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Comparison of myocardial damage among dogs at different stages of clinical leishmaniasis and dogs with idiopathic chronic kidney disease

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ABSTRACT

Canine leishmaniasis (CanL) is a systemic disease caused by the protozoan parasite *Leishmania infantum*. Myocarditis in CanL has been described previously in CanL by histopathological analysis of post-mortem specimens and by evaluation of cardiac troponin I (cTnI) levels. However, the degree of myocardial damage at different stages of CanL and the role that concurrent azotaemia plays in this myocardial injury are unknown. The aim of this study was to prospectively evaluate and compare the presence of myocardial injury in dogs at different stages of clinical CanL and in dogs with severe idiopathic chronic kidney disease (CKD) by measuring cTnI. Forty-eight dogs were included in the study, divided into four groups: (1) group A (10 healthy dogs); (2) group B (17 dogs with CanL without renal azotaemia, classified as mild to severe in the LeishVet scheme); (3) group C (11 dogs with CanL and renal azotaemia, classified as very severe in the LeishVet scheme); and (4) group D (10 dogs with idiopathic CKD). Dogs in group C had significantly higher cTnI than dogs in groups B and D, although cTnI was also elevated in these groups. Dogs in group A had normal cTnI values. Dogs in groups D and C had similar renal IRIS classification scorers. Severe lymphoplasmocytic myocarditis and a positive real time PCR of *L. infantum* DNA were observed in all dogs in group C. Dogs with very severe CanL exhibit more myocardial injury than dogs with milder CanL or dogs with idiopathic CKD.

Introduction

Canine leishmaniasis (CanL) is caused by the protozoan parasite *Leishmania infantum*. CanL is a systemic disease with variable clinical manifestations (Paltrinieri et al., 2010; Solano Gallego et al., 2011). Cardiac involvement with myocardial damage has been shown by histopathological evaluation of post-mortem specimens (Torrent et al., 2005; López Peña et al., 2009; Rosa et al., 2014) and by demonstration of raised levels of cardiac troponin I (cTnI) in affected cases (Silvestrini et al., 2012).

CTnI is a sensitive and specific biomarker for myocardial damage and it is released into the circulation in proportion to the degree of cardiac injury (Spratt et al., 2005; Burguener et al., 2006; Fonfara et al., 2010). In a retrospective study, dogs with CanL had higher levels of cTnI than normal dogs (Silvestrini et al., 2012). However, the degree of myocardial damage at different stages of the disease is unknown. Concurrent chronic kidney disease (CKD) may potentially play a role in CanL associated myocardial injury because azotaemic dogs with idiopathic CKD have also been shown to have higher cTnI concentrations than normal dogs (Porciello et al., 2008; Sharkey et al., 2009).

The aims of this prospective study were: (1) to compare the degree of myocardial injury in dogs at different stages of CanL by measuring cTnI; (2) to compare the degree of myocardial injury in dogs with idiopathic CKD versus dogs with very severe CanL by measuring cTnI; and (3) to describe the myocardial histopathological findings and the percentage of dogs with molecular evidence of *L. infantum* DNA found by PCR analysis in the myocardium of dogs with very severe CanL.

Materials and methods

Study population and selection criteria

The study was carried out at the Veterinary Teaching Hospital of the University of Extremadura, Spain, from October 2012 to January 2014. The study was reviewed and approved by the Animal Ethics Committee of the Veterinary Teaching Hospital of the University of Extremadura (protocol number 13/H07/10; date of approval 9 October 2013) and was performed in compliance with Spanish and European guidelines for research on animals (RD1201/2005 and ETS 170, respectively). Thirty-eight adult dogs with CanL or with idiopathic CKD presented to the internal medicine clinic and 10 healthy dogs owned by university staff were included in the study. All dogs included in the study completed all tests in a defined protocol and owner informed consent was obtained. Dogs with any evidence of concurrent disease, previous history of cardiac disease or dogs receiving prior therapy for CanL before

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Table 1
Cardiac troponin I (cTnI), age, haematology and biochemistry of healthy dogs (A), dogs with canine leishmaniasis (CanL) without renal azotaemia (B), dogs with CanL and renal azotaemia (C) and dogs with idiopathic chronic kidney disease with renal azotaemia not associated with CanL (D).

	Reference interval	Group A ($n = 10$)	Group B ($n = 17$)	Group C ($n = 11$)	Group D ($n = 10$)	P value*
cTnI (ng/mL)	<0.06	0.02 (0.1–0.03)	0.23 (0.20–0.24)	12.19 (2.66–14.05)	0.61 (0.23–0.62)	0.001
Age (years)		3 (2–3)	5.7 (4–8)	4.91 (4–5.5)	10.20 (7–13)	0.002
PCV (%)	39–52	46.3 (42.8–49.1)	34.4 (27.5–39.2)	34.5 (26.9–37.1)	32 (23–43.4)	0.017
Creatinine (mg/dL)	0.7–1.2	0.8 (0.7–0.9)	0.8 (0.7–0.9)	6.4 (3.5–9.4)	5.5 (2.5–8.7)	0.001
TP (g/dL)	5.1–7.3	6 (5.5–6.3)	8 (7.4–8.7)	7.2 (6.1–7.7)	6.3 (5.6–7.1)	0.001
Globulin (g/dL)	1.5–3.5	2.4 (1.8–3.1)	4.9 (3.7–5.5)	3.9 (3.1–4.8)	2.9 (2.6–3.4)	0.001
Albumin (g/dL)	2.5–3.9	3.6 (3.3–3.8)	3 (2.7–3.2)	3.3 (3.1–3.6)	3 (3–3.9)	0.036
UPC	<0.5	0.1 (0.1–0.3)	0.2 (0.1–0.3)	9.5 (3.9–11.8)	5.5 (4.5–8.9)	0.001
BP (mm/Hg)	>150	113.2 (100–200)	128.3 (110–145)	188.4 (200–205)	183.3 (183–200)	0.001
A:G	0.7–1.9	1.6 (1.2–1.9)	0.8 (0.5–0.9)	0.9 (0.6–1.1)	1.2 (1.2–1.4)	0.001
ALT (IU/L)	18–77	30.5 (14–55)	35 (18–77)	40 (21–77)	50.5 (12–76)	0.275
Na (mmol/L)	137–163	149.4 (117.6–152)	148 (142–155)	146.6 (131.5–156.5)	148.9 (130.9–172.8)	0.800
K (mmol/L)	4.2–5.7	3.9 (3.8–5.3)	4.4 (3.1–5.6)	4.6 (3.7–5.4)	4.6 (4.1–5.7)	0.116
Ca (mg/L)	9.2–13.0	9.7 (6.1–12.0)	10.2 (7.3–12.2)	10 (7.1–11)	9.9 (6.9–10.9)	0.465
P (mmol/L)	0.7–2.1	1.7 (1.2–1.9)	1.5 (1.1–2.1)	2 (1.5–2.4)	2 (1.5–2.1)	0.399

PCV, paced cell volume; TP, total protein; UPC, urine protein–creatinine ratio; BP, blood pressure; A:G, albumin to globulin ratio; ALT, alanine aminotransferase.
Values are expressed as median (interquartile range). P values represent comparisons of variables between the four groups.
* Significance was set at $P < 0.05$.

presentation were excluded from the study. The study was not blinded; investigators and clinicians were aware of the groups the dogs belonged to.

A comprehensive physical examination was carried out in each dog. Tests performed on all dogs included a complete cell blood count (CBC), biochemistry (BC), urinalysis including protein–creatinine ratio (UPC), ELISA on serum for *L. infantum*, serum cTnI concentration, blood pressure (BP) measurement, electrocardiography (ECG), thoracic radiographs and echocardiographic examination. Dogs were divided into four groups: (1) group A (10 healthy control dogs); (2) group B (17 dogs with CanL without renal azotaemia); (3) group 3 (11 dogs with CanL and renal azotaemia); and (4) group D (10 dogs with idiopathic CKD). Dogs in group B were those classified as mild to severe disease in the LeishVet scheme (Solano Gallego et al., 2011). They had general, cutaneous or ocular clinical signs, such as lymphadenopathy, dermatitis or keratoconjunctivitis, but absence of azotaemia and were in general less sick than dogs in group D. Dogs in group C included those classified as very severe in the LeishVet scheme (Solano Gallego et al., 2011). They had renal azotaemia and in general had more severe clinical signs and laboratory abnormalities. The diagnosis of CanL was achieved on the basis of appropriate clinical signs and a high serology titre (at least three-fold increase to the laboratory reference cut-off). This was often complemented with a positive visualisation on cytology of *Leishmania* amastigotes in lymph node or bone marrow, or a positive PCR in any of these tissues. In dogs with clinically suspected leishmaniasis and an inconclusive titre on serology, the diagnosis was made by visualisation of the parasite in lymph nodes or bone marrow or by a positive real time PCR in lymph node or bone marrow using as described by Belinchón-Lorenzo et al. (2013). Azotaemic dogs were staged according to the guidelines of the International Renal Interest Society (IRIS[1]). There was a predominance of mixed breed dogs (13/48), Spanish greyhounds (5/48) and American pitbull terriers (4/48), along with 18 other breeds. There were 21 males and 27 females, comprising four males and six females in group A, eight males and nine females in group B, four males and seven females in group C and five males and five females in group D.

Serum cardiac troponin I

Serum cTnI concentration was measured using an enzyme-labelled chemiluminescent immunometric assay validated for dogs, with the Siemens Immunite 1000 Troponin I immunoanalyser. A goat polyclonal anti-troponin I antibody (Laborti Veterinària Laboratories, Spain) was used and cTnI concentrations <0.06 ng/mL were considered to be normal (Pelander et al., 2002).

Leishmania infantum *serology*

An ELISA for semi-quantitative detection of specific antibodies against the total soluble antigen of *L. infantum* (obtained from *L. infantum* promastigotes MCAN/ES/1996/BCN150, zymodeme) was carried out, as described by Belinchón-Lorenzo et al. (2013).

Blood pressure measurement and cardiac evaluation

Systolic BP measurements were determined by Döppler ultrasonography following the guidelines of the American College of Veterinary Internal Medicine consensus statement (Brown et al., 2007). In order to exclude primary cardiac disease, electrocardiography (ECG), thoracic radiography and standard transthoracic echocardiography (Thomas et al., 1993) were performed. Measurements of the left ventricular diastolic dimension (LVDd), left ventricular systolic dimension (LVDs), left ventricle wall thickness in diastole (LVWd) and interventricular septum diastolic thickness (IVS) were performed from the right parasternal short axis view using M mode. The fractional shortening (%FS) was calculated as the LVDd minus the LVDs divided by the LVDd and multiplied by 100. The aortic (Ao) and left atrial (LA) diameters were measured and LA:Ao ratio was calculated by a two-dimensional method from a right-sided short axis parasternal view. Colour flow Döppler ultrasonography was used for the evaluation of transvalvular flows and the detection of valvular regurgitation. Peak systolic aortic and pulmonic velocities and peak early (E) and late (A) diastolic mitral flow velocities were also evaluated by pulse wave Doppler.

Histopathological and parasitological analysis of myocardial tissue

Ten dogs from group C were euthanased at the owner's request and post-mortem examinations was performed. Myocardial samples for histopathological analysis were taken from the left and right ventricular free walls, interventricular septum and left and right atrium. Samples were tested for the presence of *L. infantum* by real time PCR (Belinchón-Lorenzo et al., 2013) by detection and quantification of Kinetoplast minicircle DNA after deparaffinisation (Müller et al., 2003).

Statistical analysis

SPSS Statistics 21 (IBM) was used for statistical analysis. Descriptive statistics were applied and reported. The normality and homoscedasticity of serum cTnI concentration, age, packed cell volume (PCV), concentrations of creatinine, total protein (TP), albumin, globulins, albumin to globulin ratio (A:G), UPC, IRIS stage and systolic BP were tested using the Shapiro–Wilk test and Levene tests, respectively. None of the variables followed a normal distribution and they were reported as medians with interquartile ranges (IQRs). The Kruskal–Wallis test and the Mann–Whitney U test were used to examine differences in the variables studied among the four groups. Correlations between serum cTnI concentration and each variable studied were evaluated using Spearman's rank correlation test (ρ). $P < 0.05$ was considered to be statistically significant.

Results

The median (IQR) ages were 3 (2–3) years in group A, 5.71 (4–8) years in group B, 4.91 (4–5.5) years in group C and 10.2 (7–13) years in group D (Table 1). The median age of dogs in group D was statistically higher than in other groups ($P = 0.015$).

Serum cTnI concentrations in group A (median 0.02, IQR 0.01–0.03, ng/mL) were normal (<0.06 ng/mL) in all dogs. Serum cTnI concentrations of all dogs in groups B (median 0.23, IQR 0.20–0.24, ng/mL), C (median 12.19, IQR 2.66–14.05, ng/mL) and D (median 0.61, IQR 0.23–0.62, ng/mL) were >0.06 ng/mL. Significant differences were observed in serum cTnI concentrations between group A and groups B, C and D ($P = 0.001$). The median serum cTnI

[1] http://www.iris-kidney.com/guidelines (accessed 9 February 2014).