Arch Med Vet **48, 269-275 (2016)**

ORIGINAL ARTICLE

Cardiac biomarkers in dogs with visceral leishmaniasis

Biomarcadores cardíacos en perros con leishmaniasis visceral

VBC Silva[a], MG Sousa[a*], CRA Araújo[b], ABG Lima[b], R Carareto[b]

ABSTRACT. Although only a few studies have focused on the cardiac alterations in *Leishmania* spp. infected dogs, myocardial inflammation ascribed to such parasite has been documented in some animals. In spite of the increasing popularity of cardiac biomarkers in veterinary medicine in recent years, it remains unknown how most of these substances would perform in dogs with visceral leishmaniasis. In this study, we hypothesised that *Leishmania* spp. would damage cardiac myofibers resulting in increased circulating levels of cardiac specific markers in dogs. Therefore, biomarkers of myocyte injury and stress were investigated in 18 dogs with naturally-occurring visceral leishmaniasis presenting varying clinical signs, including completely asymptomatic animals. The median (min-max) concentrations of NT-proANP, NT-proBNP, cTnI, and CK-MB were 1138.0 (875-1175) pmol/L, 1160.0 (803-2034) fmol/L, 0.22 (0.15-0.51) ng/mL, and 116.7 (113-222) U/L, respectively. No differences were documented for the concentration of the natriuretic peptides and CK-MB in accordance with the severity of clinical signs, but for cTnI a significant difference was reported when comparing asymptomatic and symptomatic animals. Also, the compromise of renal tubular function seemed to interfere with the concentration of the natriuretic peptides and cTnI. In conclusion, all cardiac-specific biomarkers were elevated when compared to the normal range previously proposed for dogs. Measuring specific cardiac biomarkers has the potential to non-invasively document myocardial injuries attributable to visceral leishmaniasis.

Key words: parasitic disease, myocarditis, natriuretic peptides, troponins.

RESUMEN. A pesar de que solo unos pocos estudios se han centrado en las alteraciones cardíacas en perros infectados por la *Leishmania* spp., la inflamación miocárdica atribuida a este parásito ha sido documentada en algunos animales. Los marcadores cardiacos en medicina veterinaria han experimentado una creciente popularidad en los últimos años, sin embargo aún no se sabe cómo la mayoría de estas substancias se comporta en perros con leishmaniasis visceral. En este estudio, se presume que *Leishmania* spp. dañaría fibras cardiacas causando un aumento de los niveles circulantes de marcadores cardiacos en perros. Biomarcadores de lesión y de estrés de los miocitos fueron investigados en 18 perros con infección natural por *Leishmania* spp. y que presentaban diversos signos clínicos, incluyendo los animales totalmente asintomáticos. La mediana (mín-máx) de las concentraciones de NT-proANP, NT-proBNP, cTnI, CK-MB fueron 1138,0 (875-1175) pmol/L, 1160,0 (803-2034) fmol/L, 0,22 (0,15 -0,51) ng/mL y 116,7 (113-222) U/L, respectivamente. No se registraron diferencias para la concentración de los péptidos natriuréticos y CK-MB de conformidad con la gravedad de los síntomas clínicos, pero para la cTnI se observó diferencia significativa al comparar los animales sintomáticos y asintomáticos. Además, el compromiso de función tubular renal parece interferir con la concentración de los péptidos natriuréticos y la cTnI. En conclusión, todos los cardiobiomarcadores específicos estaban elevados en comparación con el rango normal anteriormente propuesto para perros. La cuantificación de determinados biomarcadores cardíacos tiene el potencial de identificar, de forma no invasiva, lesiones miocárdicas atribuidas a la leishmaniasis visceral.

Palabras clave: enfermedad parasitaria, miocarditis, péptidos natriuréticos, troponinas.

INTRODUCTION

Visceral leishmaniasis is a potentially fatal zoonotic disease that occurs in people and both domestic and wild animals. It is caused by the protozoa *Leishmania (Leishmania) infantun chagasi*, which is disseminated through phlebotomine sand flies. Although not frequently mentioned in literature, lesions in the cardiovascular system have been documented in *Leishmania* infections, and therefore should be considered as part of the several alterations that might be caused by such parasite. Both dogs and human beings have been reported to develop a myocarditis attributable to *Leishmania* spp., which is histologically characterised by edema, inflammatory infiltration, focal necrosis, and Anitschkow myocytes proliferation (Torrent *et al* 2005, Baneth *et al* 2006, Lopéz-Peña *et al* 2009, Alves *et al* 2010).

When the necrotic myocytes lose their integrity, macromolecules leak into the interstitial tissue, following absorption by the capillary bed and lymphatic system. Such molecules are considered biological markers or biomarkers, which are defined as substances that may be measured objectively as a surrogate for either normal or pathological processes, or even change their concentration in response to therapeutic interventions. Indeed it is desired that biomarkers could be produced by a specific tissue, being easily detected when released into the circulation (López-Sendón 2003, Reynolds and Oyama 2008).

In a prior study we investigated cardiac rhythm disturbances and ECG wave abnormalities in 105 dogs with

Accepted: 10.03.2016.

[a]Federal University of Paraná, Department of Veterinary Medicine, Paraná, Brazil.

[b]Federal University of Tocantins, College of Veterinary Medicine and Animal Science, Araguaína, Tocantins, Brazil.

*Corresponding author: MG Sousa; Rua dos Funcionários 1540, Curitiba, Paraná, Brazil; marlos98@ufpr.br

confirmed visceral leishmansiasis and found sinus arrest, right bundle branch block, and atrial premature beats in 14.3%, 4.8%, and 4.8% of the studied subjects, respectively (Sousa *et al* 2013). Also, when hearts of 30 dogs with the same disease were assessed histologically and immunohistochemically, Rosa *et al* (2013) found lymphoplasmacytic myocarditis, myonecrosis, and increased interstitial collagen in the majority of the animals, and *Leishmania* spp. could be documented in 66% of the animals, which likely points to myocardial lesions and their consequences being attributable to such parasite.

To the authors' knowledge, cardiac specific biomarkers have not been fully investigated in dogs with naturally-occurring visceral leishmaniasis, although they do have been studied in other parasitic and infectious diseases. A study by Barr *et al* (2009) investigated the serum concentration of cardiac troponin I in a model of acute myocarditis in dogs infected with *Trypanosoma cruzi*, a protozoa belonging to the same family of *Leishmania* spp.. In that study, an association was found to exist between cTnI levels and the myocardial injury seem at the histopathological assessment of the dog's hearts. Therefore, it is reasonable to hypothesize that alterations in cardiac myofibers could be documented by the circulating levels of cardiac specific markers in dogs naturally-infected by *Leishmania* spp.. Also, we sought to determine whether the clinical status of the animals could potentially interfere with the concentration of these substances.

MATERIAL AND METHODS

ANIMALS AND GROUPS

Eighteen mature mixed-breed dogs (2.7-32.1 kg; 9 males) diagnosed with visceral leishmaniasis were prospectively included in a cross-sectional observational study. The diagnosis of leishmaniasis was based on the demonstration of amastigotes of *Leishmania* spp. in lymph node aspirates, as well as a positive serology in both ELISA and IIFA. Exclusion criteria included the animal testing positive in serology for leptospirosis, toxoplasmosis, neosporosis, ehrlichiosis, babesiosis, and heartworm, Lyme and Chagas diseases, as well as the owner not voluntarily agreeing to have the animal enrolled in the investigation, which was entirely conducted within a Veterinary Teaching facility. Once the dogs were recruited into the study, clinical signs were recorded to allow their categorization in asymptomatic, olygosymptomatic (a maximum of two clinical signs), or polysymptomatic (more than two clinical signs) animals. Varying clinical signs were observed, including emaciation, lymphadenomegaly, skin lesions, ocular alterations, muscle atrophy, and onicogryphosis, but some of them had no clinical manifestations at all.

The study was entirely conducted in accordance with the guidelines outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the institutional animal experimentation ethical committee (10/1621).

QUANTIFICATION OF CARDIAC BIOMARKERS

Blood samples were drawn to obtain both the plasma and serum. Several aliquots were stored at -80ºC until the batch analyses were performed. The plasma concentrations of N-terminal pro-ANP (NT-proANP), N-terminal pro-BNP (NT-proBNP), and MB fraction of creatine kinase (CK-MB), as well as the serum concentration of cardiac troponin I (cTnI) were measured using commercially available kits (CSB-EQ027254DO, Cusabio, Wuhan, China; E90485CA, Uscn Life Sciences Technology, Missouri City, EUA; CSB-E08772C, Cusabio, Wuhan, China; 118, Labtest, Lagoa Santa, Brazil). The kits used for measurement of natriuretic peptides and cardiac troponin I were all species specific. All manufacturer recommendations were strictly followed for laboratory procedures involved with the measurements.

OTHER BIOCHEMICHAL ANALYSES

Blood and urine samples were used for quantification of creatinine concentration, urine protein-to-creatinine ratio (UPC), and the fraction excretion (FE) of sodium and potassium. These analyses were undertaken using standard laboratory methods immediately after the animals were admitted into hospital. UPC was documented as the ratio between urine protein and urine creatinine, and the FE of electrolytes was determined using the following equation: $FE = [(\text{Urine electrolyte} \cdot \text{Plasma creatinine}) / (\text{Urine creatinine} \cdot \text{Plasma electrolyte})] \cdot 100$.

STATISTICAL ANALYSES

Descriptive statistics were used to describe population characteristics. The results underwent the Shapiro-Wilk normality test, followed by either an analysis of variance for parametric data or the Kruskal-Wallis test for non-parametric data to compare animals in accordance with their presenting clinical signs. Either Pearson or Spearman test was used to look for individual correlations between the blood concentration of the several biomarkers and body weight, serum creatinine concentration, urine protein-to-creatinine ratio, and the fraction excretion of sodium and potassium. Statistical analyses were performed using statistical software Graphpad Prism, v.5.0 (California, USA), and significance was set at $P < 0.05$ for all analyses.

RESULTS

Table 1 shows the descriptive statistics of the blood concentration of the several cardiac biomarkers that were studied. Elevated concentrations of NT-proBNP (> 800 pmol/L), cTnI (> 0.03 ng/mL), and CK-MB (> 6.3 U/L) were documented in all animals enrolled in the study,

Table 1. Descriptive statistics showing the blood concentration of cardiac biomarkers in dogs with visceral leishmaniasis (n = 18).
Estadísticas descriptivas de la concentración en sangre de los marcadores cardiacos en perros con leishmaniasis visceral (n = 18).

	NT-proANP (pmol/L)	NT-proBNP (fmol/L)	cTnI (ng/mL)	CK-MB (U/L)
Minimum	823.0	803.0	0.150	113.0
25% percentile	958.5	986.0	0.190	114.1
Median	1138.0	1160.0	0.220	116.7
75% percentile	1440.0	1494.0	0.335	190.5
Maximum	1875.0	2034.0	0.510	222.8
Mean	1215.0	1284.0	0.267	141.0
Standard deviation	317.7	396.3	0.108	46.3
Lower 95% CI of mean	1057.0	1087.0	0.213	111.6
Upper 95% CI of mean	1373.0	1482.0	0.321	170.5
Coefficient of variation	26.1%	30.8%	40.5%	32.8%

CI: confidence interval.

but increased NT-proANP (> 1,000 pmol/L) was found in only 66.6% (12/18) of the dogs. However, when all subjects were considered together, the mean values of all four cardiac specific biomarkers were above the reference range for normal dogs (Montes *et al* 1987, Sleeper *et al* 2001, Ettinger and Prosek 2010, Eriksson *et al* 2014).

Although no differences were documented in the concentration of NT-proANP, NT-proBNP, and CK-MB according to the group to which the animals were assigned, there is an overt trend of increase in such concentrations as the clinical scenario worsened (table 2). For cTnI, a significant difference was attained when comparing asymptomatic and symptomatic animals (figure 1).

The results of blood and urine biochemical analyses are shown on table 3. Although no correlation existed between cardiac biomarkers and body weight, weak to moderate

Table 2. Cardiac biomarker characteristics in dogs with visceral leishmaniasis grouped according to clinical scenarios. Means, standard deviations and medians are listed.
Características de los marcadores cardiacos en los perros con leishmaniasis visceral agrupados de acuerdo con situaciones clínicas. Se detallan promedios, desviaciones estándar, y medianas.

Biomarker	Group			P
	Asymptomatic (n = 4)	Oligosymptomatic (n = 6)	Polysymptomatic (n = 8)	
NT-proANP (pmol/L)	989.0 ± 168.0 ^{A} (940.0)	1128.0 ± 257.2 ^{A} (1042.0)	1394.0 ± 337.1 ^{A} (1346.0)	0.0733
NT-proBNP (fmol/L)	1018.0 ± 265.1 ^{A} (940.0)	1146.0 ± 178.0 ^{A} (1063.0)	1522.0 ± 455.3 ^{A} (1594.0)	0.0566
cTnI (ng/mL)	0.19 ± 0.03 ^{A} (0.20)	0.22 ± 0.05 ^{AB} (0.22)	0.34 ± 0.12 ^{B} (0.33)	0.0318
CK-MB (U/L)	115.0 ± 1.4 ^{A} (114.0)	141.3 ± 49.0 ^{A} (117.2)	156.4 ± 57.4 ^{A} (116.1)	0.5188

Different letters in the same line indicate significant differences at the post hoc test.

Table 3. Blood and urine biochemical analysis in dogs with visceral leishmaniasis (n = 18).
Análisis bioquímicos de sangre y de orina en los perros con leishmaniasis visceral (n = 18).

	Mean	SD	Min-Max	CV
Serum creatinine (mg/dL)	1.25	0.43	0.60-1.90	22.5%
Urine protein-to-creatinine ratio	1.41	1.30	0.03-4.50	25.4%
Na^+ FE (%)	1.54	1.07	0.60-3.90	27.8%
Na^+ FE (%)	12.98	6.23	4.90-23.20	233.7%

SD: standard deviation; CV: coefficient of variation; FE: fraction excretion.

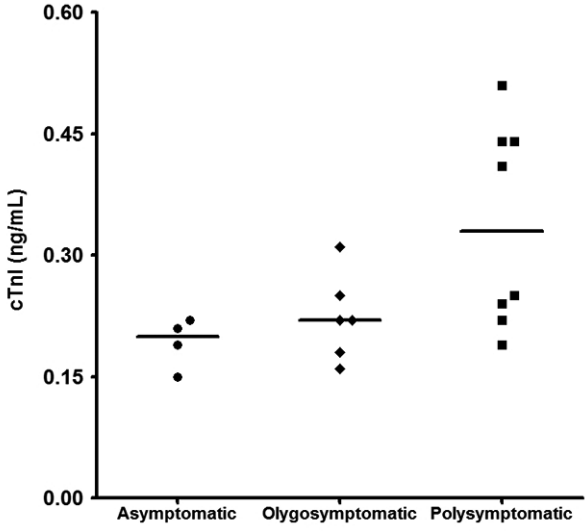

Figure 1. Individual values and medians of the blood concentration of cardiac troponin I in dogs with visceral leishmaniasis and varying clinical signs.

Valores individuales y medianas de la concentración en sangre de troponina I cardiaca en perros con leishmaniasis visceral y diversos signos clínicos.

correlations were found to exist between the Na^+ fraction excretion and the blood concentration of NT-proBNP and cTnI, as well as between the K^+ fraction excretion and the concentrations of NT-proANP, NT-proBNP, and cTnI (figure 2).

DISCUSSION

Several infectious and noninfectious diseases might result in compromise of the heart muscle, including parvovirus infection, heartworm disease, endocarditis, and dilated cardiomyopathy (Sharkey *et al* 1991, Santos 2005, Diniz *et al* 2007). Despite not being frequently documented, some studies have shown that *Leishmania (Leishmania) infantum chagasi* infected dogs may develop some sort of myocardial injury (Torrent *et al* 2005, Lopéz-Peña *et al* 2009, Alves *et al* 2010, Sousa *et al* 2013). Because studies have found contrasting results regarding the identification of *Leishmania* spp within the myocardium of these animals, it might be speculated that the basis for myocardial injury in infected subjects involves the inflammation ascribed to direct muscular injury from the parasite, as well as immune-mediated myositis (Gomes *et al* 2012, Rosa *et al* 2014)

Natriuretic peptides are biomarkers of cardiac remodeling and congestive heart failure, which have been shown, in recent years, to aid in establishing both the diagnosis and prognosis of several cardiac diseases (Maack 2006, Richards 2007). The N-terminal proANP was once demonstrated to have a greater reliability in assessing conditions such as chronic mitral regurgitation, the cardiac remodeling attributable to volume overload, heartworm disease, and atrial fibrillation (Shober 2005, Ettinger and Prosek 2010). Also, Prosek *et al* (2007) documented a sensitivity of 95.5% and specificity of 84.6% for the NT-proANP in differentiating dogs with dyspnea ascribed to either cardiac or non-cardiac conditions.

Several studies have evaluated the relationship between the blood concentration of NT-proBNP and the degree of cardiac compromise in many heart problems (DeFrancesco *et al* 2007, Serres *et al* 2009, Moonarmart *et al* 2010). It was shown that the more severe the heart failure, the higher the circulating levels of this peptide. Asymptomatic mitral valve dogs bearing increased levels of NT-proBNP developed signs of congestive heart failure more rapidly than the asymptomatic patients that had a lower blood concentration of that biomarker. Also, it was demonstrated that the augmented levels might indicate an increased probability of cardiac-related death.

NT-proBNP values ranging from 800-1800 pmol/L are highly suggestive of cardiac disease (Ettinger and Prosek 2010). Despite no significant differences in NT-proBNP concentration had been documented between the groups of this study, all animals tested within the range which might indicate cardiac involvement. Interestingly, there is a trend of increase in the blood concentration of such biomarker as the animals' clinical scenario worsened. A similar finding was reported by Lobetti *et al* (2012), who investigated the serum concentrations of NT-proBNP and cTnI in 45 dogs with babesiosis. In that study, the concentration of the biomarkers increased along with the severity of the disease.

To the authors' knowledge, no other study investigated how NT-proANP performs in dogs with either infectious or parasitic diseases. However, several studies have proven its reliability in detecting cardiac failure in dogs with heart disease (Ettinger and Prosek 2010, Eriksson *et al* 2014). In this investigation, NT-proANP was the only cardiac specific biomarker that was not elevated in all recruited dogs. Nonetheless, its concentration was found to be above the reference range (> 1.000 pmol/L) in 66.6% of the animals, being consistent with the increased concentrations of the other biomarkers.

Cardiac troponin I, which is found freely in the cytosol and accounts for approximately 2 to 4% of the total myocardial troponins, has been recognised as a highly sensitive and specific biomarker for myocardial injury in human beings (Apple *et al* 1998) as well as in animals (Schober *et al* 1999). In a study that included 42 critically ill dogs without primary cardiac compromise, cTnI was able to predict the short term death (Langhorn *et al* 2013).

Immunoassays have been validated to determine the blood concentration of cTnI in dogs, which indicated the normal range in that species to be ≤ 0.03 ng/mL (Schober *et al* 1999, Sleeper *et al* 2001). The concentration of cTnI for all animals recruited for this study was above the reference range for normal dogs. Although no differences existed between asymptomatic and olygosymptomatic dogs, an

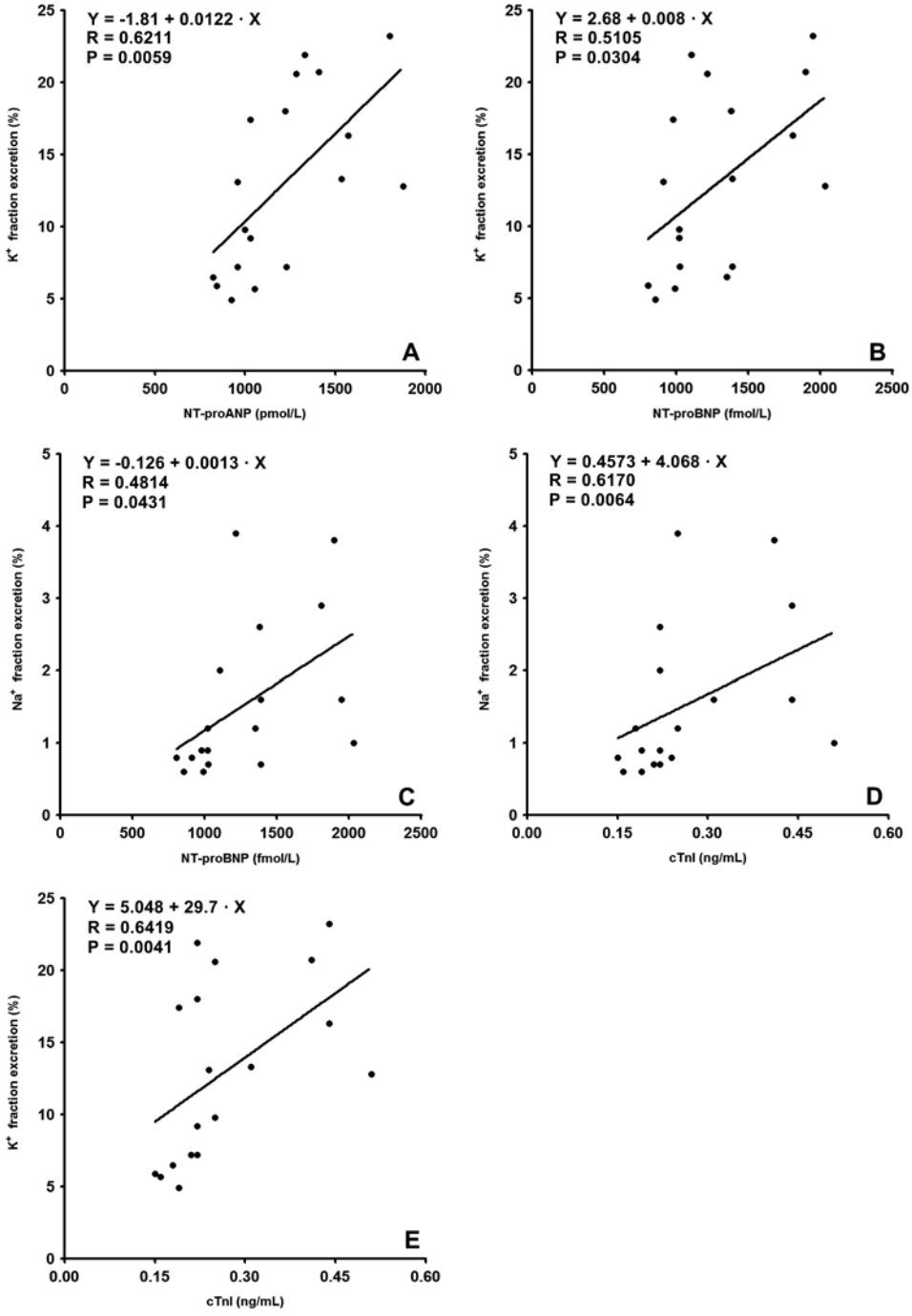

Figure 2. Scatter plots of the blood concentration of NT-proANP (A), NT-proBNP (B, C), cTnI (D, E) versus either the sodium or potassium fractional excretion in 18 dogs with visceral leishmaniasis. Best-fit lines, correlation coefficients and linear regression equations are shown.

Diagramas de dispersión de la concentración en sangre de NT-proANP (A), NT-proBNP (B, C), cTnI (D, E) y la excreción fraccional de sodio o de potasio en 18 perros con leishmaniasis visceral. Se observan los mejores ajustes de líneas, los coeficientes de correlación y las ecuaciones de regresión lineal.

increasing concentration of cTnI was seen in accordance with the decline in the clinical status of the dogs. Thus, the polysymptomatic animals presented a concentration that far exceeded the normality range, suggesting intense myocyte damage. In a similar study, Silvestrini *et al* (2012) has already documented an increased concentration of blood cTnI when comparing dogs with visceral leishmaniasis and control animals. Also, 40% of the infected

animals in their investigation had the cTnI concentration above the normal reference range, which pointed to some degree of myocardial injury. Recently, Mendes *et al* (2014) reported a case of a dog with naturally-occurring visceral leishmaniasis, in which the cTnI concentration (0.22 ng/mL) was identical to the mean value documented for the olygosymptomatic group of this investigation.

Other infectious diseases are known to result in increased levels of cTnI as well. Güneş *et al* (2014) found elevated cTnI and cTnT in dogs with parvovirus infection and distemper, which reinforces how valuable the measurement of cardiac troponins may be when myocardial injury is suspected. When dogs with heartworm disease were studied, Carretón *et al* (2014) showed a progressive increase in cTnI in accordance with the severity of the disease. Also, dogs with naturally occurring ehrlichiosis were shown to have elevated cTnI when compared to healthy controls (Diniz *et al*, 2008).

Cardiac troponins have demonstrated high sensitivity in diagnosing noninfectious conditions as well. Shaw and colleagues (2004) demonstrated its utility in differentiating idiopathic pericardial effusion and the pericardial effusion ascribed to hemangiossarcoma, with the latter presenting increased values of cTnI as compared to the former. Using a combination of clinical information, cTnI concentration and electrocardiographic findings, Diniz *et al* (2007) diagnosed two cases of myocarditis in dogs that experienced chest trauma.

Creatine kinase MB isoenzyme is released into the extracellular space by necrotic myocardial cells, which potentially allows its use as a surrogate for cardiac injury (Schober *et al* 1999, Diniz *et al* 2007). Dogs with cardiovascular diseases have been shown to present significantly higher levels of CK-MB in comparison with healthy subjects, being this condition attributable to alterations in membrane permeability owing to myocyte instability and hypoxia (Pino *et al* 2008). Both Schober *et al* (1999) and Diniz *et al* (2007) reported augmented CK-MB concentrations in dogs suspected of blunt cardiac injury. However, Diniz *et al* (2007) mentioned that CK-MB might have been released from either the myocardium or the skeletal muscle, since the animals reported in their study had concurrent muscle injury.

In dogs with visceral leishmaniasis, Santos (2013) documented an increased mean concentration of CK-MB (450.9 U/L), which was much higher than both their healthy controls and the infected dogs of this investigation. Also contrasting with the results of this study, a recent investigation in which 41 dogs with visceral leishmaniasis had been categorised into asymptomatic, olygosymptomatic, and symptomatic groups, reported greater concentrations of CK-MB in all groups (263, 488.4, and 681.2 U/L, respectively) (Godoy 2015). However, every animal of this investigation had a CK-MB concentration that far exceeded the reference range of 4.9 to 6.3 U/L previously proposed for normal dogs (Montes *et al* 1987).

In this study, we also investigated the correlation of several parameters used to assess both glomerular and tubular functions and the blood concentration of the cardiac biomarkers, since the altered renal function ascribed to *Leishmania* spp. (Costa *et al* 2003, Baneth *et al* 2008) may interfere with the concentration and clearance of those substances. The electrolyte fraction excretion, which is defined as the relationship between any given electrolyte and the creatinine elimination, may be used as a surrogate for renal tubular function (DiBartola 2000, Martinez and Carvalho 2010). Thus, the significant positive correlations between the Na^+ and K^+ fraction excretions and NT-proANP, NT-proBNP, and cTnI might be explained, at least in part, by the deterioration of renal function (Freda *et al* 2002, Raffan *et al* 2009) and not to a true myocardial injury. However, since no histopathological assessment of the hearts was performed in this study, we could not investigate whether myocardial inflammation and structural damage were present at all.

Among the several limitations of this study are the small number of dogs enrolled, which may have compromised the ability of the statistical analysis to document differences in the blood concentration of the several cardiac biomarkers between asymptomatic and symptomatic animals. Also, the absence of an echocardiographic assessment did not allow correlating any changes in myocardial function and structure with the biomarkers concentrations. Finally, since only a few infectious and parasitic comorbidities have been ruled out, our results could represent the effect of other systemic conditions over the heart.

In conclusion, this study documented elevated cTnI in all dogs, as well as increased NT-proBNP in animals with visceral leishmaniasis. The concentration of cardiac troponin I became significantly higher as the clinical scenario worsened. Although not significant, a trend of increase was also observed for the remainder of cardiac biomarkers. The quantification of specific cardiac biomarkers is likely useful to identify myocardial injury attributable to visceral leishmaniasis.

ACKNOWLEDGEMENTS

The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support of this study (grant number 309523/2012-5).

REFERENCES

Alves GBB, FA Pinho, SM Silva, MS Cruz, FA Costa. 2010. Cardiac and pulmonary alterations in symptomatic and asymptomatic dogs infected naturally with *Leishmania* (Leishmania) *chagasi*. *Braz J Med Biol Res* 43, 310-315.

Apple FS, SW Sharkey, A Falahati, M Murakami, N Mitha, D Christensen. 1998. Assessment of left ventricular function using serum cardiac troponin I measurements following myocardial infarction. *Clin Chim Acta* 272, 59-67.

Baneth G. 2006. Leishmaniasis. In: Greene CE (ed). *Infectious diseases of the dog and cat*. 3rd ed. Saunders, Philadelphia, USA, Pp 685-698.

Baneth G, AF Koulinas, L Solano-Gallego, P Bourdeau, L Ferrer. 2008. Canine leishmaniasis - new concepts and insights on an expanding zoonosis: part one. *Trends Parasitol* 24, 324-330.

Barr SC, K Warner, BG Kornreich. 2008. Serum cardiac troponin I and cardiac lesion scores in a canine model of acute myocarditis. *J Vet Int Med* 16, 626.

Carretón E, R Morchón, F Simon, MC Juste, JC Méndez, JA Montoya-Alonso. 2014. Cardiopulmonary and inflammatory biomarkers in the assessment of the severity of canine dirofilariosis. *Vet Parasitol* 206, 43-47.

Costa FAL, H Goto, LC Saldanha, SM Silva, IL Sinhorini, TC Silva, JL Guerra. 2003. Histopathological patterns of nephropathy in naturally acquired canine visceral leishmaniasis. *Vet Pathol* 40, 677-684.

Defrancesco TC, JE Rush, EA Rozanski, BD Hansen, BW Keene, DT Moore, CE Atkins. 2007. Prospective clinical evaluation of an ELISA B-type natriuretic peptide assay in the diagnosis of congestive heart failure in dogs presenting with cough or dyspnea. *J Vet Intern Med* 21, 243-250.

Dibartola SP. 2000. Clinical approach and laboratory evaluation of renal disease. In: Ettinger SJ, Feldman EC (eds). *Textbook of veterinary internal medicine.* 5th ed. Saunders, Philadelphia, USA, Pp 1600-1614.

Diniz PPVP, DS Schwartz, RC Collicchio-Zuanaze. 2007. Cardiac trauma confirmed by cardiac markers in dogs: two case reports. *Arq Bras Med Vet Zootec* 59, 85-89.

Diniz PPVP, HAS Morais, EB Breitschwerdt, DS Schwartz. 2008. Serum cardiac troponin I concentration in dogs with ehrlichiosis. *J Vet Intern Med* 22, 1136-1143.

Eriksson AS, J Häggström, HD Pedersen, K Hansson, AK Järvinen, J Haukka, C Kvart. 2014. Increased NT-proANP predicts risk of congestive heart failure in Cavalier King Charles spaniels with mitral regurgitation caused by myxomatous valve disease. *J Vet Cardiol* 16, 141-154.

Ettinger SJ, R Prosek. 2010. Biomarkers of cardiovascular disease. In: Ettinger SJ, Feldman EC (eds) *Textbook of veterinary internal medicine*. 7th ed. Elsevier, St Louis, USA, Pp 1187-1193.

Freda BJ, WHW Tang, FV Lente, WF Peacock, GS Francis. 2002. Cardiac troponins in renal insufficiency: review and clinical implications. *J Am Coll Cardiol* 40, 2065-2071.

Godoy KCS. 2015. Análise dos indicadores de lesão miocárdica em cães com leishmaniose visceral. *Tesis Maestría*, College of Veterinary Medicine, Federal University of Mato Grosso do Sul, Brazil.

Gomes AAD, MD Laurenti, GC Ferraro, MHB Camargo, DC Costa, GF Machado, SHV Perri, M Marcondes. 2012. Subclinical muscle injuries in dogs infected with *Leishmania* (Leishmania) *infantum chagasi*. *Braz J Vet Pathol* 5, 108-115.

Güneş V, F Uyanik, M Eren, M Kibar, O Aslan, AC Onmaz. 2014. The rapid analyses of cardiac troponins in dogs with dilated cardiomyopathy, distemper or parvoviral infection. *Kafkas Univ Vet Fak Derg* 20, 921-927.

Langhorn R, LG Oyama, MC King, MC Machen, DJ Trafny, V Thawley, JL Willesen, I Tarnow, M Kjelgaard-Hansen. 2013. Prognostic importance of myocardial injury in critically ill dogs with systemic inflammation. *J Vet Intern Med* 27, 895-903.

Lobetti R, R Kirberger, N Keller, F Kettner, E Dvir. 2012. NT-ProBNP and cardiac troponin I in virulent canine babesiosis. *Vet Parasitol* 190, 333-339.

López-Sendón J. 2003. Troponinas y otros marcadores de daño miocárdico. Mitos y realidades. *Rev Esp Cardiol* 56, 16-19.

López-Peña M, N Alemán, F Muñoz, D Fondevila, ML Suárez, A Goicoa, JM Nieto. 2009. Visceral leishmaniasis with cardiac involvement in a dog: a case report. *Acta Vet Scand* 51, 20-22.

Maack T. 2006. The broad homeostatic role of natriuretic peptides. *Arq Bras Endocrinol Metab* 50, 198-207.

Martinez PP, MB Carvalho MB. 2010. Participação da excreção renal de cálcio, fósforo, sódio e potássio na homeostase em cães sadios e cães com doença renal crônica. *Pesq Vet Bras* 30, 868-876.

Mendes RS, TA Gurjão, LM Oliveira, VL Santana, WL Tafuri, JRS Santos, AFM Dantas, AP Souza. 2014. Miocardite crônica em um cão naturalmente infectado com *Leishmania* (Leishmania) *infantum chagasi*: aspectos clínicos e patológicos. *Arq Bras Med Vet Zootec* 66, 79-84.

Montes AM, C Gutiérrez-Panizo, P García-Partida. 1987. CK-MB, LDG and ASAT in dogs of different ages with infarct of miocardium. *An Vet (Murcia)* 2, 101-108.

Moonarmart W, A Boswood, VL Fuentes, D Brodbelt, K Souttar, J Elliott. 2010. N-terminal pro B-type natriuretic peptide and left ventricular diameter independently predict mortality in dogs with mitral valve disease. *J Small Anim Pract* 51, 84-96.

Pino VO, EO Li, SA Alvarado, VP Fernández, RF Dávila, CC Gavidia. 2008. Determinación de los niveles séricos de enzimas cardíacas en perros adultos com enfermedad cardiovascular. *Rev Inv Vet Perú* 19, 144-147.

Prosek R, DD Sisson, MA Oyama, PF Solter. 2007. Distinguishing cardiac and noncardiac dyspnea in 48 dogs using plasma atrial natriuretic factor, B-type natriuretic factor, endothelin, and cardiac troponin-I. *J Vet Intern Med* 21, 238-242.

Raffan E, J Loureiro, J Dukes-MacEwan, S Fonfara, R James, S Swift, N Bexfield, ME Herrtage, J Archer. 2009. The cardiac biomarker NT-proBNP is increased in dogs with azotemia. *J Vet Intern Med* 23, 1184-1189.

Reynolds C, M Oyama. 2008. Biomarkers in the diagnosis of canine heart disease. *Vet Focus* 18, 2-6.

Richards AM. 2007. Natriuretic peptides. Update on peptide release, bioactivity, and clinical use. *Hypertension* 50, 25-30.

Rosa FA, JHAC Leite, ET Braga, PRR Moreira, FH Baltazar, AW Biondo, PPM Padua, RO Vasconcelos, AA Camacho, WL Ferreira, GF Machado, M Marcondes. 2014. Cardiac lesions in 30 dogs naturally infected with *Leishmania infantum chagasi*. *Vet Patholol* 51, 603-606.

Schober KE, B Kirback, G Oechtering. 1999. Noninvasive assessment of myocardial cell injury in dogs with suspected cardiac contusion. *J Vet Cardiol* 1, 17-25.

Santos ALF. 2005. Dosagem sérica da enzima creatinafosfoquinase-isoenzima MB (CK-MB) e de troponina I (cTnI) de cães eletrocardiograficamente normais e naqueles com desnível (infra e supra) do segmento ST, utilizando ensaio imunométrico por quimioluminescência. *Tesis Maestría*, College of Veterinary Medicine and Animal Science, University of São Paulo, Brazil.

Santos FP. 2013. Aspectos clínicos e histopatológicos do miocárdio de cães naturalmente infectados com leishmaniose visceral. *Tesis Maestría*, College of Veterinary Medicine, Federal University of Pampa, Brazil.

Serres F, JL Pouchelon, L Poujol, HP Lefebvre, C Trumel, T Daste, CC Sampedrano, V Gouni, R Tissier, G Hawa, V Chetboul. 2009. Plasma N-terminal pro-B-type natriuretic peptide concentration helps to predict survival in dogs with symptomatic degenerative mitral valve disease regardless of and in combination with the initial clinical status at admission. *J Vet Cardiol* 11, 103-121.

Sharkey SW, KJ Elsperger, M Murakami, FS Apple. 1991. Canine myocardial creatine kinase isoenzyme response to coronary artery occlusion. *Circulation* 84, 333-340.

Shaw SP, EA Rozanski, JE Rush. 2004. Cardiac troponins I and T in dogs with pericardial effusion. *J Vet Intern Med* 18, 322-324.

Silvestrini P, M Piviani, J Alberola, A Rodríguez-Cortés, M Planellas, X Roura, PJ O'Brien, J Pastor. 2012. Serum cardiac troponin I concentrations in dogs with leishmaniasis: correlation with age and clinicopathologic abnormalities. *Vet Clin Pathol* 41, 568-574.

Sleeper MM, CA Clifford, LL Laster. 2001. Cardiac troponin I in the normal dog and cat. *J Vet Intern Med* 15, 501-503.

Sousa MG, R Carareto, JG Silva, J Oliveira. 2013. Assessment of the electrocardiogram in dogs with visceral leishmaniasis. *Pesq Vet Bras* 33, 643-647.

Torrent E, M Leiva, J Segalés, J Franch, T Peña, B Cabrera, J Pastor. 2005. Myocarditis and generalized vasculitis associated with leishmaniosis in a dog. *J Small Anim Pract* 46, 549-552.