

Electrocardiographic evaluation and serum cardiac troponin I levels in anemic dogs with blood parasitosis

Avaliação eletrocardiográfica e níveis séricos de troponina I cardíaca em cães anêmicos com hemoparasitose

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Abstract

Blood parasitosis is a frequent cause of anemia and myocarditis in dogs. Because anemia can trigger hypoxia or hemodynamic disturbances and myocarditis secondary to blood parasitosis can develop arrhythmias, electrocardiogram changes and increased levels of cardiac biomarkers, such as cardiac troponin I, are expected in dogs with blood parasitosis. Thus, this study aimed to investigate the electrocardiographic characteristics and the relationship to troponin I levels in anemic dogs with blood parasitosis. Twenty-eight dogs with blood parasitosis (seropositive for *Ehrlichia canis*/ *Babesia canis*) and varying levels of anemia were evaluated. Dogs with very severe anemia (hematocrit below 13%) showed significant increases in heart rate when compared to dogs with severe, moderate or mild anemia. There was a predominance of respiratory sinus arrhythmia in dogs with mild, moderate and severe anemia and a high prevalence of sinus rhythm in dogs with very severe anemia, which showed a decrease in parasympathetic activity in this group. In all of the groups, electrocardiographic changes were detected showing compatibility with left and right atrial and left ventricular enlargement. Dogs with very severe anemia had a longer P wave duration. All groups of anemic dogs showed increased T-wave amplitude, suggestive of myocardial hypoxia. However, only the groups with moderate and severe anemia presented ST segment disturbances. Despite the lack of a correlation between the electrocardiographic changes suggestive of myocardial hypoxia and troponin I, there was a negative correlation ($r = -0.95$, $p = 0.05$) between hemoglobin levels and increased serum troponin. These data indicate that anemic dogs with blood parasitosis may have electrocardiographic changes suggestive of atrioventricular overload or hemodynamic disturbances, such as increased heart rate. Moreover, the negative correlation between hemoglobin and serum levels of troponin I may imply that dogs with severe and very severe anemia are at a higher risk for myocardial injury.

Key words: Cardiac biomarker, electrocardiogram; hematocrit, myocardial hypoxia

Resumo

Hemoparasitoses são causas frequentes de anemia e miocardite em cães. Em virtude da anemia poder provocar distúrbios hemodinâmicos relacionados à hipóxia e da miocardite secundária à hemoparasitose causar arritmias cardíacas, esperam-se alterações eletrocardiográficas e aumento dos níveis de

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biomarcadores cardíacos como troponina I cardíaca em cães com hemoparasitose. Por conseguinte, este estudo teve como objetivo investigar as características eletrocardiográficas de cães anêmicos por hemoparasitose e a relação da anemia com alterações eletrocardiográficas e aumento da troponina I cardíaca. Para tanto, foram avaliados vinte e oito cães com hemoparasitose, sorologicamente positivos para *Ehrlichia canis*/ *Babesia canis*, com diferentes níveis de anemia. Cães com anemia muito grave (hematócrito abaixo de 13%) apresentaram aumentos significativos da frequência cardíaca, comparados aos cães com anemia grave, moderada ou leve. Houve predomínio de arritmia sinusal respiratória em cães com anemia leve, moderada e grave e alta ocorrência de ritmo sinusal em cães com anemia muito grave, demonstrando diminuição do tônus parassimpático neste grupo. Em todos os grupos, foram detectadas alterações eletrocardiográficas sugestivas de sobrecarga ventricular e atrial esquerda, no entanto, cães com anemia muito grave, tiveram maiores durações de ondas P. Em todos os grupos de cães anêmicos, houve aumento da amplitude da onda T, sugestiva de hipóxia do miocárdio. No entanto, apenas os grupos com anemia moderada e grave apresentaram distúrbios do segmento ST. Apesar da falta de correlação entre as alterações eletrocardiográficas sugestivas de hipóxia miocárdica e os níveis de troponina I cardíacas, houve correlação negativa ($r = -0,95$, $p = 0,05$) entre os níveis de hemoglobina e o aumento da troponina sérica. Estes dados permitem inferir que cães anêmicos em decorrência de hemoparasitoses podem apresentar alterações eletrocardiográficas sugestivas de sobrecarga atrioventricular ou distúrbios hemodinâmicos, como aumento da frequência cardíaca. Ademais, a correlação negativa entre hemoglobina e os níveis séricos de troponina I cardíaca podem implicar em maior risco de lesão miocárdica em cães com anemia mais graves.

Palavras-chave: Biomarcadores cardíacos, eletrocardiograma, hematócrito, hipóxia do miocárdio

Introduction

Anemia is defined as a reduction in the number of erythrocytes and/or a subnormal concentration of hemoglobin per unit volume of blood. The decrease in red cell mass results in reduced oxygen transport and may lead to tissue hypoxia and hemodynamic and non-hemodynamic compensatory events (JONES; HUNT; KING, 2000; CHAMPION; PEREIRA NETO; CAMACHO, 2011). Examples of non-hemodynamic events include an increase in erythropoietin production and a decrease in the affinity of hemoglobin for oxygen (METIVIER et al., 2000). Hemodynamic events, in turn, are related to increased sympathetic activity with consequent increases in cardiac output and to a decrease in systemic vascular resistance due to arterial vasodilation and reduced viscosity (AIRD, 2000; METIVIER et al., 2000).

The cardiac effect of anemia can be explained by the increased metabolic demand of the heart that may exceed its supply of oxygen, leading to hypoxia and myocardial infarction as described in humans with anemia (PORTMAN; STANDAERT; NING, 1995; SZACHNIEWICZ et al., 2003). The

hemodynamic effects of anemia can be related to cardiac decompensation in patients with heart failure (HORWICH et al., 2002; KOMAJDA, 2004).

Furthermore, hemodynamic changes secondary to chronic anemia may result in cardiac remodeling because chronic hypoxia causes structural alterations in the cardiac myocyte and activation of other inflammatory systems that worsen heart failure (HORWICH et al., 2002).

There are many causes of anemia in dogs, and the severity can be classified by magnitude, time of evolution and cardiopulmonary adaptations (AIRD, 2000). Ehrlichiosis and babesiosis are frequent causes of anemia and may worsen heart function due to possible promotion of myocardial injury, such as myocarditis and the subsequent development of arrhythmias (LOBETTI; DVIR; PEARSON, 2002; LOBETTI, 2005; DINIZ et al., 2008).

The electrocardiogram is a useful test for the diagnosis of arrhythmias, but it can also be used to evaluate signs of cardiac chambers enlargement and disorders related to myocardial hypoxia (TILLEY, 1995). Furthermore, the cardiac biomarkers are being used in various clinical situations to assess the

degree of myocardial injury in heart disease in several clinical situations in which myocardial lesions can be related to non-cardiac injuries and anemia as babesiosis (LOBETTI; DVIR; PEARSON, 2002; LOBETTI, 2005) ehrlichiosis (DINIZ et al., 2008) and immune-mediated hemolytic anemia (GOW et al., 2011). Higher serum concentrations of cardiac troponin I in dogs with primary hemolytic anemia than hospitalized ill dogs or healthy dogs revealed that anemia can promote subclinical myocyte damage (GOW et al., 2011).

Because of the possibility of electrocardiographic changes resulting from hemodynamic dysfunction in conditions such as anemia and the possibility of cardiac injury caused by hemoparasitosis, this study aimed to evaluate the electrocardiographic parameters and determinate cardiac troponin I levels in anemic dogs due to natural infection by *Ehrlichia canis* and/or *Babesia canis*.

Material and Methods

Animals

Twenty-eight dogs without preexisting heart disease and with anemia due to ehrlichiosis and/or canine babesiosis were placed into four groups according to the severity of anemia. The groups were classified as mild, moderate, severe and very severe anemia, represented by a hematocrit ranging from 30 to 35% (n=7), 20 to 29% (n=7), 13 to 19% (n=7) and below 13% (n=7), respectively. The control group consisted of seven healthy dogs with a negative serology for ehrlichiosis and babesiosis. All animals of each group underwent measurements of serum troponin I levels, blood count, an electrocardiogram and a Doppler echocardiogram.

Anemic dogs were 6.43 ± 3.83 years old, weighed 10.45 ± 7.76 kg and included 14 males and 14 females. The study included 18 mongrel dogs, six poodles, one pinscher, one boxer, one beagle and one American pit bull. The dogs in the control group were 5.99 ± 2.91 years old, weighed 11.56 ± 6.31

kg, and included five females and two males, of which three were Mongrel dogs, two were poodles and two were beagles.

Hematologic examinations

Blood samples were collected by jugular venipuncture for the attainment of complete blood counts. The global count of erythrocytes, leukocytes and platelets, the hemoglobin levels and the packed cell volume average were determined by automated cell counts (ACT-8 Coulter[®] - Miami - EUA), and the differential leukocyte counts were made in blood smears stained with Rosenfeld and examined by microscopy optics (Microscope Nikon[®] Eclipse - E 200 Tokyo - Japan). The serological diagnosis of ehrlichiosis and babesiosis was performed by indirect immunofluorescent reaction (RIFI) to detect antibodies against *Babesia canis* and *Ehrlichia canis*, where the samples with titers above 1:20 for *E. canis* and 1:40 for *B. canis* were considered positive. Moreover, the determination of cardiac troponin I levels was performed by an automated quantitative method (VIDAS[®] - Troponin I Ultra - Biomeri[®] - Marcy-l'Etoile - France) with the ELFA technique (Enzyme-Linked Fluorescent Assay), and the lower limit for the dosage was 0.01 mg/L.

Electrocardiogram

A six-lead computerized electro-cardiogram (ECG-PC - TEB - São Paulo, Brazil) was used for electrocardiography recording. The dogs were positioned in right lateral recumbence, and the electrode clips were attached to the animals' skin in the standard positions as described by Tilley (1992). The paper speed was 50 mm/sec, and the electrocardiogram was standardized at 1 mV = 1 cm. The electrocardiogram analysis was obtained from lead II. We measured the following parameters: duration of P wave (Pms), P wave amplitude (PmV), duration of PR interval (PRms), duration of QRS complex (QRSms), R wave amplitude (RmV),

duration of QT interval (QTms), ST segment amplitude (ST), T wave amplitude (T), mean electrical axis, heart rate (HR) and heart rhythm.

Statistical analysis

The statistical analysis was performed using statistical software (Statistical software MINITAB 15.1 State College, USA). The distribution pattern of normality was assessed by Kolmogorov-Smirnov test. Parametric variables were analyzed using analysis of variance (ANOVA) followed by a Tukey's test (electrocardiographic parameters). The Pearson

correlation test was used to compare hemoglobin levels and electrocardiographic parameters, and the chi-square test was used to analyze categorical variables (troponin elevation and degree of anemia) using a 5% level of significance.

Results

The mean values and the standard deviations of the total erythrocyte count, the hemoglobin concentration and the hematocrit of the control group and the anemic groups, divided according to the severity of anemia, are shown in Table 1.

Table 1. Mean values \pm SD of erythrocytes, hemoglobin and hematocrit in the control group, and the mild, moderate, severe and very severe anemia groups.

	Erythrocytes $\times 10^3$	Hemoglobin (g/dL)	Hematocrit (%)
Control Group	6337.14 \pm 662.14	14.97 \pm 1.75	46.09 \pm 5.64
Mild anemia	4965.71 \pm 162.36	11.81 \pm 1.35	33.37 \pm 1.59
Moderate anemia	4040.00 \pm 415.57	9.33 \pm 1.29	27.09 \pm 2.97
Severe anemia	2444.29 \pm 452.62	5.76 \pm 0.73	17.23 \pm 1.34
Very severe anemia	1502.86 \pm 410.96	3.36 \pm 0.44	10.21 \pm 1.68

Source: Elaboration of the authors.

Only three out of the 28 anemic dogs showed no evidence of concurrently possessing anti-Erlichia and anti-Babesia antibodies. Of these dogs, two dogs from the group with severe anemia had positive serology only for *Erlichia canis*, and one dog from the group with very severe anemia had positive serology only for *Babesia canis*.

Pertaining to cardiac rhythm, there was a predominance of respiratory sinus arrhythmia in groups with mild and moderate anemia, and in both groups, 87.71% of the dogs presented this rhythm. The group with severe anemia revealed a predominance of sinus arrhythmia, involving

57.14% of the dogs. In the group with very severe anemia, 42.86% of the dogs showed sinus arrhythmia. The second-most common rhythm was sinus rhythm, appearing in 12.29% of the dogs in groups with mild and moderate anemia and 42.86% of the dogs in groups with severe and very severe anemia. Only one dog in the group with severe anemia showed junctional rhythm.

The heart rate in dogs with very severe anemia was higher than the other groups, and there were no significant differences between groups with mild, moderate and severe anemia. The heart rate values and other electrocardiographic parameters are shown in Table 2.

Table 2. Mean values and standard deviations of electrocardiographic parameters in dogs (n = 28) with different degrees of anemia caused by *Ehrlichia canis* and *Babesia canis* infections in relation to the control group.

	Control Group (n=7)	Anemia			
		Mild (n=7)	Moderate (n=7)	Severe (n=7)	Very severe (n=7)
P (ms)	41.86±2.54 ^a	45.57±4.61 ^a	44.28±6.13 ^a	44.72±5.62 ^a	55.71±6.72 ^b
P(mV)	0.20±0.02 ^a	0.23±0.10 ^a	0.15±0.06 ^a	0.18±0.09 ^a	0.19±0.05 ^a
PR (ms)	90.43±10.5 ^a	109.14±6.4 ^a	95.71±20.1 ^a	90.43±6.6 ^a	87.00±23.1 ^a
QRS (ms)	54.57±4.61 ^a	56.71±7.18 ^a	59.14±5.61 ^a	51.43±7.74 ^a	60.14±10.7 ^a
R (mV)	1.10±0.27 ^a	1.20±0.25 ^a	1.61±0.18 ^a	1.04±0.84 ^a	1.39±0.49 ^a
QT (ms)	189.0±8.6 ^a	216.7±26.0 ^b	208.9±32.2 ^a	184.9±14.5 ^a	188.7±23.7 ^a
HR (bpm)	122.1±22.5 ^a	104.9±18.0 ^a	105.9±16.9 ^a	130.0±23.4 ^a	135.6±12.8 ^b
Axis (°)	73.86±11.48 ^a	72.86±15.99 ^a	67.86±13.75 ^a	40.57±68.05 ^a	63.43±15.91 ^a

Pms = P wave duration (milliseconds); PmV = P wave amplitude (millivolt); PRms = PR interval duration (milliseconds); QRSms = QRS complex duration (milliseconds); RmV = R wave amplitude (millivolt); QTms = QT interval duration (milliseconds); HRbpm = heart rate beats/minute; Axis (degree) = electrical cardiac axis. Values (mean ± standard deviation) with the same letters in the same row for each group (control, mild, moderate, severe and very severe) are not statistically different (P > 0.05).

Source: Elaboration of the authors.

The analysis of wave amplitudes and durations in DII according to reference values for dogs (TILLEY, 1992; WOLF; CAMACHO; SOUZA, 2000) showed that 85.71% (24/28) of the anemic dogs produced at least one altered

electrocardiographic parameter. Table 3 shows the percentage of electrocardiographic abnormalities in anemic dogs classified according to the severity of anemia in mild, moderate, severe and very severe groups.

Table 3. Percentage of electrocardiographic abnormalities in anemic dogs (n = 28) divided according to the severity of anemia in four groups of seven dogs (mild, moderate, severe and very severe) and caused by natural infection with *Ehrlichia canis* and *Babesia canis*.

ECG abnormalities	Mild (%)	Moderate (%)	Severe (%)	Very severe (%)
Increase in P-wave amplitude	42.86	14.29	28.57	14.29
Increase in P-wave duration	28.57	28.57	42.86	100
Increase in QRS duration	28.57	28.57	14.29	42.86
Increase in R-wave amplitude	14.29	85.71	28.57	57.14
Abnormalities in PR interval	0	0	0	0
Abnormalities in QT interval	57.14	14.29	14.29	42.86
ST segment depression	0	42.86	14.29	0
ST segment elevation	0	0	14.29	0
Increase in T-wave amplitude	14.29	14.29	14.29	14.29
RV axis deviation	0	0	14.29	0
Cardiac rhythm disturbance*	0	0	0	14.29

* Junctional Rhythm; ECG = Electrocardiographic; RV = right ventricular.

Source: Elaboration of the authors.

However, when compared to the control group, only the values of P-wave amplitudes in the group

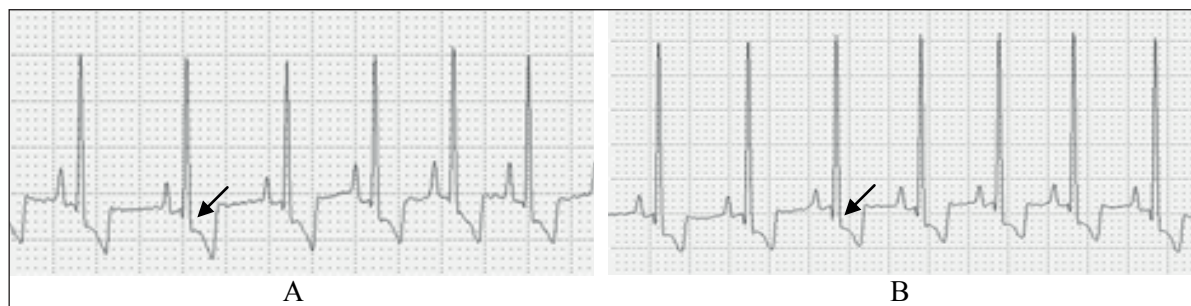
with severe anemia and the QT intervals in the group with mild anemia were significantly increased. There

was an association between the degree of anemia and an increased P wave duration ($p = 0.003$).

Only 10.71% (3/28) of the anemic dogs showed increased concentrations of troponin I, with two of these dogs having very severe anemia and one having severe anemia. There was a negative

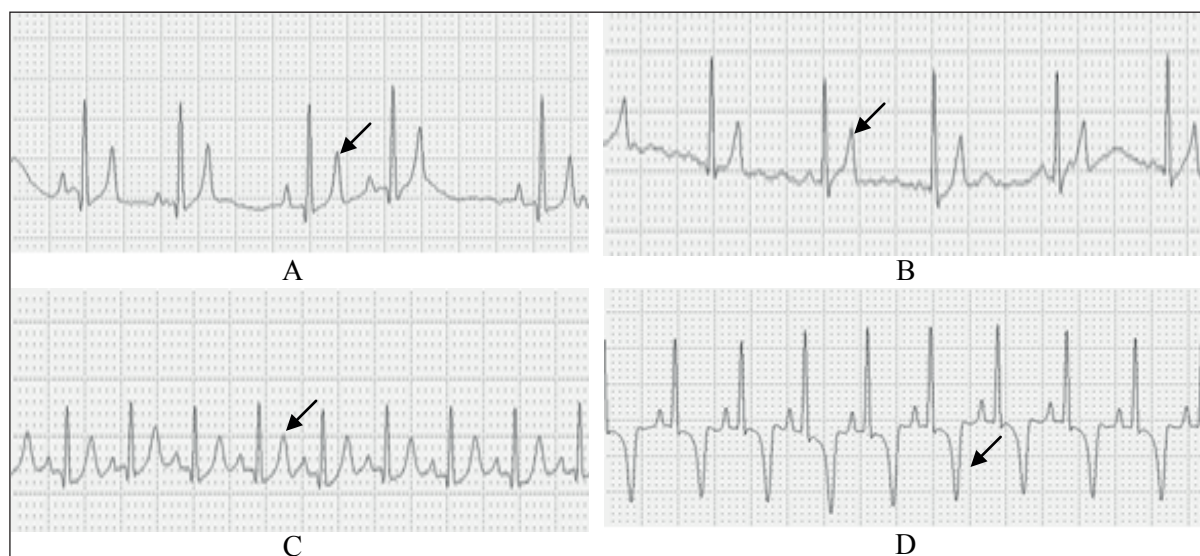
correlation between the level of hemoglobin and an increased troponin I concentration ($r = -0,950$, $p = 0,05$). However, there was no association between increased levels of troponin I and electrocardiographic parameters suggestive of myocardial hypoxia, such as ST-segment elevation (Figure 1) or T-wave changes (Figure 2).

Figure 1. Electrocardiographic recordings showing ST-segment depression in dogs with moderate (A) and severe (B) anemia. Lead DII, 50 mm/s, sensibility, N.



Source: Elaboration of the authors.

Figure 2. Electrocardiographic recordings showing increases in T-wave amplitude in dogs with mild (A), moderate (B), severe (C) and very severe (D) anemia. Lead DII, 50 mm/s, sensibility, N.



Source: Elaboration of the authors.

Discussion

Among the cardiac rhythms assessed by electrocardiography, sinus arrhythmia occurred most frequently (85.71%) in dogs with mild and

moderate anemia. In dogs with severe and very severe anemia, the occurrence of this rhythm was not as significant, likely due to the sympathetic activity surpassing the activities of the parasympathetic

nervous system. In response to hypoxemia, it was observed that patients with anemia may have generalized peripheral vasodilation leading to hypotension, and as a compensatory mechanism, the vasodilation activates the sympathetic nervous system, allowing vasoconstriction to maintain a normal blood pressure range (MARTINS et al., 2012). Apart from vasoconstriction, sympathetic activation promotes an increased heart rate and positive inotropism (HORWICH et al. 2002; ANAND et al., 2004). In this study, there was no occurrence of sinus tachycardia; however, although the heart rate was within normal limits in all groups, the mean values of heart rates in dogs with very severe anemia were statistically higher than all the other dogs. The heart rate is increased in anemia due to hypoxia-stimulated chemoreceptors and increased sympathetic activity (METIVIER et al., 2000).

Electrocardiogram characteristics compatible with atrioventricular overload are perhaps related to an increased plasma volume, high levels of myocardial wall stress and left ventricular hypertrophy that occur in response to anemia because tissue hypoxia and peripheral vasodilation related to anemia lead to a hemodynamic response, such as sympathetic activation (METIVIER et al., 2000; MARTINS et al., 2012), activation of the renin angiotensin aldosterone system and increased production of the antidiuretic hormone (HORWICH et al., 2002; ANAND et al., 2004). These mechanisms promote water retention, which, when combined with myocardial hypoxia and oxidative stress, explain why anemia may precipitate congestive heart failure, even in normal individuals (ANAND et al., 2004).

In this study, left atrial enlargement was related to the degree of anemia severity, and all dogs with very severe anemia showed increases in the P-wave duration. The same can be observed in relation to left ventricular overload because dogs with very severe anemia showed increases in the QRS complex more frequently (48.86%) compared to other groups.

Because every animal included in this study had not heart disease, electrocardiographic findings consistent with atrial or ventricular overload may be related to compensatory hemodynamic changes. Furthermore, electrocardiographic traces that suggest left ventricular overload, such as changes in the QRS complex and the R wave, corroborate the findings on left ventricular hypertrophy previously described in dogs with anemia (SCHEEL; WILLIAMS, 1985).

Although only one dog had right ventricular electrical axis deviation, there was no consensus about the effects of anemia in the cardiac axis because there are reports of right axis deviation (MANOACH et al., 1971), left axis deviation (ROSENTHAL; RESTIEAUX; FEIG, 1971) and absence of ventricular axis deviation related to anemia (SINGH; SOOD, 1996).

The group of dogs with mild anemia showed a significant increase in the mean values of the QT interval; however, there is no clinical relevance because all values were within the reference values (TILLEY, 1992).

Abnormalities in the ST-segment (ST depression and elevation) were found in 17.86% of all anemic dogs, and only animals in groups with moderate and severe anemia showed these electrocardiographic abnormalities. It is presumed that this may be due to the preservation of tissue oxygenation and the absence of myocardial hypoxia in dogs with mild anemia because ST abnormalities are related to damage in ventricular repolarization. However, the absence of ST abnormalities in dogs with very severe anemia does not rule out the possibility of impaired myocardial perfusion and oxygenation because in this group, an alteration in the T-wave amplitude occurred, which also indicates impaired repolarization (TILLEY, 1992), and some dogs showed increases in troponin I concentrations, indicating myocardial damage (LINDE, et al., 2006). Currently, measurements using cardiac biomarkers have been used to assess the degree

of cardiac injuries, and troponin I is considered to be a biomarker with high sensitivity (O'BRIEN; LANDT; LADENSON, 1997). Cardiac troponin I is located exclusively in the myocardium where it is the only existing form, and its sensitivity and specificity for acute myocardial infarction are 97% and 95%, respectively (LINDE et al., 2006). The increase in cardiac troponin I levels, especially in dogs with advanced degrees of anemia, may indicate the possibility of cardiac injury both by hypoxia and myocarditis, as described in dogs with myocarditis and hemoparasitosis (LOBETTI; DVIR; PEARSON, 2002; SHAH; MCGOWAN, 2003; DINIZ et al., 2008).

The cardiac injury associated with anemia results from a reduction in red cell mass because erythrocytes provide oxygen to myocardial cells and promote the exchange of antioxidants that prevent oxidative stress and programmed cell death (SZACHNIEWICZ et al., 2003). Dogs with other etiologies for anemia showed higher levels of cardiac troponin I (GOW et al., 2011). Moreover, with an increase in sympathetic activation, the time of diastole is reduced, and this may impair coronary perfusion and increased myocardial oxygen consumption (CRUVINEL; CASTRO, 2003).

In the study, the association between the levels of troponin I and anemia were verified, showing the possibility of cardiac lesions related to anemia. Ralli, Horwich and Fonarow (2005) indicated that anemia is a factor for increased mortality by heart failure in humans, and they found increased levels of troponin I in 50.9% of the anemic patients. This should be verified in dogs with cardiomyopathy.

The study has some limitations, such as the evaluation of 28 patients divided into four groups of seven dogs only. Other limitations are related to atrial and ventricular measurements of enlargement, since the electrocardiogram is not the gold standard exam for measuring cardiac chambers. Therefore, thoracic radiography and

doppler echocardiography could increase the values of the results of this study. The laboratory tests that could be performed are PCR to confirm the diagnosis of blood parasitosis and the electrolytic evaluation to exclude its influence on electrocardiographic parameters as ST segment and T wave. Despite these limitations, the results of this study are relevant and further investigations can be performed to confirm the influences of hypoxia related to anemia on cardiovascular parameters in dogs, in several experimental models, since anemia is a very common medical condition in small animals.

Conclusions

Dogs with anemia may develop electrocardiographic changes consistent with sympathetic activation, such as increased heart rate and a decreased prevalence of respiratory sinus arrhythmia. Electrocardiographic changes suggestive of atrial enlargement, such as increased duration and amplitude of the P wave, are likely related to increased preload and cardiac remodeling. Despite changes in repolarization suggestive of myocardial hypoxia, as the amplitude of the T wave increased, an elevation in the ST segment was not very frequently found in this study. However, there were increases in the levels of troponin I in 10.71% of the dogs. It is noteworthy that there was an association between increased troponin I and the level of severity of anemia, showing a risk of cardiac damage in anemic dogs.

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