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Research article
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# Determination of abdominal aortic elasticity changes in naturally infected dogs with canine visceral leishmaniasis

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**ABSTRACT:** The aim of the study was to detect and assess early-stage hypertension in dogs with Canine Visceral Leishmaniasis (CanL), by determining the changes in elasticity occurring on the abdominal aorta, in addition to the use of oscillometric measurements. A total of 44 animals, consisting of 32 animals diagnosed with CanL and 12 healthy dogs, were evaluated. The diseased animals were categorized into four different groups based on the stages of CanL. The dogs with CanL and the healthy dogs were assessed using Doppler ultrasonography to determine the changes in abdominal aorta elasticity according to their respective stages. CanL -infected animals were identified in different stages, including Stage I, Stage II, and Stage III. It was found that there were no significant changes in the blood pressure values measured through oscillometric method based on the results of abdominal aorta elasticity. However, a significant decrease in abdominal aorta elasticity ($p<0.05$) was observed in infected animals at Stage III compared to healthy animals and in infected animals at Stage II compared to Stage III, according to the clinical staging of the disease. It was determined that the abdominal aortic elasticity of CanL-infected animals tends to decrease as the disease stage progresses, and these changes can directly affect the systemic circulation.

***Keyword:*** Aortic Elasticity; Canine Visceral Leishmaniasis; Hypertension; Stage

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## INTRODUCTION

Dogs play a crucial role as carriers in the epidemiological cycle of Leishmania. Canine Leishmaniasis (CanL) can affect any organ or tissue with non-specific clinical signs, commonly observed in various diseases (Baneth et al., 2008; Koutinas and Koutinas, 2014). The clinical signs observed during examinations, as well as abnormalities in hematological and serum biochemical tests, can determine the clinical staging and prognosis of dogs with CanL (Solano-Gallego et al., 2011).

In the active phase of the disease, kidney failure, resulting from immune complex deposition, is the most common cause of death in dogs infected with *L. infantum* (Solano-Gallego et al., 2011; Koutinas and Koutinas, 2014). The deposition of immune complexes causes thickening of the glomerular basement membrane, leading to protein loss in the urine and a gradual impairment of glomerular filtration (Plevraki et al., 2006; Aresu et al., 2010).

Systemic hypertension (SH) is common in kidney diseases, and a cycle may develop where hypertension causes kidney damage, and kidney damage, in turn, leads to hypertension (Kobayashi et al., 1990; Brown, 2001). The prevalence of SH in dogs with Leishmaniasis can reach up to 61.5%, potentially leading to renal vascular damage, kidney failure, and death (Cortadellas et al., 2006; Amann et al., 2006; Schiffrin et al., 2007; Jacob et al., 2005).

Early detection of kidney damage allows for the early diagnosis of the disease, preventing its progression and increasing survival time. Identifying new biomarkers for the early and accurate diagnosis of asymptomatic kidney disease plays a crucial role in the prognosis, treatment, and control of the disease (García-Martínez et al., 2015). In clinical practice, non-invasive estimation of blood pressure using Doppler and oscillometric devices is essential for the early diagnosis of hypertension, thus minimizing target organ damage.

The changes in the abdominal aorta during systole and diastole, caused by blood ejection from the left ventricle, reflect the elastic properties of the aortic wall. Chronic systemic hypertension leads to structural changes characterized by smooth muscle cell hypertrophy and increased collagen in the arterial walls. This thickeness and stiffness of the aortic wall result from systemic hypertension (Laurent and Boutouyrie, 2015).

In this study, in addition to measuring blood pressure using oscillometric methods, the elasticity of the abdominal aortic walls, which expand during systole and contract during diastole, will be assessed using non-invasive ultrasonographic methods. The aim is to evaluate changes in aortic elasticity related to CanL-induced SH and to provide insights into the pathogenesis, stages, and complications of Leishmaniasis.

## MATERIALS AND METHODS

### Study Design and Population

A total of 44 dogs (aged 1-8 years), including 32 CanL-infected animals presented to the Small Animal Clinic of Aydın Adnan Menderes University for examination and 12 healthy dogs presented for vaccination or control, were included in the study.

Exculision criteria for the animals used in the study were as follows:

i) Age less than 1 year or more than 8 years,
ii) Absence of metabolic (hyperadrenocorticism, diabetes mellitus, hypothyroidism) and protozoal diseases other than CanL,
iii) Non-pregnant or non-lactating,
iv) Absence of chronic heart failure, dilated cardiomyopathy, pericardial effusion, or degenerative valve disease,
v) No exposure to antihypertensive drugs or drugs that induce systemic hypertension,

For healthy dogs, the inclusion criteria were as follows:

i) Over 1 year old, fully vaccinated, regularly treated for parasites, clinically and hematologically healthy, and not affected by Leishmaniasis or other protozoal diseases.

### Diagnosis of CanL and Group Assignment

The diagnosis of CanL in infected dogs was made using compatible clinical and laboratory findings along with a positive rapid test kit (4DX SNAP®, IDEXX Laboratories, Westbrook, Maine, USA). The CanL-infected dogs were divided into four groups according to IRIS criteria based on serum creatinine levels (Solano-Gallego et al., 2011). Including the healthy control group, a total of five groups were formed for the study (Table 1).

### Sample Collection and Laboratory Analysis

Blood analyses were performed for both diagnosis and the grouping of infected dogs. Blood samples collected in tubes containing EDTA were used for

**Table 1.** Distribution of the animals into groups within the scope of the study.

| | GROUPS | SERUM CREATINE LEVEL (mg/dl) | Applications |
|---|---|---|---|
| Infected Group | Group-1 | <1.4 | Doppler ultrasonography and blood pressure measurement |
| | Group-2 | 1.4-2.8 | Doppler ultrasonography and blood pressure measurement |
| | Group-3 | 2.9-5 | Doppler ultrasonography and blood pressure measurement |
| | Group-4 | >5 | Doppler ultrasonography and blood pressure measurement |
| Healthy Group | Group-5 | <0.8 | Doppler ultrasonography and blood pressure measurement |

complete blood count analysis and for CanL diagnosis with rapid test kits. Plasma obtained from samples with lithium heparin was used to measure routine biochemical parameters, including glucose (mg/dL), ALT (IU/L), AST (IU/L), ALP (IU/L), GGT (IU/L), albumin (g/dL), total protein (g/dL), total bilirubin (mg/dL), urea (mg/dL), and creatinine (mg/dL). Complete blood count analyses were performed using the Abacus Vet5 (Diatron®, Hungary), and biochemical analyses were conducted using the Amishield Biochemistry Analyzer (Gazelmed, Istanbul).

**Blood Pressure Measurement**

Blood pressure measurements were taken after ensuring the patients were kept in a calm environment to eliminate stress and obtain accurate results. The Petmap Graphic II oscillometric blood pressure device (Ramsey Medical, Inc., USA) was used for the measurements. The appropriate cuff size for each dog's weight was placed on the tail region, and measurements were taken. At least five repeated measurements were taken from both healthy and infected dogs. The average systolic and diastolic blood pressure values were recorded, and the dogs were classified as normotensive (SBP <140 mmHg), pre-hypertensive (SBP 140-159 mmHg), hypertensive (SBP 160-179 mmHg), and severely hypertensive (SBP ≥180 mmHg).

**Ultrasonographic Measurements**

All dogs underwent ultrasonographic evaluation at the imaging unit of the Small Animal Clinics of Adnan Menderes University Veterinary Faculty. The dogs were fasted for at least 3 hours before the ultrasound procedure. The abdominal aorta was imaged using a 4-8 MHz multi-frequency transducer with Doppler ultrasound (Esaote®, Florence, Italy). Measurements were taken in the transverse section, where only the abdominal aorta was visible in a circular view.

**Determination of Aortic Elasticity**

Aortic elasticity was calculated from two different aortic sections: the first caudal part of the left renal artery (K AoSt) and the second cranial part of the external iliac arteries (I AoSt). For each location, the maximal systolic diameter of the aorta (AoDs) and the minimal diastolic diameter of the aorta (AoDd) were measured. The aortic stiffness (AoSt), defined as the percentage change in aortic diameter between systole and diastole, was calculated using the formula: $AoSt = ([AoDs - AoDd] / AoDd) \times 100$. The average of three consecutive measurements of AoDs and AoDd was recorded for each dog. These values were used to evaluate changes in aortic elasticity and assess the potential development of hypertension (Figure 1).

**Statistical Analyses**

Descriptive statistics were performed for the demographic and numerical data obtained in the study, and the results were tabulated. It was determined that the data did not follow a normal distribution, and non-parametric test techniques were used to compare the groups. In this context, the Mann-Whitney U test was used to compare the main blood pressure groups, while the relationship between abdominal aortic elasticity and the sub-hypertensive groups, as well as the relationship between CanL and infection levels was analyzed using the Kruskal-Wallis ANOVA test. In all analyses, p-values less than 0.05

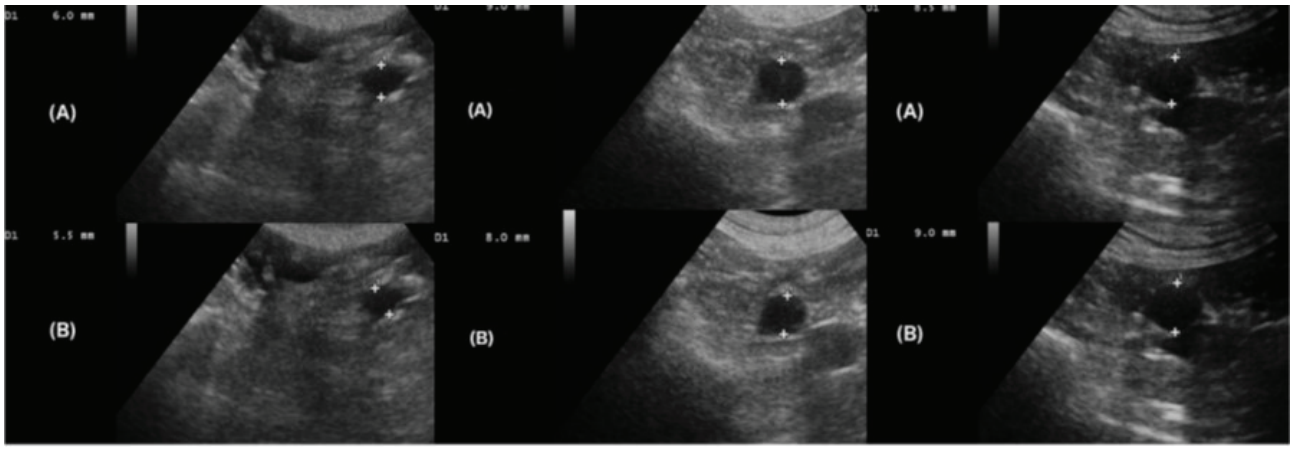


**Figure 1.** Ultrasonographic assessments for evaluating aortic elasticity. (A): Systolic diameter, (B): Diastolic diameter.

were considered statistically significant. The analyses and graphs were generated using the GraphPad 9.2.0 software (USA).

## RESULTS

Among the dogs with Leishmaniasis, 28 were identified as hypertensive, while 4 were recorded as normotensive. The average aortic elasticity in hypertensive CanL dogs was 10.09 ± 4.3, while in normotensive dogs, this value was 9.22 ± 3.2, with no statistically significant difference observed between the groups (p>0.05) (Figure 2).

Upon evaluating the infected dogs based on hypertension, it was determined that 4 dogs were normotensive, while the distribution across the pre-hypertensive, hypertensive, and severely hypertensive groups consisted of 10, 9, and 9 dogs, respectively (Figure 3). The findings indicate that the majority of CanL-infected animals exhibited clinical signs of systemic hypertension, with a significant proportion of the patients falling into the pre-hypertensive, hypertensive, or severely hypertensive categories.

It was determined that there was no statistically significant difference in the measurements of abdominal aortic elasticity among the systemic hypertension subgroups (p>0.05). However, although

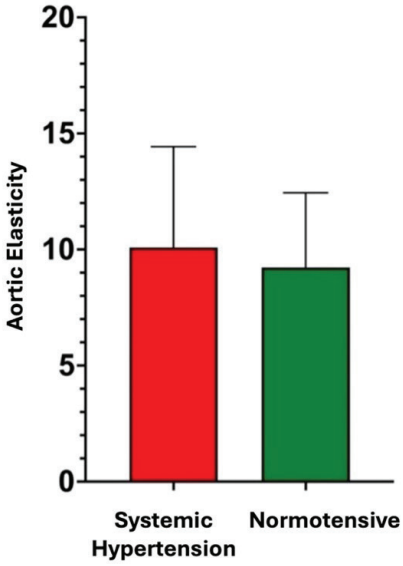


**Figure 2.** Aortic elasticity levels according to the main blood pressure groups.

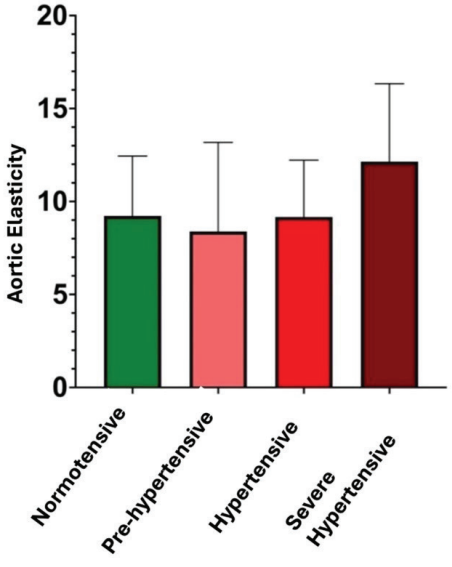


**Figure 3.** Aortic elasticity levels of dogs with CanL based on systemic hypertension.

not statistically significant, an increasing trend in abdominal aortic elasticity was observed as blood pressure levels increased (Table 2).

It was determined that the abdominal aortic elasticity of dogs infected with CanL showed a decreasing trend according to the stage of infection. The reductions observed in the measurements of healthy dogs and dogs at Stage III, as well as the changes between Stage II and Stage III, were found to be statistically significant ($p<0.05$) (Figure 4, Table 3).

## DISCUSSION

Systemic hypertension is a persistent increase in arterial blood pressure commonly caused by renal and endocrine disorders in dogs and cats (Acierno et al., 2020). SH can cause severe damage to target organs such as the kidneys, eyes, central nervous system, heart, and vascular system. In dogs with existing renal damage, SH is one of the symptoms encountered with varying prevalence depending on the type, stage, and affected region of the disease (Acierno et al., 2020; Ware et al., 2021). Therefore, the early and accurate diagnosis of SH is crucial (Acierno et al., 2020).

It is well known that the kidneys are affected in almost all dogs with visceral leishmaniasis. This renal damage can be caused by interstitial nephritis or the accumulation of parasitic antigens in the glomeruli. This condition can lead to glomerulonephritis, tubulointerstitial nephritis, renal failure, and amyloidosis. In a study involving 55 positive dogs, glomerulonephritis was detected in 100% of the animals, even though 13 of them were asymptomatic (Costa et al., 2003; Baneth et al., 2008).

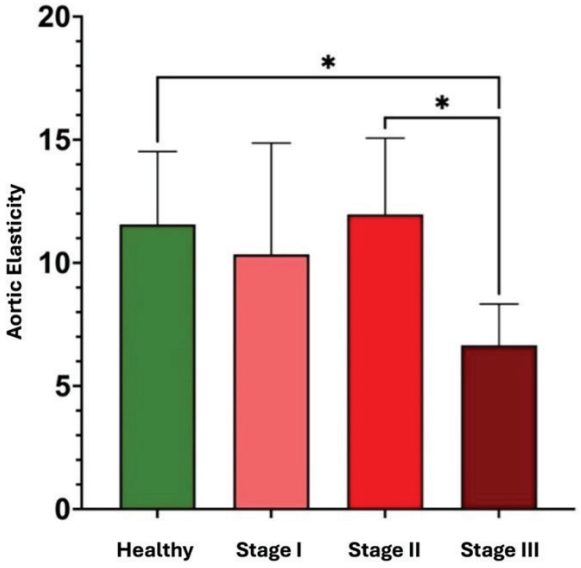


**Figure 3.** Aortic elasticity levels in healthy dogs and those infected with leishmaniasis at different stages.

In association with Leishmaniasis, 61.5% of dogs with glomerular renal damage were also found to have systemic hypertension (Cortadellas et al., 2006). In our study, a similar finding was observed, with 85% of the CanL-infected dogs showing systemic hypertension.

The aorta, as the main distributing artery, reduces the pressure fluctuations generated by left ventricular ejection and transforms pulsatile flow into continuous blood flow. During systole, the Ao expands, storing elastic energy, which is then used to propel blood

**Table 2.** Comparison of abdominal aortic elasticity of dogs with CanL based on the systemic hypertension subgroups

| | Normotensive | prehypertensive | Hypertensive | Severe Hypertensive |
|---|---|---|---|---|
| N | 4 | 10 | 9 | 9 |
| Aortic Elasticity | 9.2±3.2 | 8.2±4.7 | 9.1±3 | 12.14±4.1 |

*($p<0.05$)*

**Table 3.** Aortic elasticity changes in animals infected with CanL at different stages and in healthy groups

| | Healthy Group | Stage I | Stage II | Stage III |
|---|---|---|---|---|
| N | 12 | 20 | 6 | 6 |
| Aortic Elasticity | 11.56±2.9[a] | 10.35±4.5 | 11.97±3.1[a] | 6.65±1.6[b] |

[a.b]: Values expressed with different letters on the same row are statistically significantly different.

through the circulation during diastole. The elastic properties of the Ao are determined by the elastin fibers, smooth muscle, and collagen in its walls (Klabunde, 2011; Orsi et al., 2004). Chronic SH causes hypertrophy and increased collagen in arterial walls, resulting in increased Ao wall thickness and stiffness (Chobanian, 1992; Laurent and Boutouyrie, 2015). Increased Ao stiffness indicates reduced elasticity. Studies in cats have shown a positive correlation between hypertension and aortic root dilation, with more severe aortic dilation observed in hypertensive cats (Nelson et al., 2002; Kim et al., 1996). In hypertensive dogs, aortic arch dilation and fluctuations in the size and shape of the thoracic descending aorta have been detected (Holland et al., 2022a,b). Additionally, an increased aorta-to-caudal vena cava ratio and decreased abdominal aortic elasticity have been observed in hypertensive dogs (Holland et al., 2022a; Corda et al., 2020). In healthy dogs, the size of the abdominal aorta has been standardized (Darnis et al., 2018). When evaluating studies conducted on dogs and cats, it is noted that these evaluations have generally focused on determining changes in the elasticity of the abdominal aorta in relation to the presence and degree of systemic hypertension. However, there is a lack of literature on the relationship between systemic hypertension and the diseases that cause it. Systemic hypertension is considered the most significant disease affecting the aortic wall in humans (Tsai et al., 2005). Investigating not only the effects of systemic hypertension on vascular elasticity but also the diseases that cause systemic hypertension in dogs and cats will allow for more accurate approaches in determining the prognosis of affected animals. In our study, it was determined that the changes in abdominal aortic elasticity in CanL-infected animals showed a decreasing trend in parallel with the clinical staging of the infection. These changes were significantly lower in Stage II and III patients, and the reductions observed in Stage III patients were statistically significant compared to the healthy control group. This finding suggests that, as the disease progresses, the abdominal aorta loses its elasticity, which may be due to an inability to undergo aortic remodeling. Importantly, the early-stage alterations in aortic elasticity detected in this study indicate that vascular wall involvement may begin before the clinical onset of systemic hypertension. This suggests that assessing abdominal aortic elasticity could serve as an early diagnostic indicator, particularly in asymptomatic or mildly proteinuric CanL cases. Early detection of such vascular changes may allow timely clinical interventions and improve overall prognosis.

## CONCLUSION

This study concluded that as the clinical staging of CanL progresses, the infection leads to a loss of elasticity in the abdominal aorta. It was determined that this parameter should be considered in clinical diagnostic procedures, particularly in advanced-stage of CanL. However, our findings also indicate that changes in aortic elasticity are not limited to advanced disease stages and may be detectable earlier in the course of infection. Therefore, ultrasonographic evaluation of abdominal aortic elasticity may be considered as a potential non-invasive screening tool for the early detection of vascular changes prior to the development of systemic hypertension.

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