Research Article

Evaluation of Hematological, Biochemical, and Echocardiographic Findings in Dogs Infected with *Dirofilaria spp.*

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Abstract

Canine dirofilariasis is a significant disease that associate with heart and cardiopulmonary complications. In endemic regions, the co-occurrence of other vector-borne infections further complicates diagnosis and treatment. Additionally, the lack of standardized diagnostic tools to assess disease progression represents a critical gap in veterinary literature. In this study echocardiographic, hematological, and biochemical findings of dogs infected with heartworm alone and those co-infected with other vector-borne diseases were compared. Furthermore, the study aimed to evaluate the usability of these parameters in determining the prognosis and severity of the disease. This study included 12 dogs diagnosed with *Dirofilaria spp.* infection, categorized into two groups: mono-infected (n=7) and co-infected (n=5). Dirofilaria antigens and additional co-infecting agents were detected using the Knott test and SNAP 4Dx Plus, blood samples were collected for complete blood count (CBC) and serum biochemistry analysis. Each dog underwent an echocardiographic evaluation. While most parameters were similar between the mono-infected and co-infected groups, platelet (PLT) counts and mean corpuscular hemoglobin concentration (MCHC) values were lower, and liver enzyme levels were higher in the co-infected group. Although echocardiographic parameters were generally similar, the mono-infected group showed higher left atrial dimensions and ventricular volumes, while the co-infected group exhibited slightly elevated fractional shortening (FS) and ejection fraction (EF) values. These findings suggest that co-infection may influence both platelet counts and liver enzyme levels. This study indicates that co-infections in dogs with dirofilariasis may lead to lower PLT and MCHC levels, accompanied by higher liver enzyme levels, which could impact disease management approaches.

Keywords: Canine dirofilariasis, Echocardiography, Vector borne

Introduction

Canine dirofilariasis, commonly known as heartworm infection, could cause cardiac disorder and has also been mentioned as one of the the top ten causes of mortality, particularly in tropical and temperate regions (Kim, 2011; Rath et al., 2014). Caused by the nematode *D.immitis*, this parasitic disease primarily targets the cardiopulmonary system, leading to structural and functional impairments such as cardiomegaly, pulmonary artery enlargement, and congestive heart failure. Clinical manifestations may range from asymptomatic to severe conditions with symptoms including weight loss, lethargy, exercise intolerance, and chronic cough, depending on the infection load (Maxwell et al., 2014).

In advanced stages, the disease often results in right ventricular enlargement, increased pulmonary artery pressure, and tricuspid regurgitation, as observed through echocardiography (Browne et al., 2005; Oldach et al., 2018). These structural changes complicate the progression of dirofilariasis and can lead to right ventricular insufficiency (atrioventricular canal dilation, tricuspid valve insufficiency, and subsequent right atrial enlargement) and other severe complications (Atkins et al., 1988; Venco et al., 2014; Falcón-Cordón et al., 2019).

The diagnosis of dirofilariasis is complex, often involving a combination of epizootiological data, clinical signs, pathoanatomical findings, and laboratory diagnostics (Strickland, 1998; Hoch & Strickland, 2008; Romano et al., 2021; Yermolenko et al., 2022). Hemolarvascopy and other blood tests are commonly employed to detect dirofilaria in blood samples; however, these approaches may have limited efficacy, particularly in cases where immature nematodes are present (Magnis et al., 2013; Ionica et al., 2017; Genchi et al., 2021). More advanced techniques, such as rapid immunochromatographic tests and genetic assays, offer enhanced sensitivity, enabling detection of both mature and immature dirofilaria species (Albonico et al., 2014; Borthakur et al., 2015). Imaging tools like radiography and echocardiography are invaluable in assessing cardiopulmonary complications related to dirofilariasis, providing crucial insights into the extent of cardiopulmonary involvement (Venco et al., 1996; Little et al., 2018; Corda et al., 2022).

In endemic regions, co-infections with other vector-borne diseases add further complexity to the diagnosis and management of dirofilariasis. Other vector-borne diseases, such as ehrlichiosis, babesiosis, and anaplasmosis, frequently occur alongside heartworm infections (Radzijevskaja et al., 2020; Ramos et al., 2022). The co-occurrence of these pathogens complicates both clinical presentation and treatment strategies, posing a unique challenge for veterinary practitioners, especially in areas with high prevalence rates of these vector-borne diseases (Otranto et al., 2009).

While *D. immitis* infections are prevalent in both domestic and wild carnivores, the morphological and functional impact of this parasite on cardiovascular structures remains insufficiently studied (Matos et al., 2023; Rafailov et al., 2022). Specifically, there is a lack of standardized echocardiographic criteria that can reliably assess the progression of dirofilariasis in relation to infection intensity, complicating disease monitoring and prognosis determination. Furthermore, the effects of concurrent vector-borne infections on the progression and clinical management of dirofilariasis are not fully understood, highlighting a critical gap in the current veterinary literature.

This study aims to address these gaps by comparing echocardiographic, hematological, and biochemical findings in dogs infected solely with heartworm and those co-infected with heartworm and other vector-borne diseases. Through this approach, we aim to identify specific echocardiographic parameters and hematological markers that can aid in the assessment of disease severity and progression, potentially leading to more tailored treatment protocols for canine dirofilariasis in endemic regions.

Materials and Methods

This study included 12 dogs diagnosed with *Dirofilaria spp.* infection, categorized into two groups: mono-infected (n=7) and co-infected (n=5). These animals were selected from clinical cases presented at the Aydın Adnan Menderes University Faculty of Veterinary Medicine Small Animal Clinic due to symptoms indicative of dirofilaria infection. The co-infected group consists of dogs that carry *D. immitis* along with single or multiple infections of *Leishmania spp., Anaplasma spp.,* or *Ehrlichia spp.*

Sample Collection and Blood Analysis

Peripheral blood samples were collected from the *Cephalic vein* of each dog using sterile 5 mL syringes, ensuring minimal stress and discomfort during handling. After collection, blood samples were immediately transferred to EDTA tubes for complete blood count (CBC) analysis and into plain tubes for serum biochemistry. CBC parameters, including White Blood Cell (WBC) count, Neutrophils (NEU), Lymphocytes (LYM), Monocytes (MON), Eosinophils (EOS), Red Blood Cell (RBC) count, Hemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Red Cell Distribution Width (RDW), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelet Count (PLT), were assessed to identify any hematological abnormalities linked to dirofilaria infection. For serum biochemistry, samples were centrifuged at 1500 x g for 10 minutes to separate the plasma, which was then analyzed for key biochemical markers such as liver and kidney function tests (Blood Urea Nitrogen (BUN), Creatinine (CRE), Total Protein (TP), Albumin (ALB), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST)) to monitor organ involvement.

For *Dirofilaria spp.* antigen detection, Knott's modified test was employed to detect microfilariae in the blood, while a SNAP 4Dx Plus (IDEXX Laboratories, Westbrook, ME, USA) was used for serological confirmation. Both tests were conducted in line with the manufacturer's protocols and allowed the differentiation between single and co-infections.

Echocardiographic Examination

All dogs underwent an echocardiographic examination to evaluate the structural and functional cardiac changes associated with dirofilaria infection. Each animal was positioned in right lateral recumbency, with minimal restraint to reduce stress and avoid interference with cardiac measurements. The examinations were conducted using Mindray M5 (Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China) multifrequency ultrasound machine with spectral and color Doppler capabilities to capture real-time images and measurements. The left atrium-to-aorta ratio (LA/Ao) was measured in the right parasternal short-axis view, with values above the normal range (>1.6) flagged as indicative of cardiac enlargement. M-mode Echocardiography was performed on the right parasternal short-axis view at the level of the papillary muscles. The following parameters were measured for each dog: end-systolic volume (ESV) and end-diastolic volume (EDV) were calculated to assess the chamber size and ventricular volumes during cardiac cycles; stroke volume (SV) was derived by subtracting ESV from EDV, indicating the blood volume ejected per beat; ejection fraction (EF) and fractional shortening (FS) values were computed to evaluate the contractile function of the left ventricle, with EF serving as an indicator of global systolic function and FS as a measure of left ventricular shortening.

Statistical Analysis

Statistical analyses were performed using SPSS version 26 (IBM Corp, Armonk, NY, USA). The data were first assessed for normality using the Shapiro-Wilk test. Based on the results, appropriate statistical tests were selected. For comparisons between mono-infected and co-infected groups regarding echocardiographic, hemogram, and biochemical parameters, the Independent Samples t-test was used when the data were normally distributed. In contrast, the Mann-Whitney U test was applied for non-normally distributed data. In cases where multiple groups were analyzed, One-Way Analysis of Variance (ANOVA) was conducted for normally distributed data, and the Kruskal-Wallis test was utilized for non-normally distributed data. Correlation analyses among echocardiographic, hemogram, and biochemical parameters were performed using Pearson correlation for normally distributed data, and Spearman correlation for non-normally distributed data. All statistical tests were considered significant at a p-value of less than 0.05.

Results

The hematological, biochemical, and echocardiographic parameters of the mono-infected and co-infected groups are detailed in Tables 1-3. In terms of hematological findings, there were no statistically significant differences between the two groups for WBC, NEU, LYM, MON, EOS, RBC, HGB, HCT, MCV, MCH, or RDW. However, MCHC and PLT values were significantly lower in the co-infected group compared to the mono-

infected group, with p-values of 0.030 and 0.003, respectively. This suggests that co-infected animals may have alterations in coagulation and oxygen transport capacities.

Table 1: Hematological parameters in mono-infected and co-infected dogs with dirofilariasis.

	Group	Mean ± Std. Deviation	P value
WBC	*Mono-infected*	12.80 ± 2.40	0.639
	Co-infected	18.39 ± 17.72	
NEU	*Mono-infected*	8.87 ± 2.23	0.432
	Co-infected	12.85 ± 14.08	
LYM	*Mono-infected*	2.25 ± 0.75	0.876
	Co-infected	2.87 ± 1.88	
MON	*Mono-infected*	0.79 ± 0.39	0.755
	Co-infected	1.58 ± 1.85	
EOS	*Mono-infected*	0.81 ± 0.71	0.876
	Co-infected	1.07 ± 0.89	
RBC	*Mono-infected*	6.07 ± 0.78	1.000
	Co-infected	6.03 ± 1.38	
HGB	*Mono-infected*	14.72 ± 2.56	0.530
	Co-infected	13.62 ± 2.15	
HCT	*Mono-infected*	40.81 ± 4.58	0.876
	Co-infected	40.09 ± 8.35	
MCV	*Mono-infected*	67.42 ± 3.15	1.000
	Co-infected	66.68 ± 3.98	
MCH	*Mono-infected*	24.32 ± 3.70	0.268
	Co-infected	22.92 ± 2.70	
MCHC	*Mono-infected*	360.85 ± 48.99	0.030
	Co-infected	290.32 ± 146.04	
RDW	*Mono-infected*	13.62 ± 1.13	0.268
	Co-infected	14.46 ± 1.00	
PLT	*Mono-infected*	301.42 ± 165.90	0.003
	Co-infected	90.20 ± 35.61	
MPV	*Mono-infected*	10.02 ± 3.20	0.202
	Co-infected	11.30 ± 2.18	
PDW	*Mono-infected*	14.17 ± 2.23	0.343
	Co-infected	19.68 ± 9.64	
PCT	*Mono-infected*	0.28 ± 0.09	0.106
	Co-infected	0.77 ± 0.44	

Table 2: Comparison of Biochemical Parameters in Mono- and Co-Infected Dogs with Dirofilariasis

	Group	Mean ± Std. Deviation	P value
BUN	*Mono-infected*	39.01 ± 16.14	0.755
	Co-infected	49.78 ± 51.83	
CRE	*Mono-infected*	1.85 ± 0.45	0.639
	Co-infected	1.45 ± 0.81	
TP	*Mono-infected*	6.59 ± 1.01	0.432
	Co-infected	6.96 ± 0.54	
ALB	*Mono-infected*	2.84 ± 0.68	0.149
	Co-infected	3.36 ± 0.11	
ALT	*Mono-infected*	87.14 ± 63.36	0.149
	Co-infected	128.00 ± 65.39	
ALP	*Mono-infected*	242.85 ± 190.46	0.202
	Co-infected	892.20 ± 1464.36	
AST	*Mono-infected*	68.14 ± 44.51	0.432
	Co-infected	109.80 ± 87.73	

Table 3: Comparison of Echocardiographic Parameters in Mono-Infected and Co-Infected Dogs with Dirofilariasis

	Group	Mean ± Std. Deviation	P value
LA	*Mono-infected*	2.54 ± 0.54	0.755
	Co-infected	2.3 ± 0.43	
AO	*Mono-infected*	1.82 ± 0.51	0.639
	Co-infected	2.00 ± 0.59	
LA/AO	*Mono-infected*	1.42 ± 0.14	0.149
	Co-infected	1.23 ± 0.26	
IVSd	*Mono-infected*	0.91 ± 0.22	0.432
	Co-infected	1.08 ± 0.24	
LVPWd	*Mono-infected*	0.85 ± 0.07	0.530
	Co-infected	0.95 ± 0.37	
LVIDs	*Mono-infected*	2.17 ± 0.48	0.106
	Co-infected	1.59 ± 0.29	
EDV	*Mono-infected*	52.53 ± 22.14	0.073
	Co-infected	30.95 ± 14.39	
SV	*Mono-infected*	35.25 ± 14.18	0.106
	Co-infected	23.98 ± 10.74	
FS	*Mono-infected*	37.61 ± 2.45	0.149
	Co-infected	43.84 ± 7.29	
LVIDd	*Mono-infected*	3.47 ± 0.69	0.073
	Co-infected	2.85 ± 0.54	
IVSs	*Mono-infected*	1.53 ± 0.33	0.149
	Co-infected	1.13 ± 0.29	
LVPWs	*Mono-infected*	1.38 ± 0.27	0.755
	Co-infected	1.31 ± 0.31	
ESV	*Mono-infected*	17.28 ± 8.10	0.073
	Co-infected	6.95 ± 4.43	
EF	*Mono-infected*	70.15 ± 2.78	0.268
	Co-infected	77.09 ± 9.08	

For the biochemical parameters, there were no significant differences between the groups for BUN, CRE, TP, ALB, ALT, ALP, AST. However, it

is noteworthy that the mean values of ALT and ALP tended to be higher in co-infected dogs, suggesting a trend towards increased liver enzyme levels, which could imply greater hepatic involvement in these animals.

Echocardiographic parameters also showed mostly overlapping results between the two groups. The LA Dimension, AO Dimension , and the LA/AO ratio did not differ significantly. However, the mono-infected group displayed a trend towards a higher LA/AO ratio, which may suggest a degree of atrial enlargement in these individuals. Furthermore, both the EDV and ESV were generally higher in mono-infected dogs, although these differences did not reach statistical significance ($p = 0.073$). Similarly, the FS and EF were slightly elevated in the co-infected group, though these were not statistically significant. These findings highlighted that while the majority of parameters did not show significant differences between the mono-infected and co-infected groups, the observed reductions in PLT and MCHC in co-infected dogs might be clinicallv relevant indicators of co-infection.

Figure 1. Detection of Dirofilaria spp. using Microscopic and Rapid Test Kits

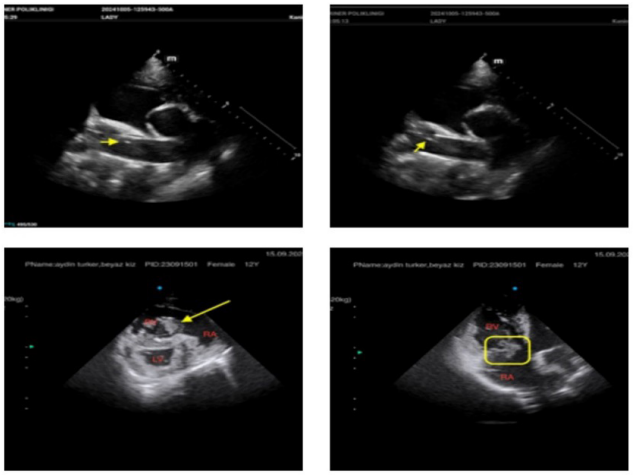

Figure 2. Visualization of Dirofilaria spp. parasite Using Echocardiography

Discussion

Canine dirofilariasis, commonly known as heartworm disease, is a significant cause of cardiovascular disorders and one of the leading causes of death in dogs (Kim, 2011). Dirofilariasis is a parasitic disease caused by the nematode *D. immitis*, which targets the cardiopulmonary system and leads to structural and functional abnormalities such as cardiomegaly, pulmonary artery dilation, and congestive heart failure.

Clinical signs can vary widely depending on the parasite load, ranging from asymptomatic to severe symptoms including weight loss, lethargy, exercise intolerance, and chronic cough (Maxwell et al., 2014). In this study, dogs were divided into mono-infected and co-infected groups. In the mono-infected dogs, clinical signs such as coughing (n=5), lethargy and anorexia (n=6), and exercise intolerance (n=4) were observed, while in the co-infected group, additional signs such as nasal bleeding (n=2), mucosal pallor (n=3), hematuria (n=1), and ascites (n=1) were noted.

In dogs with dirofilariasis, CBC plays a crucial role in detecting the clinical effects of the infection. Eosinopenia is considered an indicator of acute infection, while eosinophilia, particularly in cases involving pulmonary involvement, is associated with chronic infections (Lilliehöök et al., 2000). Thrombocytopenia may be related to increased platelet activity or immune-mediated platelet destruction in heartworm infections (Niwetpathomwat et al., 2007). Neutrophilia,

presence of monocytes, and activated monocytes are common findings in dogs with heartworm infections (Nelson & Couto, 2015). Anemia, commonly observed in heartworm infections, is associated with the movement of the parasite through red blood cells and blood vessel walls, induced by trauma (Attiyah & Alani, 2016; Madril et al., 2020). In our study, no significant differences were found between mono-infected and co-infected groups in hematological parameters such as WBC, NEU, LYM, MON, EOS, RBC, HGB, HCT, MCV, MCH, and RDW. However, a significant decrease in MCHC and PLT values was observed in the co-infected group compared to the mono-infected group ($p=0.030$ and $p=0.003$), indicating potential changes in clotting and oxygen-carrying capacity in co-infected dogs. Thrombocytopenia observed in other studies is believed to result from increased platelet consumption due to damage caused by the parasite to vascular endothelial cells (Su et al., 2004). The lower PLT count in the co-infected dogs, compared to mono-infected dogs, may be associated with additional stress on the hematopoietic system, leading to clotting problems. The decrease in MCHC suggests a reduction in hemoglobin concentration in red blood cells, weakening oxygen-carrying capacity. Eosinophils play a role in the immune response, surrounding the parasites and metabolizing infection-related substances. Although eosinophilia is rarely seen in pulmonary dirofilariasis, it may increase as the infection progresses, especially with metazoan parasites, including heartworms (Behm & Ovington, 2000; Ciferri, 1982; Werner et al., 1984). Consistent with these observations, higher eosinophil counts were seen in co-infected cases, indicating an increased immune response.

In the pathogenesis of the infection, intravascular hemolytic anemia is observed due to the mobility of microfilariae and the damage they cause to red blood cells (Kitagawa et al., 1989). Contrary to our findings, a study conducted in 2023 comparing the hematological and clinical findings of dogs co-infected with dirofilaria, babesia, or both did not report a significant decrease in hematological parameters in co-infected animals (Wężyk et al., 2023). This discrepancy may stem from host immune modulation, differences in the virulence of co-pathogens, the severity of infections, and the timing of sample collection (Wężyk et al., 2023). Naturally, in microfilaremic dogs, mild to moderate anemia, thrombocytopenia, leukocytosis, neutrophilia, eosinophilia, and monocyte elevation are common hematological abnormalities (Bowman, 2003).

Considering the natural course of infections, it is difficult to determine the duration for which these animals have been infected. However, clinical signs of the disease, physical activity, host immune response, parasite load, and infection duration can all lead to changes in hematological parameters (Nelson et al., 2014). Studies have highlighted significant kidney damage in dogs infected with *D. immitis* (Abramowsky et al., 1981; Morchón et al., 2012; Simón et al., 2012). In these infections, ALP elevation is reported as the only altered parameter in dogs (Niwetpathomwat et al., 2007). The increased ALP activity, coupled with normal AST and ALT levels, minimizes the likelihood of hepatocellular damage and instead points to chronic stress induced by elevated endogenous glucocorticoid levels (Fernandez, 2007). Nevertheless, concurrent increases in AST, ALT, and ALP activities may indicate potential liver damage. In our study, no significant differences were found between the two groups in biochemical parameters such as BUN, CRE, TP, ALB, ALT, ALP, and AST. However, the higher average ALT and ALP values in the co-infected group are noteworthy, suggesting that liver function may be more severely affected in co-infected dogs. This trend in liver enzyme elevation could indicate mild liver damage caused by the infection, leading to higher enzyme levels, and aligns with previous studies reporting elevated enzyme levels in dogs with concurrent infections (Niwetpathomwat et al., 2006).

In the diagnostic approach to heartworm disease, the importance of echocardiographic examination has been emphasized, highlighting its value in assessing pulmonary pressures and secondary effects on the right heart (Venco et al., 2014). In our study, most echocardiographic parameters were similar between the two groups, and no statistically significant differences were observed. This suggests that echocardiographic changes due to dirofilaria infection may be more subtle and may become more pronounced as the disease progresses. Additionally, the similarity in echocardiographic findings may reflect the variability in the clinical presentations of the animals. Other studies have shown a significant relationship between the severity of dirofilariasis

and echocardiographic findings (Pajas & Acorda, 2018; Su et al., 2004). However, our findings showed that concurrent infections have a clearer impact on cardiac health. In mono-infected dogs, the mean interventricular septal diastolic thickness (IVSd) was 0.91 ± 0.22 cm, while in co-infected dogs, this value was 1.08 ± 0.24 cm. Although no statistically significant difference was found (p = 0.432), the increase in IVSd in co-infected individuals may indicate a myocardial response to hemodynamic stress, which could lead to early myocardial remodeling. In mono-infected dogs, the mean stroke volume (SV) was 35.25 ± 14.18 ml, while in co-infected dogs, this value was 23.98 ± 10.74 ml (p=0.106). This reduction may indicate a decrease in cardiac output due to the increased workload caused by concurrent infections. Specifically, the significant reduction in SV and the increase in IVSd in co-infected dogs are in line with changes observed in advanced dirofilaria cases (Pajas & Acorda, 2018). This correlation underscores that the severity of dirofilaria infection, combined with the presence of co-infections, leads to significant hemodynamic changes, emphasizing the need for careful monitoring of cardiac function in dogs with concurrent infections

This study has several important limitations. First, the inability to measure the pulmonary artery and right ventricular outflow tract during echocardiography limits the comprehensive assessment of the cardiovascular status of patients with suspected dirofilaria infection. This limitation complicates the understanding of the systemic effects of the disease. Additionally, the sample size used in this study is limited, which introduces uncertainty in the generalizability of the findings. The limited sample size may have reduced the statistical power of this study, potentially obscuring subtle yet clinically relevant changes in echocardiographic parameters.

In conclusion, this study demonstrates that co-infections can lead to significant systemic effects in dogs with dirofilariasis. Hematological, biochemical, and echocardiographic evaluations reveal differences in certain parameters, particularly in the co-infected group. The lower PLT and MCHC values, along with the observed increase in liver enzymes, suggest that the disease may have broader systemic effects in this group. Therefore, considering co-infection in dogs diagnosed with dirofilariasis is crucial for managing the disease and preventing potential complications.

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