have the formation of coincidental antibodies as autoantibodies or cryoglobulines that can contribute to hypergammaglobulinemia.[12,13,14] The dog of case 1 may have developed an antibody response with antibody classes that do not have cross reacted with the IFAT test.

Multiple myeloma was ruled out by exclusion of osteolytic bone lesions and absence of Bence-Jones proteinuria. Concurrent E. canis infection was ruled out by serological examination.

Serum HVS consists of a number of clinical signs resulting from an increase in blood viscosity. As viscosity increases, blood flow through the microvasculature is impaired, leading to tissue hypoxia. Blood viscosity is affected by a number of factors, of which plasma proteins are a major component. The effect of a protein on plasma viscosity depends on its molecular weight and structure. Most commonly, HVS results from monoclonal increase in the largest globulin, IgM, which can also bind red blood cells and lead to roleaux formation. However, HVS can also be secondary to increased levels of IgA because of IgA dimer formation; otherwise, it can also be due to a very high concentration of IgG, which tend to form aggregates or, more rarely, can be associated a polyclonal gammopathy.[6,7] In the cases reported here, a monoclonal spike in c-globulin accounted for approximately 70% of total serum protein. Due to technical reasons, we did not perform immuno-electrophoresis or high-resolution electrophoresis, which are both sensitive methods for identification of the type of antibody causing the monoclonal gammopathy, so we are unable to comment on which specific immunoglobulin was contributing to the HVS.[15] Additionally, similar results could be created by a polyclonal gammopathy with restricted migration during electrophoresis or a shift in the gamma peak due to the faster migration of the IgA. In dogs, multiple myeloma is the most common cause of monoclonal gammopathy, but other possible causes include macroglobulinemia, lymphoproliferative disease, or infectious diseases, such as L. infantum and E. canis.[6,15] CanL is a non-neoplastic disease that frequently leads to dysproteinemia. In dogs infected with Leishmania, increases in levels of IgG1, IgG2, IgM, IgA, and IgE, as well as both IgG- and IgM-type paraproteins, have all been reported.[16,17] A retrospective study of 18 cases of monoclonal gammopathies in dogs confirmed that most were associated with lymphoproliferative tumours. However, non-myelomatous monoclonal gammopathies have been identified in cases of CanL in this and other studies.[3,10,18] A presumptive diagnosis of canine HVS due to serum dysproteinemia

was associated with IgA myeloma, chronic lymphocytic leukemia, Ehrlichia canis infection, and Bartonella henselae infection.[9,10,19,20] However, serum viscosity was only measured in two reports: the first reporting canine Waldestro¨m macroglobulinemia and the other multiple myeloma.[9,17] In one study of epistaxis and CanL, hyperviscosity was documented and positively correlated with hypergammaglobulinemia in a significantly higher proportion of dogs with epistaxis compared to dogs without epistaxis.[21] Two cases of CanL were reported in which blood hyperviscosity was associated with hyperproteinemia, causing ataxia and hind-limb edema in one case and facial edema in the other.[18]

In Leishmania-infected dogs, central nervous system disease may be caused by an inflammatory reaction at the blood–brain barrier and a failure of the blood–cerebrospinal fluid filtration with anti-Leishmania antibodies being found in cerebrospinal fluid.[22,23] There are reports in the literature of brain lesions in dogs with leishmaniasis caused by the deposition of both antigen and immunoglobulins resulting in perivascular lymphoplasmacytic infiltration, meningitis, gliosis, and choroiditis.[22] Recently, Ikeda et al. reported histopathological alterations and the presence of the amastigote form of L. chagasi in nervous tissue of dogs with visceral leismaniasis, with and without signs of generalised central nervous system involvement, with no correlation between nervous tissue lesions and clinical signs.[24]

Despite these studies, there are very few reports of neurolog- ical signs as the primary presenting feature of CanL. Lethargy, paresis, and cervical and spinal rigidity were described in two dogs with granulomatous meningitis associated with CanL and another case report describes one dog with acute paraplegia associated with vasculitis due to L. infantum infection.[2,3] Recently, paraparesis and ataxia were described in a dog with myelopathy induced by an extradural inflammatory granuloma caused by Leishmania spp.[4] A second recent study found tetraplegia and bilateral Horner's syndrome in a Leishmania-infected dog.[5] Leishmania infection was confirmed with anti-Leishmania antibodies, Leishmania antigen in the cerebrospinal fluid, and a mass adhering to the cervical spinal nerves 7 and 8 containing a Leishmania amastigote.[5]

While Leishmania parasites have the potential to infect the central nervous system and cause either primary lesion-associated or secondary inflammatory-associated central nervous system disease, it is unlikely this was the case in either of the two dogs in this report. This is supported by normal neurologic examina- tions in both of the cases. While histopathology would help to confirm this position, necropsies were not performed in either of the currently reported cases. Nonetheless, a recent histopathology report by Ikeda et al. did not find any correlation between clinical signs and nervous tissue lesions in Leishmania infections.[24]

Seizure episodes may also be caused by metabolic or cardiac disorders, but in the dogs described in the present report, cardiac examinations were normal and blood tests showed no metabolic or laboratory abnormalities other than anemia, hyperproteinemia, and hypergammaglobulinemia. The anemia was too mild to have caused the cerebral hypoxia responsible for the seizures. Serum hyperviscosity was, however, demonstrated in both dogs described in this report. Serum hyperviscosity is a recognized cause of seizures in dogs and serum hyperviscosity could also explain the other clinical signs, including lethargy and exercise intolerance, in the dogs described in this report.[6]

Hyperproteinemia (total protein usually above 10 g/dL) and monoclonal gammopathy are compatible with blood hyperviscosity, which can result in tissue hypoperfusion and hypoxia.[6] In both cases reported here, viscosity was greatly increased, almost five times the normal value.

Seizures and lethargy resolved completely, with a marked reduction in serum protein and globulin levels following antimonial therapy, a specific treatment registered for CanL.[1]

Conclusion

This report is of value as it both raises awareness of the potential for seizures as a primary presenting complaint of CanL and suggests a possible pathogenesis for CanL-induced seizures. Serum viscosity measurements could be used by the clinician as an accurate assay to prevent the onset of signs of HVS, such as retinal hemorrhage, seizures, or bleeding, when hypergammaglobulinemia is present.

FOOTNOTES

1. [a] Sodium Dodecyl Sulphate; PolyAcrylamide Gel Electrophoresis, Bio Rad Laboratoires s.r.l. Segrate (MI), Italy
2. [b] Glucantime; Merial, Milan, Italy
3. [c] Zyloric; Glaxosmithkline s.p.a., Verona , Italy

REFERENCES

1. Solano-Gallego L, Koutinas A, Miro` G, et al. Directions for the diagnosis, clinical staging, treatment and prevention of canine leishmaniasis. Vet Parasitol 2009;165:1–18.
2. Vin˜uelas J, Garcia-Alonso M, Ferrando L, et al. Meningeal leishmaniasis induced by Leishmania infantum in naturally infected dogs. Vet Parasitol 2001;101:23–7.
3. Font A, Closa, JM, Mascort J. Monoclonal gammopathy in a dog with visceral leishmaniasis. J Vet Intern Med 1994;8:233–35.
4. Caudouro A, Favole P, Lorenzo V, et al. Paraparesis Caused by Vertebral Canal Leishmaniotic Granuloma in a Dog. J Vet Intern Med 2011;25:398–99.
5. Marquez M, Pedregosa JR, Lo` pez J, et al. Leishmania amastigotes in the central nervous system of naturally infected dog. J Vet Diagn Invest 2013;25:142–6.

6. Forrester SD, Relford RL. Serum hyperviscosity syndrome: its diagnosis and treatment. Vet Med 1992;48–61.

7. Mehta J, Singhal S. Hyperviscosity syndrome in plasma cell dyscrasias. Semin Thromb Hemost 2003;29:467–71.

8. Johnson MC. Immunologic and Plasma Protein Disorders. In: Willard M, Tvedten H, eds. Small Animal Clinical Diagnosis by Laboratory Methods. 5th ed. St Louis: Elsevier Saunders; 2012:278–93.

9. Antognoni MT, Birettoni F, Miglio A, et al. Monoclonal gammopathy associated with multiple myeloma and visceral leishmaniasis in the dog: A comparison of two cases. Vet Res Comm 2010;34(suppl 1):97–101.

10. Giraudel JM, Pages JP, Guelfi JF. Monoclonal gammopathies in the dog: A retrospective study of 18 cases (1986-1999) and literature review. J Am Anim Hosp Assoc 2002;38:135–47.

11. Ferrer L, Aisa MJ, Roura X, et al. Serological diagnosis and treatment of canine leishmaniosis. Vet Rec 1995;20:514–516.

12. Mettler M, Grimm F, Capelli G, et al. Evalutation of enzyme-linked immunosorbent assay, an immunoflorescent–antibody test, and two rapid tests for serological diagnosis of symptomatic and asymptomatic Leishmania infections in dogs. J Clin Microbiol 2005;43:5515–5519

13. Day M. Immunoglobulin G subclass distribution in canine leishman- iosis: A review and analysis of pitfalls in interpretation. Vet Parasitol 2007;147:2–8.

14. Saridomichelakis MN. Advances in the pathogenesis of canine leishmaniosis: epidemiologic and diagnostic implications. Vet Derm 2009;471–489.

15. Geigy C, Riond B, Bley CR, et al. Multiple myeloma in a dog with multiple concurrent infectious diseases and persistent polyclonal gammopathy. Vet Clin Pathol 2013; 42:47–54.

16. Reis AB, Teixeira-Carvalho A, Vale AM, et al. Isotype patterns of immunoglobulins: Hallmarks for clinical status and tissue parasite density in Brazilian dogs naturally infected by Leishmania (Leishmania) chagasi. Vet Immunol Immuno-pathol 2006;112:102–16.

17. Gentilini F, Calzolari C, Buonacucina A, et al. Different biological behaviour of Waldestro¨m macroglobulinemia in two dogs. Vet Comp Oncol 2005; 3:87–97.

18. Blavier A, Keroack S, Denerolle P, et al. Atypical forms of canine leishmaniasis. Vet J 2001;162:108–20.

19. Leifer CE, Matus RE. Chronic lymphocytic leukemia in the dog: 22 cases (1974-1984). J Am Anim Hosp Assoc1986;189:214–17.

20. Tabar MD, Maggi RG, Altet L, et al. Gammopathy in a Spanish dog infected with Bartonella henselae. J Small Anim Pract 2011;52:209–12.

21. Petanides TA, Koutinas AF, Mylonakis ME, et al. Factors associated with the occurrence of epistaxis in natural canine leishmaniasis (Leishmania infantum). J Vet Intern Med 2008;22:866–72.

22. Garcia-Alonso M, Nieto CG, Blanco A, et al. Presence of antibodies in the aqueous humor and cerebrospinal fluid during Leishmania infection in dogs: Pathological features at the central nervous system. Parasite Immunol 1996;18:539–46.

23. Lima VMF, Concalves ME, Ikeda FA, et al. Anti-leishmania antibodies in cerebrospinal fluid from dogs with visceral leishmaniasis. Braz J Med Biol Res 2003;36:485–89.

24. Ikeda FA, Laurenti MD, Cobertt CE, et al. Histological and immunohistochemical study of the central nervous system of dogs naturally infected by leishmania (Leishmania) chagasi. Braz J Vet Res Anim Sci 2007;44:5–11.