Evaluation of cardiovascular injury in dogs coinfected with visceral leishmaniasis and monocytic ehrlichiosis by echocardiographic examination and selected biomarker measurements

Canberk Balıkçı[1]*, Erdem Gülersoy[1], Songül Erdoğan[2], İsmail Günal[1], Adem Şahan[1], Hasan Erdoğan[2] and Kerem Ural[2]

[1]*Department of Internal Medicine, Faculty of Veterinary Medicine, University of Harran, Şanlıurfa,Turkiye.*
[2]*Department of Internal Medicine, Faculty of Veterinary Medicine, University of Aydın Adnan Menderes, Aydın, Turkiye.*

**Corresponding author at: Department of Internal Medicine, Faculty of Veterinary Medicine, University of Harran, Şanlıurfa, Turkiye*
E-mail: canberkbalikci@gmail.com.

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Summary
Visceral Leishmaniasis (VL) and Monocytic Ehrlichiosis (ME), which are an important zoonotic diseases of dogs, causing multiple organ dysfunction and has a poor prognosis when not interfered. In this study, it was aimed to investigate the cardiovascular injury that develops in dogs that co-infected with VL and ME with cardiovascular biomarkers and echocardiographic parameters. The animal material of this study was consisted of 14 owned dogs in total; 7 diseased dogs which were determined to be co-infected with VL and ME according to the results of clinical examination and rapid test kits, and 7 healthy dogs, which were determined to be healthy as a result of the same examinations. As a result of echocardiographic examinations, decreased left ventricular cytolic and diastolic diameters (LVIDs, LVIDd), fractional shortening (FS) and increased ratio of left atrium to left aortic root diameter (LA/Ao) values were determined in the Co-infected Group compared with the Healthy Group. Also, as a result of biomarker analysis, higher cTnI) D-dimer and NT-proBNP levels were detected in the Co-infected Group. In conclusion, considering studies of dogs infected with VL and/or ME alone, it was concluded that similar cardiovascular injury develops in dogs co-infected with VL and ME.

Introduction

Visceral Leishmaniasis (VL) is a zoonotic disease of dogs which its importance is emphasized by the World Health Organization (WHO), has a poor prognosis if not intervened due to the damage it causes in various tissues and organs. Since dogs are both reservoir and host in the transmission of the disease, VL is considered as an emerging public health threat (Sollano-Gallego *et al*. 2011, Torres-Guerrero *et al*. 2017). Studies have reported that the most important pathological changes are on the cardiovascular system besides various organ and system disorders (Ural *et al.* 2017, Balikci and Ural 2018). Monocytic Ehrlishiosis (ME), which is similar to canine VL (Canine Visceral Leishmaniasis, CVL) infection, is another vector-borne disease of dogs. ME, in which ticks play a role in the transmission of the disease, has been reported mostly in tropical and subtropical regions (Lin and Rikihisa 2003). Like CVL, significant cardiovascular injuries have been reported in dogs with ME infection (Diniz *et al.* 2008).

As a result of the literature scan, no study was found that evaluated the cardiovascular injury

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in dogs naturally co-infected with ME and VL. Therefore, in this study, it was aimed to evaluate the cardiovascular injury with echocardiographic examinations (including LVIDs, LVIDd, EF and FS parameters) along with selected biomarkers such as cardiac troponin I (cTnI), D-dimer and N terminal pro b-type natriuretic peptide (with NT-proBNP) in dogs naturally co-infected with VL and ME.

Materials and methods

Animals

The animals involved in this study consisted of 14 mixed breed dogs in total, aged 2-4 years, which were brought for diagnosis/treatment or routine check-up/vaccination. As a result of clinical and laboratory examinations with rapid diagnosis test kit results, 7 dogs (3 males and 4 females, non-neutered, mean age of 3.71 (2,4) years, 20 (7-28) kilograms of body weight) which were determined to be co-infected with VL and ME, were enrolled in the Co-infected Group. Also, as a result of the same clinical, laboratory and rapid diagnostic test kit examinations, 7 dogs (2 male and 5 female, non-neutered, mean age of 2.86 (2, 4), 15 (10, 18) kilograms of body weight) which were determined to be healthy were enrolled in the Control Group.

Inclusion/exclusion criteria

In order to rule out the diseases (*Dirofilaria sp., Anaplasma phagocytophilum, Anaplasma platys and Borrelia burgdorferi*) that may cause similar clinical findings similar to VL and ME infections, rapid diagnostic test kits (SNAP 4Dx Plus®, Idexx, USA) were applied to the dogs of the Co-infected Group of the study. Dogs with any comorbid disease were excluded from the study. The same examinations and tests were applied to the dogs of the Control Group. All examination and test results were negative in context of differential diagnosis.

Clinical examinations

In accordance with the anamnestic data obtained as a result of face-to-face interviews with the animal owners, all of the dogs were living at home, were taken to a walk 2-3 times a day and were fed with commercial dry dog food. Heart rate, respiratory rate, body temperature and capillary refill time (CRT) measurements, evaluation of dehydration status and palpable lymph nodes were assessed in all the dogs within the scope of clinical examinations. In addition, auscultation of the lungs and heart (mitral valve, in line with the costochondral junction of the left 5th intercostal space; aortic valve, just above the costrochondral junction of the left 4th intercostal space; pulmonary valve, just above the sternum in the left 2nd-4th intercostal space region; tricuspid valve, right 3rd-5th intercostal space near the costrochondral junction) was performed as previously reported (Pace, 2017).

Collecting blood samples

Venous blood samples (5-10 mL) were obtained by vena cephalica venepuncture with minimal restraint in order to not cause stress to all dogs included in the study. The blood samples were taken into tubes without anticoagulant, centrifuged at 5000 rpm for 5 minutes and their serum was extracted. Some of the serum samples (3-5 mL) were used for rapid diagnostic test kits.

Centrifugation and rapid diagnostic test kit applications were performed within 10-15 minutes after blood sampling. The remaining serum samples were stored in the freezer at -80 °C until the day of biomarker measurements.

Rapid diagnostic test kit applications

Confirmation of CVL and canine ME (CME) infections in dogs which were enrolled in the Co-infected Group based on clinical findings such as anorexia, lethargy, exercise intolerance, and arrhythmia, was made by rapid ELISA test kits (SNAP 4Dx Plus®, Idexx, USA) applications from the serum samples of the dogs of the Co-infected Group in accordance with the manufacturer's instructions.

CVL and CME infections were confirmed according to the rapid diagnostic test kit results along with clinical findings (Athanasiou *et al.* 2014). The same tests were applied to the dogs of the Control Group and all were found to be negative.

Echocardiographic examinations

Two-dimensional motion (M) and bright (B) mode ECO examinations of all the dogs were performed using a portable ECO device (Mindray M5®, China) by the same personnel. ECO, M and B mode examinations were performed from the right parasternal long and short axis (4th-6th intercostal space) on the right side.

Within the scope of In ECO examination, categorical parameters such as left ventricular cytolic and diastolic diameters (LVIDs, LVIDd), ejection fraction (EF), fractional shortening (FS) (Testuz *et al.* 2013) and the ratio of left atrium to left aortic root diameter (LA/Ao), which is the indicator of left atrial enlargement (Tai and Huang 2013) were evaluated.

Biomarker measurements

Serum cTnI, D-dimer and NT-proBNP concentrations were measured using a commercial analysis system (Wondfo Finecare® Fluorescent Immunoassay) at Adnan Menderes University Faculty of Veterinary Medicine Department of Internal Medicine laboratory. The linear range (min-max) of the Finecare® Fluorescent Immunoassay was 0.1-50 ng/mL, 0.1-10 mg/L and 18-35000 pg/mL for cTnI, D-dimer, and NT-proBNP, respectively. Measurements below the lower detection limit (0.1 ng/mL, 0.1 mg/L and 18 pg/L for cTnI, D-dimer and NT-proBNP, respectively) were used for statistical analysis. Considering the evaluation of pmol/L in studies of NT-proBNP measurements in dogs, measurement was assessed based on the conversion factor of 1 pg/mL = 0.118 pmol/L (Weber 2006).

Statistical analysis

All data were evaluated using SPSS 21.00 (SPSS for Windows®) statistical software. One sample Kolmogorov-Smirnov test was applied to determine whether all data were parametric or non-parametric. Parametric data were evaluated as median (min, max) with Mann Whitney U, Kruskal-Wallis test. In addition, Receiver Operating Characteristic curve (ROC) analysis was used to distinguish healthy dogs from the co-infected ones using cardiovascular injury biomarkers. Within the scope of ROC analysis, Area Under Curve (AUC), standard error (std. error), sensitivity, specificity and Observed Power parameters were evaluated.

Moreover, Pearson correlation test was performed to determine possible correlations between LVIDs, LVIDd, EF, FS and LA/Ao (Tai and Huang 2013) with other clinical and echocardiographic parameters. Statistical significance was accepted as $p<0.05$ for all the data.

Results

Clinical examination results

As a result of clinical examinations, among the dogs of the Co-infected group, two dogs had high body temperature (40.0 °C and 40.4 °C) and four had tachycardia (162, 170, 178 and 185 beats/min). In addition, lymphadenopathy (n: 7, 100%), hypothyrcosis (n: 7, 100%), exercise intolerance (n: 6, 85.7%) and onychogriposis (n: 4, 57.1%) were determined as the most important clinical findings in the dogs of the Co-infected Group. The percentage distribution of symptoms along with all clinical examination findings of the Co-infected Group are presented in Table I.

Table I. *Percent distribution of clinical findings and symptoms of the Co-infected Group.*

Clinical Findings	Percentage Distribution of the Symptoms
Fever	n=2 (%28.6)
Lymphadenopathy	n=7 (%100)
Weight loss*	n=5 (%71.4)
Onychogryposis	n=4 (%57.1)
Hypotrichosis	n=7 (%100)
Periocular alopecia	n=1 (%14.3)
Epistaxis	n=2 (%28.6)
Exercise intolerance *	n=6 (%85.7)
Arrhythmia	n=3 (%42.9)

* Based on body condition score and daily diet intake (Brooks et al., 2014).

Echocardiographic examination results

Within the scope of echocardiographic examinations, LVIDs, LVIDd, EF, FS and LA/Ao parameters were evaluated. No statistical difference was determined in the comparison of the Control and Co-infected groups ($p>0.05$). Echocardiographic examination findings of the Control and Co-infected groups are presented in Table II.

Table II. *Echocardiographic Examination Findings of the Control and Co-infected groups.*

Parameters	Control Group median (min, max)	Co-infected Group median (min, max)	P value
LVIDs (cm)	1.96 (1.83, 3.22)	2.1 (1.03, 3.68)	0.966
LVIDd (cm)	2.91 (2.49, 4.23)	3.39 (2.52, 4.37)	0.671
EF %	58 (48, 74)	65 (35.3, 92)	0.560
FS %	30 (24, 42)	36 (16.4, 63)	0.445
LA / Ao	1.26 (1.05, 1.45)	1.3 (1, 2.06)	0.345

LVIDs: left ventricular internal systolic dimension, LVIDd: left ventricular internal diastolic dimension, EF: Ejection fraction, FS: Fractional shortening, LA/Ao: Ratio of left atrium to aortic root.

Biomarker measurement results

Within the scope of biomarker measurements, the levels of cTnI, Nt-proBNP and D-Dimer were higher in the Co-infected Group compared to the Control Group ($p<0.05$). All biomarker measurement findings are presented in Table III.

Table III. *Biomarker Measurement Findings of the Control and Co-infected groups.*

Parameters	Control Group median (min, max)	Co-infected Group median (min, max)	P value
cTnI (ng/ml)	0.09 (0.09, 0.09)	0.1 (0.09, 0.2)	0.038
NT-proBNP (pmol/L)	61.9 (61.9, 62.9)	251.89 (61.9, 1939.68)	0.035
D-Dimer (mg/L)	0,09 (0.09, 0.09)	1.3 (0, 5.1)	0.025

cTnI: Cardiac troponin I, NT-proBNP: N-terminal pro b-type natriuretic peptide.

ROC analysis results

ROC analyzes (CI: 95%) were performed to evaluate the efficacy of CTnI, NT-proBNP and D-dimer parameters, which were statistically different, in the determination of the cardiovascular injury and the distinguishment of healthy dogs from the diseased ones. As a result of the comparative ROC analysis, it was determined that CTnI had high AUC (0.857), sensitivity (71.4%) and specificity (100%); NT-proBNP had high AUC (0.898), sensitivity (85.7%) and specificity (100%) and D-dimer had very high AUC (0.929), sensitivity (85.7%) and specificity (100%). Comparative ROC analysis results and ROC curves are presented in Table IV and Table VI, respectively.

Table IV. *ROC analyzes findings*

Parametreler	AUC	Std. Error	P value	Asymp. % 95 CI		Cut-off	Sensitivity	Specificity	Observed Power
				Lower Bound	Upper Bound				
cTnI (ng/ml)	0.857	0.112	0.025	0.638	1.000	0.095	71.4%	100%	57.1%
NT-proBNP (pmol/L)	0.898	0.101	0.013	0.699	1.000	71.585	85.7%	100%	59%
D-Dimer (mg/L)	0.929	0.082	0.007	0.767	1.000	0.15	85.7%	100%	65.3%

cTnI: Cardiac troponin I, NT-proBNP: N-terminal pro b-type natriuretic peptide, AUC: Area under curve, Std. error: Standard error, CI: Confidence interval.

Pearson correlation test results

Pearson correlation tests of the parameters which were evaluated within the scope of the echocardiographic examination and the body weights of all the dogs were performed. As a result of the test, a strong positive correlation was found between LVIDs and LVIDd, a reasonable negative correlation between LVIDs and EF, a reasonable negative correlation between LVIDs and FS and a strong positive correlation between EF and FS were observed.

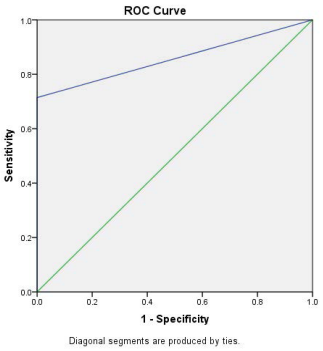
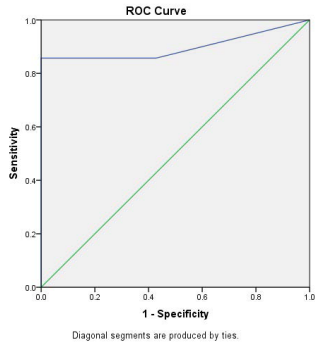
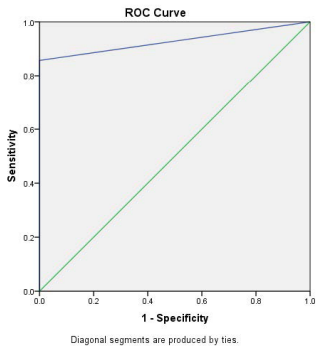
No correlations were observed between the other parameters. Test results are presented in Table V.

Table V. *Pearson correlation test results of echocardiographic parameters with body weight*

Parametreler	Body weight (kg)	LVID %	LVIDd %	EF %	FS %	LA / Ao
Body weight (kg)	1	.334 .244	.353 .215	.083 .777	.177 .545	.464 .095
LVIDs (cm)		1	.845** .000	-.591* .026	-.596* .024	.192 .510
LVIDd (cm)			1	-.178 .542	-.185 .527	.382 .178
EF (%)				1	.983** .000	.331 .248
FS (%)					1	.313 .276
LA / Ao						1

Kg: Kilogram, cm: Centimeter, LVIDs: left ventricular internal systolic dimension, LVIDd: left ventricular internal diastolic dimension, EF: Ejection fraction, FS: Fractional shortening, LA/Ao: Ratio of left atrium to aortic root, **. Correlation is significant at the 0.01 level (2-tailed), *. Correlation is significant at the 0.05 level (2-tailed).

Table VI. *ROC curves of statistically different biomarkers*

Parametreler	Receiver Operating Characteristic Curves
CTnI	
NT-proBNP	
D-Dimer	

Discussion

In this study, cardiovascular injury in dogs co-infected with VL and ME was evaluated with echocardiographic examinations along with selected biomarkers such as cTnI, NT-proBNP and D-dimer for the first time. Based on ROC analyzes, it was concluded that compared to previous reports in dogs infected with VL and/or ME alone, more severe cardiovascular injury develops in dogs naturally co-infected with VL and ME and in addition, evaluation of cTnI, D-dimer and NT-proBNP biomarkers along with ECO examinations are useful in demonstrating the injury Visceral Leishmaniasis is a vecto-borne zoonotic and life-threatening disease which causes systemic findings such as loss of appetite, weakness, exercise intolerance, lymphadenopathy, keratoconjunctivitis, onychogriposis and hyperthermia in dogs (Poli *et al.* 1997, Baneth *et al.* 2008, Symeonidou *et al.* 2021, Gradoni *et al.* 2022).

Also, depending on the period of the disease arrhythmia, epistaxis, nasal depigmentation, skin erosion and severe ulcerations may develop (Solano-Gallego *et al.* 2011, Balikci and Ural 2018). Similar to VL, abnormalities in many organs and systems including kidney, liver, lymphoid tissue and respiratory system along with haematological abnormalities such as thrombocytopenia, anemia, and vasculitis manifesting as cutaneous and mucosal petechiae and ecchymoses and especially epistaxis have been reported as prominent clinical findings in ME infection (De Castro *et al.* 2004, Pugliese *et al.* 2022). In the present study, the clinical findings (Table I) of the Co-infected Group were consistent with previous reports (Poli *et al.* 1997, De Castro *et al.* 2004, Baneth *et al.* 2008, Solano-Gallego *et al.* 2011, Sainz *et al.* 2015). It was an expected result that clinical findings such as lymphodenopathy, hypotrichosis, exercise intolerance and weight loss would have a higher incidence rate in cases of co-infection due to postulation of a synergistic pathological effect between the pathogens.

Although more severe reduction of the platelet aggregation and haemostatic disorders were reported in cases of co-infection (Cortese *et al.* 2009), the lesser incidence rates of epistaxis and arrhythmia in the present study were thought to be related to the stage of the disease and/or the time of admission to the hospital and the formation of organ damage depending on the severity of the immune-mediated response.

Evaluation of left atrial (La) dimensions in dogs provides significant clinical information for assessing the stage of an existing disease, effect of the treatment protocol and prognosis (Safian *et al.* 2022). In a study, it was reported that the La/Ao >1.5 cut-off value is an indicator of left atrial enlargement, and the La/Ao >2 cut-off value is an indicator of more severe left atrial enlargement (Tai and Huang 2013). Also, it was reported that La/Ao < 1.5, 1.5 ≤ La/Ao ≤ 1.8 and La/Ao > 1.8 values can be used to classify left atrial enlargement (Safian *et al.* 2022). In addition, in an echocardiographic study, it was reported that the normal FS value should be >30% in healthy dogs (Dukes-McEwan *et al.* 2003, Vörös *et al.* 2009). Moreover, in several studies of dogs with heart damage and/or diseases such as mitral valve abnormalities, myxomatous mitral valve disease and dilated cardiomyopathy, it was reported that the FS value is statistically significantly reduced (Cheng *et al.* 2021, Chompoosan *el al.* 2021, Elsharkawy *et al.* 2022, Tidholm and Häggström 2022).

In addition, there are studies reporting correlation between body weight and echocardiographic parameters (Cornell *et al.* 2004, Vörös *et al.* 2009, Tidholm and Häggström 2022). It was reported that an increase in the La/Ao ratio along with decrease in LVIDd, LVIDs and FS values support the presence of cardiomyopathy in cases of VL infection in dogs (Ural *et al.* 2017). In a study of 50 dogs infected with ME, the FS value was reported to be low in 25 dogs and the LVIDd value was reported to be low in 10 dogs (Diniz *et al.* 2008). It has been reported that consistent echocardiographic results could not be achieved in experimentally infected dogs with ME (Kalogianni *et al.* 2016).

In addition, pulmonary hypertension due to cardiomegaly (hypovolemic left ventricle and flattening of interventricular septum) has also been reported by echocardiographic examination in a dog with suspected ehrlichiosis (Toom *et al.* 2016). However, there are no reported LA/Ao ratio in dogs mono-infected with ME. In the present study, two of the dogs in the Co-infected Group had low LVIDd, LVIDs and FS values and three of them had high LA/Ao ratio. These findings were interpreted as a result of cardiomyopathy in cases of VL and ME coinfection. Yet, more echocardiographic studies are needed to investigate the presence of heart damage/disease in dogs monoinfected with ME. It is common to observe a strong positive correlation between LVIDs and LVIDd, as disruptions in myocardial contractility caused by myocarditis may affect both the diastolic and systolic phases due to changes in ventricular internal diameters (Diniz *et al.* 2008, Vörös *et al.* 2009, Hamabe *et al.* 2015, Ural *et al.* 2017, Cheng *et al.* 2021, Chompoosan *el al.* 2021, Elsharkawy *et al.* 2022, Tidholm and Häggström 2022).

In addition, it was reported that pathological increases in ventricular internal diameters may cause decreases in EF and FS values (Turgut 2017). Recent studies in dogs with heart diseases such as dilated cardiomyopathy and myxomatous mitral valve disease report similar correlations between LVIDs and EF and between LVIDs and FS (Cheng *et al.* 2021, Elsharkawy *et al.* 2022). In the present study, the negative correlation between LVIDs and EF and between LVIDs and FS was consistent with previous reports (Hamabe *et al.* 2015, Turgut 2017). Since FS and EF are useful parameters in evaluation of the systolic performance of the heart, decreases in systolic performance cause changes in both parameters.

That is why, a strong positive correlation was reported between FS and EF (Cheng *et al.* 2021, Elsharkawy *et al.* 2022, Gugjoo *et al.* 2014). Since the evaluation of systolic function and therefore EF and FS is fundamental in diseases that cause hemodynamic unstableness, evaluation of the correlation of EF and FS in the present study may indicate left ventricular dilatation (Diniz *et al.* 2008).

In Canine Visceral Leishmaniasis (CVL) and CME, myocarditis was reported to be a significant finding (Silva *et al.* 2016, Filippi *et al.* 2019, Casamián-Sorrosal *et al.* 2021). cTnI is an important biomarker to demonstrate the myocardial injury (Langhorn *et al.* 2013, Pereira *et al.* 2022) and its high levels were reported in CVL infection (Silva *et al.* 2016, Balikci and Ural 2018, Casamián-Sorrosal *et al.* 2021). Similar to CVL, myocarditis was also reported in CME and high cTnI levels were determined to be useful in demonstrating the presence of myocarditis (Diniz *et al.* 2008, Kalogianni *et al.* 2016, Filippi *et al.* 2019).

Compared to dogs which were mono-infected with CVL and/or CME, the level of cTnI of the present study was significantly higher ($p=0.038$) compared with both previous reports (Diniz *et al*. 2008, Kalogianni *et al.* 2016, Silva *et al.* 2016, Balikci and Ural 2018) and the Control Group of the present study except for one report which study on CVL mono infection (Casamián-Sorrosal *et al.* 2021). The higher cTnI levels of the coinfected dogs in the present study may be associated with greater damage in cases of coinfection and the development of pathological synergy between the agents. However, aforementioned high levels of cTnI (Casamián-Sorrosal *et al.* 2021) may be related to the fact that the study population was formed of dogs which had been determined to be euthanized.

Another biomarker used in the evaluation of myocardial injury and for quantitative assessment of various cardiac diseases in dogs is NT-proBNP (Brennan *et al.* 2022). It was reported that the level of NT-proBNP <900 pmol/L is not associated with cardiac stress whereas >1800 pmol/L is associated with severe cardiac damage. Furthermore, NT-proBNP values between 900-1800 pmol/L was reported to suggest cardiac damage (Baisan *et al.* 2016). There are studies reporting that Nt-proBNP can be used for the diagnosis of dilated cardiomyopathy, the prognosis of myxamatous mitral valve disease, and the differentiation of cardiac and non-cardiac respiratory distress (Prosek *et al.* 2007, Fine *et al.* 2008, Oyama *et al.* 2008, Oyama *et al.* 2009, Brennan *et al.* 2022). In previous studies, which were conducted in dogs which mono-infected with VL, positive correlations between the severity of the infection and the level of NT-proBNP were reported (Silva *et al.* 2016, Balikci and Ural 2018, Casamián-Sorrosal *et al.* 2021). Also, in a study of mono-infected dogs with ME, higher NT-proBNP levels in the diseased dogs compared to the healthy ones were reported, but its increase was observed to be lower than 900 pmol/L (Dogan 2019). Besides, no increase in serum NT-proBNP levels was reported in dogs which mono-infected with ME.

The normal NT-proBNP levels were reported to associate with the absence of any stress on the heart muscle due to normal pressure in the heart chambers (Filippi *et al.* 2019). In the present study, NT-proBNP levels were above 1800 pmol/L in 3 dogs of the Co-infected Group. Considering previous studies (Silva *et al.* 2016, Balikci and Ural 2018, Casamián-Sorrosal *et al.* 2021), this finding was interpreted as a result of higher effect of CVL over serum NT-proBNP levels than CME. Moreover, the high serum NT-proBNP level of the Co-infected Group compared with the Control Group of the present study was thought to develop as a result of severe infection and cardiac damage. The reason why high NT-proBNP levels could not be detected in the present study may be related to the absence of any stress on the heart muscle and the normal pressure of the heart chambers in dogs infected with chronic ME (Filippi *et al.* 2019). Therefore, studies with a larger number of animals in dogs with ME can be used to compare abnormal findings that are not detected in cases of coinfection.

D-Dimer is a highly sensitive indicator of fibrinolysis and activated coagulation and can be used in the diagnosis and follow-ups of cases of venous thromboembolism and disseminated intravascular coagulopathy (DIC) (Boyé *et al.* 2021). In studies of dogs which mono-infected with VL, it was reported that DIC may develop secondary to infection and high D-Dimer levels can be observed (Honse *et al.* 2013, Balikci *et al.* 2015, Balikci and Ural 2018). A similar increase was also reported in dogs which mono-infected with ME (Diniz *et al*. 2008, Erdogan *et al.* 2018, Atikyilmaz and Cingi 2021). In the present study, higher D-Dimer levels of the Co-infected Group were interpreted as a predisposing factor of VL and ME coinfection to the development of DIC ($p<0.05$) and more severe reduction of the platelet aggregation (Cortese *et al.* 2009). In addition, D-Dimer levels of the present study were numerically higher compared to the previous levels in cases of mono-infection (Honse *et al.* 2013, Balikci *et al.* 2015). This finding was also interpreted as more severe haemostatic disorders such as the presence of thromboembolism and DIC (Atikyilmaz and Cingi 2021) develop in cases of coinfection (Cortese *et al.* 2009). In the present study, as a result of comparative ROC analysis of biomarker levels associated with cardiovascular abnormalities, high diagnostic accuracy for cTnI and NT-proBNP and very high diagnostic accuracy for D-Dimer levels were determined. Based on the ROC analysis of the present study, it was determined that the selected biomarkers in the present study may be useful in distinguishing healthy dogs from the diseased ones and in demonstrating the cardiovascular injury.

There are some limitations in this study. First, disease period staging based on the presence of antigen-antibody (acute, active and exposing infection) has not been made. Second, it is the low number of animals that may affect the results of the comparative ROC analysis. For these reasons, studies which include more dogs are needed on naturally co-infected dogs with disease stage classified.

In this study, cardiovascular injury that develops as a result of VL and ME in dogs that naturally co-infected, was evaluated with echocardiographic examinations and selected biomarker measurements. Low LVIDd, LVIDs, FS and high LA/Lo values, as well as high cTnI, D-dimer and NT-proBNP levels were determined in dogs naturally co-infected with VL and ME.

As a result, considering the studies of dogs infected with VL and/or ME alone, it was concluded that severe cardiovascular injury develops in dogs co-infected with VL and ME and the evaluation of cTnI, D-dimer and NT-proBNP was useful in demonstrating the injury.

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