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Case Report

Canine leishmaniasis associated with pericardial effusion in a 4-year-old dog

P. Sebastián-Marcos, DVM, MSc[a,*], G. Santarelli, DVM, MSc[a], S. Gómez, DVM, PhD[b], M. J. Fernández-del Palacio, DVM, PhD[a,c]

[a] *Division of Veterinary Cardiology-Pulmonology, Veterinary Teaching Hospital, Veterinary Faculty, University of Murcia, Campus Espinardo, 30100, Murcia, Spain*
[b] *Department of Compared Anatomy and Pathological Anatomy, Veterinary Faculty, University of Murcia, Campus Espinardo, 30100, Murcia, Spain*
[c] *Department of Animal Medicine and Surgery, Veterinary Faculty, University of Murcia, Campus Espinardo, 30100, Murcia, Spain*

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Abstract A 4-year-old crossbreed dog presented with a two-day history of lethargy and abdominal effusion. Physical examination and echocardiography revealed pericardial effusion with cardiac tamponade. Pericardiocentesis was performed. Intracytoplasmic *Leishmania* amastigotes were found on cytological examination of the pericardial fluid. The animal was treated with N-methylglucamine antimoniate and allopurinol. After an initial favorable response, cardiac tamponade reoccurred one month later. The dog died during a pericardiectomy four months after the initial diagnosis. Histology confirmed the presence of chronic pericarditis. The presence of *Leishmania* amastigotes on cytological examination of pericardial effusion suggests a possible association between canine leishmaniasis and chronic pericarditis. This finding also supports the importance of cytological examination of pericardial fluid in areas endemic for canine leishmaniasis.

A 4-year-old intact female crossbreed dog (27.5 kg) was referred to the Veterinary Teaching Hospital of the University of Murcia for evaluation of a one-week history of progressive lethargy, apathy, and abdominal distension due to peritoneal effusion. Decreased appetite and weight

* Corresponding author.
E-mail address: patricia.sebastian@um.es (P. Sebastián-Marcos).

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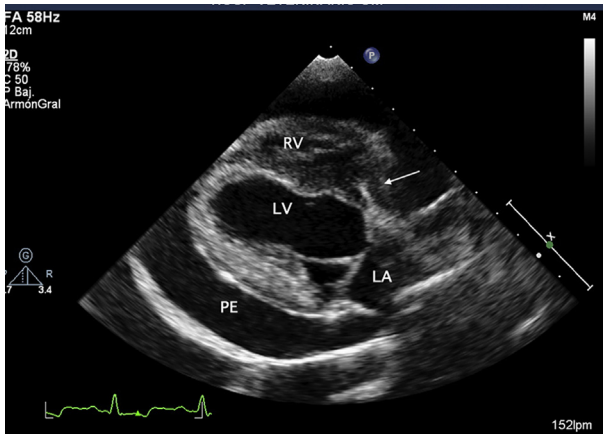

Fig. 1 Two-dimensional right parasternal long-axis echocardiographic view showing large volume of pericardial effusion and cardiac tamponade with collapse of the right atrium (arrow). LV: left ventricle, RV: right ventricle, PE: pericardial effusion, LA: Left Atrium.

loss were also reported. Deworming and vaccination were up to date, and there was no history of previous relevant diseases. A complete blood count performed by the referring veterinarian was within normal range. The most remarkable biochemistry data showed hypoalbuminemia (2.2 g/dL; range 2.5—3.6 g/dL), hyperglobulinemia (5.4 g/dL; range 2.6—3.8 g/dL), and increased aspartate aminotransferase (90 IU/L; range 5—50 IU/L). The abdominal fluid, also sampled by the referring veterinarian, was compatible with a modified transudate, and the dog was discharged on furosemide (1.5 mg/kg PO q 8 h).

On presentation, the dog was quiet but alert and responsive. Body condition score was 4/9. Mucous membranes were slightly pale, with a capillary refill time of 2 s. The patient had marked abdominal distension with a palpable fluid wave, as well as jugular distension. Respiratory rate and pattern were normal. All peripheral lymph nodes were slightly enlarged, and mild exfoliative dermatitis was noted affecting the tip of both ears and the tail. On cardiac auscultation, the heart sounds were muffled, with a regular rhythm and a heart rate of 120 beats per minute. A pulsus paradoxus was palpable.

Based on the physical examination, there was a strong suspicion of pericardial effusion (PE) with chronic cardiac tamponade. Brief transthoracic echocardiography[d] revealed a large volume of anechoic pericardial fluid. Diastolic collapse of the right atrium and ventricle confirmed cardiac tamponade (Fig. 1). There was no evidence of masses or structural abnormalities in the cardiac chambers and great vessels. A six-lead electrocardiogram[e] showed sinus rhythm and reduced QRS complex voltage.

Pericardiocentesis was performed under sedation (butorphanol 0.3 mg/kg administered intravenously), and 400 mL of hemorrhagic fluid was removed and analyzed. The fluid was characterized as an exudate, with a protein count of 5.7 g/dL and 27,870 cells/μL. Cytological examination of smears stained with Wright—Giemsa method revealed reactive macrophages with intracytoplasmic structures compatible with *Leishmania* amastigotes (Fig. 2) and neutrophils. There was also an unidentified population of round cells, with marked basophilic cytoplasm and multiple vacuoles. The presence of a concomitant neoplasia was suspected, and pericardial lymphoma was included in the differential diagnosis of the PE. A flow cytometry study could not be performed because of inadequate storage of the remaining fluid, and therefore, a fluid smear was submitted for clonality determination by polymerase chain receptor rearrangement assay. A polyclonal population was detected, compatible with a reactive process, and therefore, lymphoma was considered unlikely.

An enzyme-linked immunosorbent assay[f] performed on serum revealed a sample to a positive ratio of 3.52, considered positive as per the cut-off included in the kit instructions (>1.5). The panel of acute phase proteins included albumin (2 g/dL; range: 2.5—3.6 g/dL), haptoglobin (2.75 g/dL; normal: <3 g/dL), C-reactive protein (38.8 μg/mL; normal <12 μg/mL), and ferritin (896 μg/L; range: 60—190 μg/L). Overall, these values indicate the presence of inflammation. Considering the results of the serology and the moderate increase in C-reactive protein and ferritin, the patient fits in the category of dogs with clinical leishmaniasis [1]. Urinalysis was unremarkable.

Based on the aforementioned results, the dog was discharged on furosemide (0.5 mg/kg PO q 12 h for three days). On recheck, five days after the initial presentation, the dog appeared much brighter, with a cardiac physical examination within normal limits. Echocardiography confirmed absence of PE or other obvious abnormalities. Based on the diagnostic findings and the stability of the dog, treatment for canine leishmaniasis (CL)

[d] iE33 ultrasound system, Philips Medical Systems, Andover, MA, USA.

[e] Megacart, Siemens-Elema AB, Electrocardiography Division, Solna, Sweden.

[f] Leiscan® Leishmania ELISA Test, Esteve Veterinaria, Laboratorios Dr. Esteve SA, Barcelona, Spain.

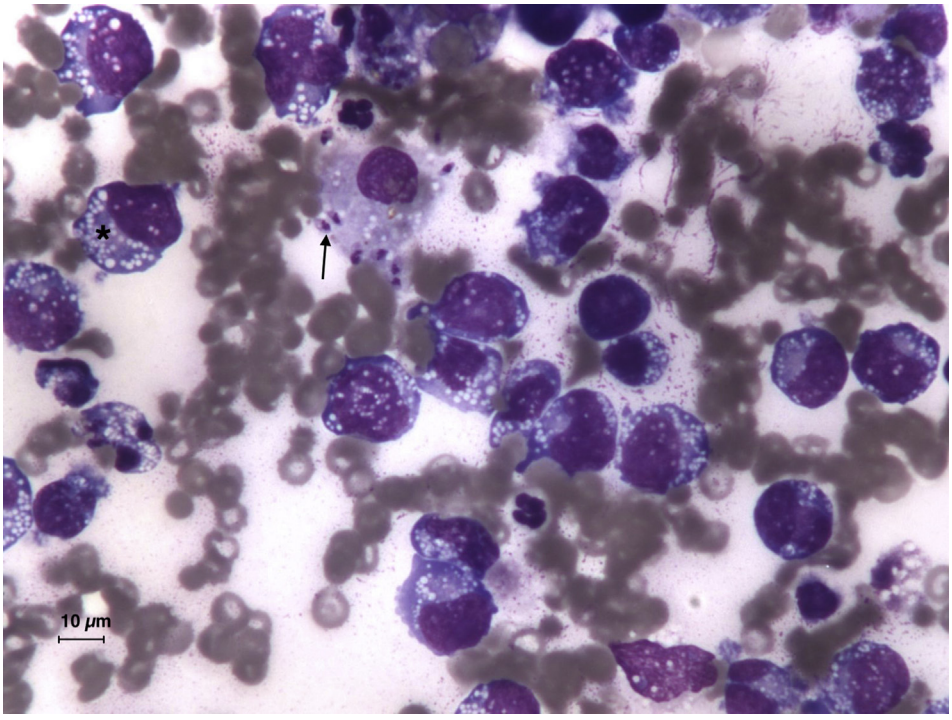

Fig. 2 Cytology of a pericardial fluid smear showing active macrophages with intracytoplasmic *Leishmania* amastigotes (arrow), neutrophils, and an unidentified population of mononuclear round cells with basophilic cytoplasma and multiple vacuoles (asterisk) (Wright—Giemsa stain, 100x).

with N-methylglucamine antimoniate (50 mg/kg SC q 12 h for 28 days) and allopurinol (10 mg/kg PO q 12 h for six months) was initiated.

One week after initial treatment, the animal continued to be cardiovascularly stable. A repeat echocardiographic examination showed normal cardiac structures and function and absence of PE. Evidence of constrictive pericarditis was not present based on the absence of detectable respiratory variations of peak mitral E-wave velocity (lower velocities in inspiration than expiration) on pulsed-wave Doppler, normal interventricular septal motion on two-dimensional and M mode echocardiography, and septal E′ velocity lower than lateral mitral annulus E' velocity on tissue Doppler. Repeat complete blood count and evaluation of the blood smear showed evidence of a mild non-regenerative anemia (hematocrit 32%; range 38—58%). On biochemistry, hypoalbuminemia (1.8 g/dL; 2.5—3.6 g/dL) and hyperglobulinemia (6.5 g/dL; 2.6—3.8 g/dL) were still present and attributed to the short time since the treatment was started.

One month after the initial presentation, PE with cardiac tamponade recurred. Physical examination revealed tachycardia with a heart rate of 150 beats per minute, slightly pale mucous membranes with weak femoral pulses, jugular distention, muffled heart sounds, and ascites. Pericardiocentesis was repeated and 270 mL of hemorrhagic fluid was removed. On cytology, macrophages with intracytoplasmic *Leishmania* amastigotes were visualized as previously. However, the abnormal population of basophilic round cells previously observed was not present. Large mononuclear basophilic cells showing anisocytosis and anisokaryosis were seen and interpreted as reactive mesothelial cells. Pericardiectomy was proposed to the owners, who provisionally declined the surgical procedure and elected to continue with the antiparasitic treatment.

Frequent reevaluations were scheduled. The dog remained clinically asymptomatic for another six weeks when PE with cardiac tamponade recurred. Pericardiocentesis was repeated, and the owners gave their consent for pericardiectomy. Pericardial effusion recurred before surgery, and despite preoperative pericardiocentesis, the dog showed signs of severe hemodynamic compromise and did not survive the procedure.

On gross postmortem examination, the pericardium was thickened and slightly opaque, and the parietal layer was highly vascularized. The epicardium showed multiple small (1 mm) whitish nodules on its surface but otherwise appeared normal. The right and left ventricular myocardium also appeared normal. No other significant

abnormalities were observed other than congestion of abdominal organs. Myocardial and pericardial samples were fixed in 10% buffered formalin and sent for pathological evaluation. Paraffin sections were stained by hematoxylin and eosin, Masson's trichrome, and immunohistochemistry with a polyclonal antibody specific for *Leishmania infantum* [2]. Histopathological analysis demonstrated features of chronic inflammation, including moderate epicardial (Fig. 3) and pericardial (Fig. 4) thickening with fibrovascular proliferation and mononuclear infiltration, predominantly lymphocytic. Immunohistochemistry staining was performed on several pericardial samples, as well as on the left and right atrial and ventricular myocardial specimens. The presence of amastigotes was not demonstrated in any of them.

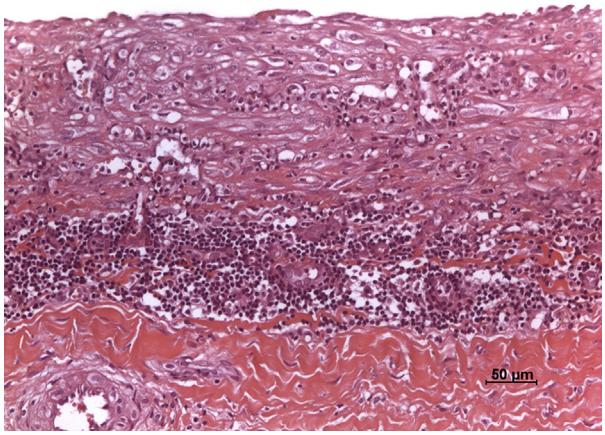

Fig. 4 Fibrovascular proliferation with mononuclear infiltration (predominantly lymphocytic) of the pericardium (Hematoxylin and eosin stain, 200x).

Discussion

To the author's knowledge, the dog described in this study is the second case of PE associated with CL reported in the veterinary literature. Pericardial disorders represent approximately 8% of the caseload in cardiac referral centers [3]. In dogs, PE is the most common manifestation of acquired pericardial disease, and the most frequent cause of PE is neoplasia (particularly hemangiosarcoma and chemodectoma), followed by idiopathic PE [4]. Other causes include the following: cardiovascular diseases (e.g. congestive heart failure and atrial perforation), trauma, metabolic disorders, coagulopathies, toxicities, and infectious diseases [3,4].

Among the infectious causes, bacterial infections secondary to migrating foreign bodies seem to be most frequently reported [5,6]. Other causative organisms described include fungal agents such as *Aspergillus niger* [7], *Inonotus tropicalis* [8], and *Coccidioides immitis* [9,10]. Viral infections have been investigated as a possible underlying condition for idiopathic PE [11], but its pathogenesis remains unclear.

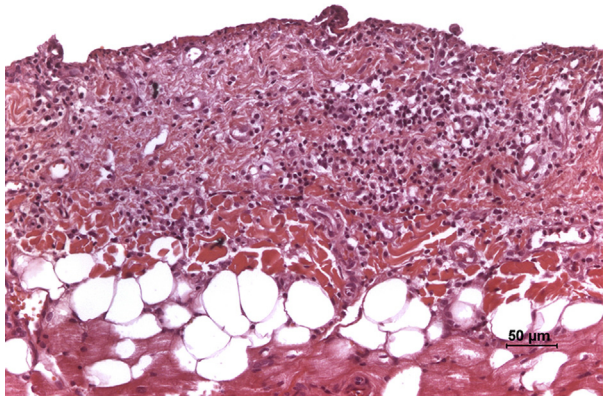

Fig. 3 Fibrovascular proliferation with mononuclear infiltration (predominantly lymphocytic) of the epicardium (Hematoxylin and eosin stain, 200x).

Parasitic infections as a primary cause of PE are very rare in veterinary medicine. A previous clinical case of cardiac tamponade associated with visceral CL has been reported, showing both pericardial and myocardial involvement [12].

Canine leishmaniasis includes all the disease manifestations caused by protozoa of the genus *Leishmania*. This worldwide vector-borne disease affects 88 countries and is spread by female phlebotomine sand flies [13]. Dogs are the main reservoir of this parasite. This is a systemic disease, and although the most frequent clinical manifestation is cutaneous lesions, any other organ may appear affected [14]. Studies estimate that >2.5 million of dogs are infected with *L. infantum* in southwestern Europe [15].

Myocardial lesions that have been described in dogs with CL are characterized by lymphocytic—plasmacytic myocarditis, myonecrosis, increased interstitial collagen, lepromatous-type granulomatous myocarditis, fibrinoid vascular changes, and vasculitis [16—18]. The severity of the lesions seems to correlate with the number of infecting parasites [17]. Cardiac troponin I has been suggested as an indirect method to evaluate myocardial injury in dogs with CL [19,20]. A recent study has suggested that myocarditis might be related to immunological alterations that are not dependent on the presence of amastigote forms in the cardiomyocytes [21].

In the present case, PE was the most relevant clinical abnormality. As in the case previously reported [12], intracellular amastigotes in the macrophages were directly visualized on stained smears of pericardial fluid, leading to the suspicion that PE could be a manifestation of CL. A positive serology supported the presence of the disease. Diagnosis of CL is based on several criteria [22]. Although serological tests such as the enzyme-linked immunosorbent assay method are recommended as the first approach in a suspected case [14], direct visualization of parasites has the advantage of providing an immediate conclusive diagnosis [22]. The overall diagnostic utility of PE cytology has been recently reported to be only 7.7% in a large population of dogs, but it rises to 20.3% if the hematocrit of the sample is $<$10%. In the same study, the diagnostic yield of the test varied as per the etiology with 40% of diagnostic samples classified as infectious effusions [23]. The present case supports routine pericardial fluid cytological analysis of PE.

Cytological findings of the PE fluid, in this case, were similar to the previously documented case, with a predominance of reactive mesothelial cells and polymorphonuclear neutrophils and macrophages with intracytoplasmic *Leishmania* amastigotes. A difference in this case was the presence of a poorly differentiated round cell population on the first cytological analysis. Although it is known that mesothelial surfaces react by producing dysplastic and hyperplastic mesothelial cells which can be difficult to differentiate from neoplastic cells [24], these cells appeared dissimilar to mesothelial cells. Owing to an initial concern of concurrent round cell neoplasia (most likely lymphoma), a polymerase chain receptor rearrangement assay was performed. The test result decreased the likelihood of neoplasia. These abnormal cells were not found on subsequent fluid analyses performed after recurrence of PE. Evidence of malignancy was not found on postmortem examination. The origin of these round cells is unclear, but it is hypothesized that they could have represented an altered lymphoid population related to *Leishmania* infection with their disappearance possibly secondary to the antiparasitic treatment.

Treatment of PE with cardiac tamponade consists of pericardiocentesis. Pericardiectomy is indicated when constrictive pericarditis becomes apparent or if relapses are frequent. In addition, treatment of the primary or underlying cause is required if it can be identified [3,4]. In this case, we considered the possibility that CL could be the underlying cause of the PE and initiated treatment with N-methylglucamine antimoniate and allopurinol after the dog was stabilized through pericardiocentesis. As per the current guidelines from the Leishvet group [14], this dog was categorized as stage II, with a prognosis varying from good to guarded. Survival in this case was nearly four months from the initial diagnosis, whereas, in the previous report, the dog died after four days of hospitalization and treatment initiation [12]. Successful pericardiectomy in this case might have resulted in a more significant survival benefit.

The fact that PE recurred after this dog finished the treatment with N-methylglucamine antimoniate suggests that inflammatory chronic pericarditis persisted, despite completing a recommended therapeutic protocol for visceral CL. Postmortem examination confirmed moderate chronic pericarditis. Severe chronic pericarditis may evolve into constrictive pericarditis [10]. Abnormal echocardiographic findings indicative of effusive—constrictive pericarditis were not present antemortem in this dog.

Although myocardial lesions have been described in dogs associated with CL [16,17], including in the previously reported case of PE [12], in this case, *Leishmania* amastigotes were not found on histopathology and myocardial involvement was limited to the epicardium. We hypothesize that amastigotes were not observed due to the efficacy of the leishmanicidal treatment; however, persistence of the established chronic inflammatory state of the pericardium resulted in repeated accumulation of fluid. Non-causal association between *Leishmania* and PE was also considered, and the presence of a concomitant idiopathic pericarditis could not be excluded based on histopathology. Other potential causes such as neoplasia or effusive-constrictive pericarditis were ruled out by postmortem examination.

In conclusion, this case suggests an association between CL and PE in dogs living in endemic areas. The possibility of *Leishmania* amastigotes appearing in the pericardial fluid of dogs with PE emphasizes the importance of routine cytological examination of pericardial fluid.

Conflicts of Interest Statement

The authors do not have any conflicts of interest to disclose.

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