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P-wave dispersion and renal biomarkers in canine visceral leishmaniasis stage III and IV infected dogs

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Abstract: Canine visceral leishmaniasis is a protozoan disease affecting most vital organs and also causing myocardial and/or renal damage. P-wave dispersion, a newly used non-invasive ECG indicator, is used to follow atrial arrhythmias, atrial fibrillation, valvular disease in both human and veterinary medicine. The purpose of this study involving Canine Visceral Leishmaniasis (CVL)-infected dogs in stage III and IV was to verify whether the P-wave dispersion was related to the renal failure. For this purpose, a total of 17 dogs of different ages, both sexes, comprising eleven animals with CVL (at stage III and IV) diagnosed using a rapid ELISA (enzyme-linked immunosorbent assay) test and serological IFAT (immunofluorescence antibody titres) and six healthy dogs used as controls were enrolled. Significant differences between the P-wave dispersion (regarding the mean values) of the control, stage III and IV-infected groups ($P = 0.003$) were detected as follows: 21.8 ± 0.5, 20.6 ± 1.2 and 25.0 ± 0.4 ms, respectively. The P-wave dispersion value was moderately longer in the stage IV-infected group compared with the control one ($P = 0.022$), however, the aforementioned relationship was determined as significant between the stage IV and III-infected groups ($P = 0.003$). Regarding all of the biomarkers of the renal function monitored within this study, there were differences between the control and stage IV-infected group for the creatinine ($P = 0.002$), the control and stage IV-infected group for the urine protein/creatinine ratio ($P < 0.001$), and the stage III and stage IV-infected groups for the urine protein/creatinine ratio ($P < 0.001$). In conclusion, an increased P-wave dispersion was not associated with renal failure. It might be related to the limitation of our study including small sample sizes in the groups. Therefore, the cardiac indicators were not analysed. For more accurate results, it is desirable to perform further studies including a larger sample size along with an investigation of the cardiac and renal indicators.

Keywords: last stage infection; non-invasive ECG indicator; renal failure

Canine Visceral Leishmaniasis (CVL), caused by the prevalent agent *Leishmania infantum* is a zoonotic protozoan disease transmitted by phlebotomine sand flies as biological vectors (Baneth et al. 2008). CVL, a multisystemic disease affects most organs; skin lesions are the most frequently encountered clinical findings (Solano-Gallego et al. 2004; Baneth et al. 2008). CVL-associated myocarditis has been reported already with myocardial damage that involves parasitic lymphocytic infiltration, and necrosis. However, the related clinical changes have been less frequently stated (Font et al. 1993; Lopez-Pena et al. 2009; Rosa et al. 2014; dos Santos et al. 2015).

Some biomarkers, electrocardiographic (ECG) and echocardiographic findings are being used for the diagnosis of asymptomatic cardiac alterations in CVL-infected dogs (Lopez-Pena et al. 2009; Silva et al. 2016; Ural et al. 2017). P-wave dispersion (P_d), a relatively newly used non-invasive ECG indicator defined as the difference between the widest and the narrowest P-wave duration may be used to assess

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the risk of, e.g., supraventricular arrhythmias in dogs (Noszczyk-Nowak et al. 2008). This indicator is used to evaluate atrial arrhythmias and atrial fibrillation in human medicine (Dilaveris and Gialafos 2001); it is a novel tool in veterinary medicine to follow a chronic valvular disease (Noszczyk-Nowak et al. 2011), in obese dogs with and without mitral valve disease (Dittrich et al. 2018), and atrial fibrillation (Noszczyk-Nowak 2012). To our knowledge, only one study including P_d alteration associated with CVL has been reported with increasing P_d values in polysymptomatic dogs (Nakipoglu and Ural 2018).

Renal failure has especially been encountered in the progressive last stages of CVL-infected dogs (Solano-Gallego et al. 2011) and none of the studies indicated whether the P_d were related to renal failure. For this purpose, we aimed at studying whether the P_d were related to the renal failure in CVL-infected dogs that had a stage III and IV infection.

MATERIAL AND METHODS

Animals and laboratory analysis

The study included different ages, breeds and both sexes of dogs with several clinical signs related to CVL (ocular lesions, anorexia, cachexia, onychogryphosis, lymphadenopathy, dermatitis). The diagnosis was confirmed by a positive serological rapid ELISA test (Snap Leishmania Test; IDEXX Laboratories, Barcelona, Spain) (Ferroglio et al. 2007) along with the positive results obtained using an indirect immunofluorescence antibody titre (IFAT) above 1/64 (Paltrinieri et al. 2016). The control animals were enrolled based on the negative rapid ELISA and IFAT results mentioned above. A total of 17 dogs, comprising eleven with CVL and six healthy animals were enrolled.

Along with the complete clinical examination, blood samples were collected from *v. cephalica antebrachii* into serum tubes (8 ml) for the IFAT, serum urea and creatinine analysis along with urine samples (10 ml) for detecting the urine creatinine, protein and UPC (urine protein/creatinine) ratio analysis.

Renal biomarkers included the serum BUN (blood urea nitrogen) with creatinine (Crea), urine Crea and urine protein/creatinine (UPC) ratio were analysed to both evaluate the kidney failure and classify the CVL stage III and IV. Besides classification into the CVL stage, various clinical findings were identified with positive antibody concentrations. The staging of the groups was determined with UPC > 1 or serum creatinine 0.5–0.71 mmol/l (stage III) and UPC > 5 with marked proteinuria, serum creatinine 0.71–1.78/> 1.78 mmol/l (stage IV). UPC < 0.1 was accepted for the negative control dogs (Solano-Gallego et al. 2011).

No dogs infected with other parasites such as *Ehrlichia canis*, *Dirofilaria immitis*, *Anaplasma phagocytophilum*, *Anaplasma platys* and *Borrelia burgdorferi* (Snap 4Dx Test; IDEXX Laboratories, Barcelona, Spain) causing kidney disease or animals with previously administered drugs (antibiotic, glucocorticosteroids, drugs for leishmaniasis treatment) were accepted for the study. The dogs were provided by volunteer owners after they were informed about the study and the CVL.

Evaluation of P-wave dispersion

A standard 12-lead surface ECG (50-mm/s, 10- mV/cm, and 100-Hz) was recorded and the ECG signals were saved automatically with a BTL MT08 device. All the electrodes were placed as described by Noszczyk-Nowak et al. (2008).

Then P duration was measured with the distance between the beginning of the P-wave deflection from the isoelectric baseline and the returning of the P-wave offset to the isoelectric baseline on the six ECG leads (I, II, III, aVR, aVL, aVF) at five cardiac cycles. P_d was assessed based on the mean value obtained by the differences between P_{min} and P_{max} values of the P-wave from the five measurements (Noszczyk-Nowak et al. 2008; Noszczyk-Nowak et al. 2011; Okutucu et al. 2016; Kollu et al. 2018). The P_d dispersion was calculated based on the measurements using computerised methods with a software program (BTL Cardiopoint, Ankara, Turkey).

Statistical analysis

A statistical analysis was carried out using the SPSS statistical program (v22; SPSS Inc., Chicago, IL, USA). An ANOVA (analysis of variance) test was used to analyse the P_d and the renal biomarker differences between the groups with the selected 95% CI (confidence interval) and $P < 0.05$ as significant.

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Table 1. The descriptive data of the P_d, BUN, Crea and UPC ratio values in the CVL infected dogs with the last stages

Group	P_d (ms)			BUN (mmol/l)		Crea (mmol/l)		UPC	
	n	mean ± SEM	min-max	mean ± SEM	min-max	mean ± SEM	min-max	mean ± SEM	min-max
Control	6	21.8 ± 0.55[a]	20–23.8	5.28 ± 0.67	3.57–7.5	0.04 ± 0.06[a]	0.03–0.07	0.1 ± 0.00[a]	0.1–0.1
Stage III	5	20.6 ± 1.25	18.2–25.2	9.14 ± 1.026	7.14–11.78	0.15 ± 0.01[b]	0.12–0.17	2.4 ± 0.25	2–3
Stage IV	6	25.0 ± 0.40[b]	23.6–26	11.78 ± 2.84	4.64–25	0.18 ± 0.04[b]	0.11–0.36	7.8 ± 0.98[b]	5–10

[a,b]The values indicated by the different letters in the columns are statistically significant ($P < 0.001$)

BUN = blood urea nitrogen; Crea = creatinine; P_d = P-wave dispersion; UPC = urine protein/creatinine

RESULTS

The P_d mean values (in ms) of the control, stage III and IV CVL-infected dogs were determined as 21.8 ± 0.55, 20.6 ± 1.24, and 25.0 ± 0.40 ms, respectively (Table 1). The differences among the groups were significant ($P = 0.003$). The P_d value was moderately longer and negative in the stage IV infected group compared with the control ($P = 0.022$), however, this relationship was determined as more significant between the stage IV and III infected groups ($P = 0.003$).

The descriptive analysis of the renal biomarkers (BUN, Crea and UPC ratio) is presented in Table 1. As the distribution of these indicators was evaluated according to the groups (Figure 1), there were differences between the control and stage IV infected group for the creatinine ($P = 0.002$), the control and the stage IV infected group for the UPC ratio ($P < 0.001$) and the stage III and the stage IV infected group for the UPC ($P < 0.001$). Conversely, there was no significant correlation between the P_d and the renal biomarkers within the groups.

DISCUSSION

In this study, the P_d was assessed with an ECG based on previous studies (Noszczyk-Nowak et al. 2008; Noszczyk-Nowak et al. 2011; Okutucu et al. 2016; Kollu et al. 2018) and the P_d mean values (in ms) differed significantly between the groups with 21.8 ± 0.55 (control), 20.6 ± 1.24 (stage III) and 25.0 ± 0.40 (stage IV) ms. In one of the studies performed on healthy dogs, the mean P_d was recorded as 16.9 ± 9.4 (Noszczyk-Nowak et al. 2008). In another study in a different stage, the CVL-infected dogs' P_d was recorded at 22.76 ± 3.12 ms in the control (Nakipoglu and Ural 2018). Our present results obtained for the control group were similar to those in the latter study (Nakipoglu and Ural 2018).

According to our results, the P_d value was moderately longer and negative in the stage IV infected group compared with the control ($P = 0.022$), and between the stage IV and III infected groups ($P = 0.003$). Similar to a previous study in which significant differences occurred between the polysymptomatic group and the controls ($P = 0.026$) (Nakipoglu and

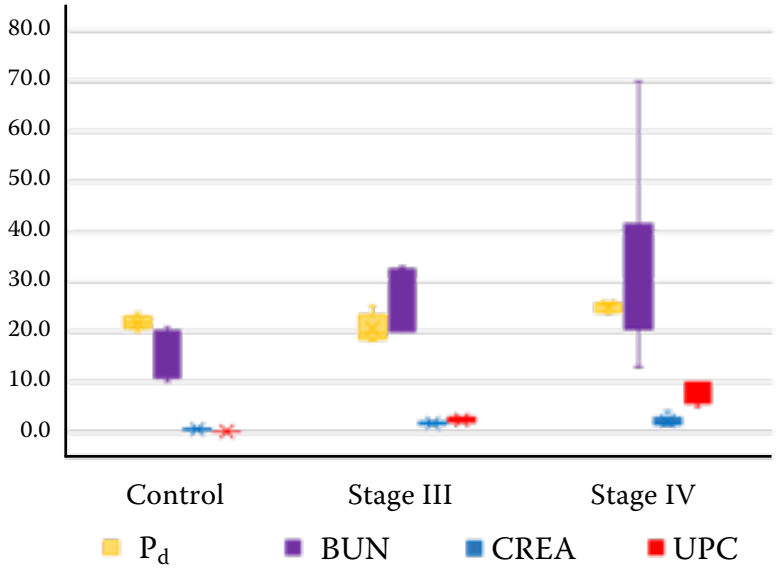

Figure 1. Box plot graphics regarding the P_d, BUN, Crea and UPC in the control and Leishmaniasis groups. The results are shown as box and whisker plots (the boxes extend from the 25th to the 75th percentile; and the whiskers represent 95% confidence intervals)

BUN = blood urea nitrogen; Crea = creatinine; P_d = P-wave dispersion; UPC = urine protein/creatinine

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Ural 2018), there were significant differences among the groups of our study. This finding may be related to the myocardial effect of the CVL infection in the dogs, especially in the last stage of illness (dos Santos et al. 2015; Ural et al. 2017).

In the present study, significant alterations among the groups were as follows: between the control and the stage IV infected group for the creatinine ($P = 0.002$), between the control and the stage IV infected group for the UPC ratio ($P < 0.001$) and the stage III and the stage IV infected group for the UPC ratio ($P < 0.001$). On the other hand, no significant correlations between the P_d and the renal biomarkers within the groups were evident.

Taking into account the important role of the ECG data for the interpretation of the myocardial injury in the CVL-infected dogs (Lopez-Pena et al. 2009), there is a lack of detailed literature on P_d alterations in these dogs. To our knowledge, only one study including a P_d alteration associated with the CVL reported increasing P_d values in polysymptomatic dogs (Nakipoglu and Ural 2018). Although renal failure eventuates, especially in the progressive last stages of the CVL infected dogs (Solano-Gallego et al. 2011), none of the studies indicated whether the P_d were related to the renal failure.

CVL may impair the renal structure with the tubular and glomerular damage (Zaragoza et al. 2003; Solano-Gallego et al. 2011), and besides, the proteinuria might be detected during the infection (Solano-Gallego et al. 2011). There were alterations in the blood creatinine and the UPC ratio between the stage IV and control groups, whereas it was not found between the stage III and the control groups, it was solely detected between the stage III and IV infected dogs among the UPC ratios. According to the authors of this study, it is assumed that it might be related to the microalbuminuria in most of the CVL infected patients, although normal creatinine levels might be detected (Elnojomi et al. 2010).

It was reported that if the P_d values exceed > 63.0 ms, it might be related to the decreased GFR (glomerular filtration rate) and the progression of the renal end point (Su et al. 2012). Different studies supported these results indicating that the renal damage increases the cardiovascular mortality (Khan et al. 2006; Huang et al. 2014) or increases the demand of the dialysis in the end stage of the chronic renal disease (Huang et al. 2014) with higher P_d values. On the other hand, the P_d values of haemodialysed patients without an atrial fibrillation were determined as higher than those of the healthy patients (Atar et al. 2007). In conclusion, we diagnosed an increased P_d as not associated to the renal failure unlike several human medicine studies (Khan et al. 2006; Su et al. 2012; Huang et al. 2014). It might be related to the limitation of our study including the small samples size in the groups and the cardiac indicators that were not followed. For more accurate results, further studies are warranted including larger sample sizes along with investigating the cardiac indicators.

Conflict of interest

The authors declare no conflict of interest.

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