

## PATHOMORPHOLOGICAL FINDINGS IN DOGS WITH SPONTANEOUS HEARTWORM DISEASE

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### ABSTRACT

Study reveals the pathomorphological findings of 6 dogs, 3 males and 3 females, diagnosed with Heartworm disease. By necropsies were found a total number of 144 adult stages (74 male and 70 female) of the nematode *Dirofilaria immitis*, located in *a. pulmonalis*, right ventricle and atrium and *sulcus vena cavae*. The gross pathology shows right-sided enlargement of the heart, dilated pulmonary arteries, and single petechial hemorrhages in the lungs. Histopathologically, degenerative changes in the pulmonary arteries, thickening of the pulmonary valves, interstitial reactions in the lungs and protein precipitates in the kidneys were found.

**Key words:** *Dirofilaria immitis*, dogs, gross and histopathological findings.

### Introduction

Heartworm disease continues to be one of the most common parasitic diseases in dogs in Bulgaria. That's why in recent years, the disease has been studied by a number of Bulgarian scientists (Panayotova-Pencheva et al. 2016; Radev et al. 2016; Iliev et al. 2017, Borisov et al. 2017; Rafailov, 2020).

Necropsy of cardio-vascular system are main technique for reaching the definitive diagnosis of Heartworm disease. Postmortem finding is the only method to count an exact number of adults in the infected animal and to determine the parasites gender. This method often eliminates cases of false antemortem diagnoses.

The main postmortem changes are found in the cardiovascular system. The main lesions are dilatation of the right ventricle and diffuse thickening of the wall of the pulmonary arteries (Pasca et al, 2012). Microscopically, the most obvious changes are found in the heart and blood vessels as well as in the lungs, liver and kidneys (Ceribasi & Simsek 2012).

The grade of heart alterations is not always directly related to the number of adult parasites, but nevertheless, with a massive infestation, the probability of obvious changes is higher. Initially, the adult nematodes are located in the pulmonary artery and with the increase in their number can be found in the right ventricle, followed by the right atrium and finally in severe infestation, are found in the vena cava. Pulmonary hypertension with subsequent hypertrophy of the right ventricle and right heart failure are observed as a consequence of vascular damage and impaired blood flow in the pulmonary artery (Zachary, 2017). In the segments of the pulmonary artery containing parasites, endarteritis is initially detected, by infiltration of the intimal layer with eosinophils and subsequent proliferation of abnormal fibrous tissue. Grossly, a granular or irregular appearance of the endothelial surface of the vessel is found. In severe infection as a result of mechanical irritation, erosions and tissue proliferations in the pulmonary arteries can be observed, accompanied by accumulations of foamy macrophages, eosinophils, plasma cells and, less commonly, neutrophils in the intimal layer (Boreham, 2018).

As a result of the Heartworm disease, interstitial edema is found in the muscular layer of the heart, which is observed by the accumulation of edematous fluid around the myocardial cells. In some cases, disseminated hemorrhages in epicardial adipose tissue have been detected (Ranjbar-Bahadori, 2010). The histological alterations of the myocardium include necrotic changes and cellular vacuolization (Carreton et al., 2012).

Pathomorphological changes as dilatation, hypertrophy, thrombosis, myointimal proliferation of arterial vessels, infarctions, hemorrhages, hemosiderosis, chronic inflammatory reactions, fibrosis are observed in the lung depending on the degree of infestation (Grandi et al., 2007). In some cases, alveolar edema with focal cellular alterations can be found in the lung parenchyma (Dillon et al., 1995). It is possible to be detected granulomatous inflammatory reactions accompanied by perivascular connective proliferations when there are dead adult parasite stages in the lungs (Ceribasi & Simsek 2012). With severe involvement of the lung structure, pulmonary and pleural collapse has also been reported (Atwell et al., 1986).

Pathology in kidney includes chronic membranoproliferative glomerulonephritis and interstitial nephritis accompanied by cortical and medullary alterations (Grauer et al., 1987, Ceribasi & Simsek 2012). In the microscopical examination of the renal tissue are observed inflammatory cellular infiltration of macrophages, lymphocytes and plasma cells, which determines the formation of focal mononuclear interstitial nephritis. Most of the glomeruli are edematous. An increased number of mononuclear cells is found in the mesangial matrix. In rare cases, eosinophilic amyloid substances may be identified. In cases of proteinuria, hyaline droplets are observed in the urinary tract and renal tubules (Nakagaki et al., 1990; Ranjbar-Bahadori, 2010).

The aim of the presented study was to determine the topography, pattern and extent of pathomorphological changes in Fourth-grade Heartworm disease in dogs.

### **Materials and methods**

Six necropsies were performed on three male and three female dogs with clinically manifested and antemortem diagnosed Heartworm disease. All animals are of mixed breed. The youngest was eight and the oldest eleven years old. Patients were presented with clinical signs of heart failure, cough, shortness of breath, fatigue, anorexia and ascites. A quick IDEXX 4Dx TEST KIT was performed for the presence of *Dirofilaria immitis* antigens. The tests were positive. The modified Knott test was used to detect the presence of *D. immitis* microfilariae in the blood of the six animals. Echocardiography, echography and radiography, and paraclinical tests - complete blood count, biochemical blood profile and urine tests were performed. Based on the results of all examinations, the chance of concomitant cardiovascular or pulmonary disease was minimized. The AHS (American Heartworm Society) classification, modified by Kostadinov (2016), was used to determine the degree of disease development.

The necropsies were performed according to the classical necropsy technique for carnivores (Stoev et al., 2016).

Tissue materials from pulmonary arteries, myocardium, lungs, and kidneys were taken for morphological analysis during the necropsies. The samples were fixed in 10% buffered neutral formalin solution. After dehydration by ascending alcohol, the materials were cleared in xylene and embedded in paraffin. Sections with a section thickness of 5 µm were prepared and stained with Hematoxylin-Eosin (Bancroft & Gamble, 2002). Microscopic examination and photography were performed with a light microscope with a built-in Euromex BioBlue camera.

## Results

The examined animals were classified as Fourth-grade Heartworm disease. After unsuccessful therapy, they died.

During the necropsies, 37 male and 32 female adult *D.immitis* were found in *a. pullmonalis*. In the right ventricle and atrium, 36 male and 37 female adults were detected, and in *sulcus vena cavae*, one specimen of each gender. Summarized, 74 males and 70 females, a total of 144 adult stages were found. Table №1 shows the number of parasites, their gender distribution and predilection site in each of necropsied dogs.

Gross pathology shows distinct cardiomegaly in all examined animals. General right-sided enlargement of the hearts was detected with clearly visible and dilated pulmonary arteries.

**Table 1: Number adult *D.immitis*, gender and predilection site in individual dogs.**

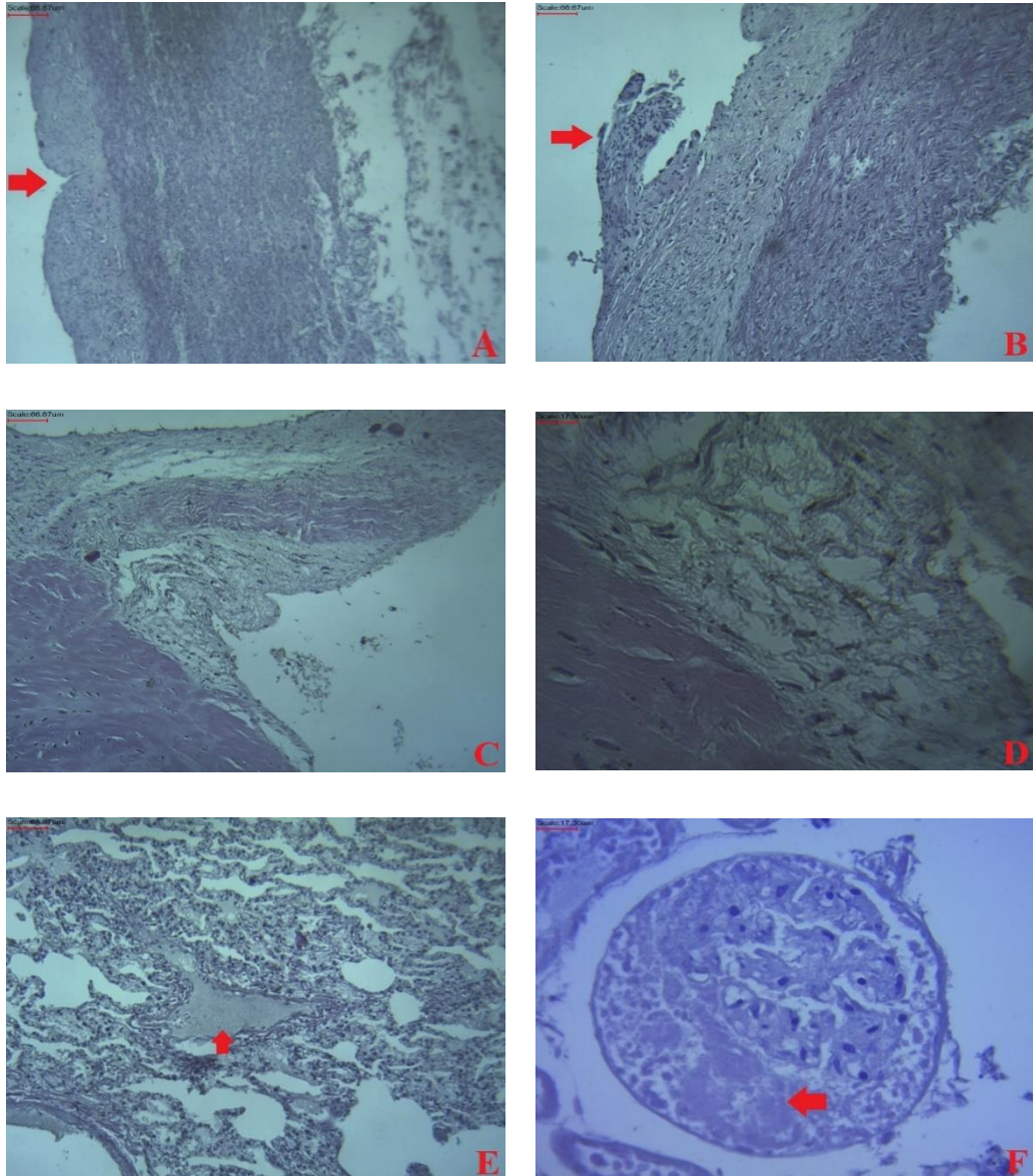
DOG №:	Number of adults in <i>a.pullmonalis</i>		Number of adults in right ventricle and atrium		Number of adults in <i>sulcus vena cavae</i>	
	males	females	males	females	males	females
1	5	3	6	6	0	0
2	6	7	6	5	0	0
3	8	7	7	5	0	0
4	6	4	4	6	1	1
5	5	5	6	8	0	0
6	7	5	7	7	0	0

Histo-pathologically, the main alterations were found in the pulmonary arteries. Thickening of the vessel trunk accompanied by degenerative changes in the endothelium was found in all examined animals (Fig. 1A). Similar lesions were observed at a.pulmonary main branches. Villous proliferations of the intimal layer were found in four of six animals along the pulmonary artery (Fig. 1B). Connective tissue with radiant fibroblasts was observed in the villous protrusions. In some places, the growth of connective tissue affected the medial layer of the vessel. These changes were found in all necropsied dogs.

Significant alterations in the pulmonary valve were detected in four of the dogs. Thickening of the cusps and a fine granular structure were found in the gross examination. Microscopically, a thickening of the valve was observed (Fig. 1C), based on proliferated connective tissue. Myxomal type metaplasia was found in two of the animals (Fig. 1D).

During the pathomorphological examination of the lungs, no grossly visible changes were found, except for single petechial hemorrhages on the serosal surface. Diffuse interstitial reactions with mononuclear infiltration, fibrous proliferation, and fluid accumulation in some of the alveolar spaces were observed histologically, in five of the animals (Fig. 1E).

No grossly visible lesions were detected in the kidneys during the examinations. Microscopically, protein deposits were found in half of the dogs in some of the glomerules (Fig. 1F).



**Figure 1: Histopathological findings in blood vessels and organs in a dog with 4<sup>th</sup> grade Heartworm disease.**  
**A.** Main branch of pulmonary artery. Thickening of the artery wall accompanied by degenerative changes in the endothelium (arrow). (x100; H&E); **B.** Left pulmonary artery. Thickened vessel wall with villous protrusions of the intimal layer (arrow). (x100; H&E); **C.** Pulmonary valve. Thickened valve structure. **D.** (fragment from Fig. 1C) Myxomal metaplasia of the valve structure. (x400; H&E); **E.** Lung parenchyma. Interstitial reaction with infiltration of mononuclear cells and accumulation of fluid in the alveolar space (arrow). (x100; H&E); **F.** Kidney parenchyma. Accumulation of protein structures in the glomerular structure (arrow) (x100; H&E).

## Discussion

As in a number of studies (Boreham, 2018; Ceribasi & Simsek, 2012; Kramer et al., 2011) and in the present, the main pathomorphological findings, during necropsies of dogs, were found in the heart, lungs and kidneys. These results were due to the fact that necropsies were performed on animals in the Fourth grade of Heartworm disease. The classification of the infection rate is mainly determined by the number of adult nematodes, as well as their predilection site.

The main vascular abnormalities which were found by us in dogs with Heartworm disease, confirm previous studies (Ceribasi & Simsek, 2012; Boreham, 2018). Enlargement of the pulmonary arteries was due to thickening of the vessel wall and dilatation. These changes were a consequence of the presence of adult parasites and the occurrence of pulmonary hypertension. Dilatation and morphological changes in the structure of the pulmonary artery lead to thickening and functional rigidity of the vessel. The loss of elasticity, which under normal conditions compensates for the increased systolic volume and pressure, leads to a significant increase in systolic and diastolic pressure in the pulmonary artery (Zachary, 2017).

Like the other studies (Boreham, 2018), the main histopathological lesion of the pulmonary artery that was found during the necropsies was an intimate proliferative reaction, and in some animals the medial layer of the vessel was also affected. Vascular proliferation is thought to be mainly due to mechanical irritation from the presence of adults, which leads to an inflammatory reaction too. Chronologically, in the early stages of infestation, vacuolation and swelling of the subendothelial connective tissue are firstly observed, together with the presence of a small number of plasma cells and eosinophils. With the progression of endarteritis, fibrous growths affecting the intimal and medial layers lead to villous protrusions in the lumen of the vessel.

As a result of the affecting of the pulmonary circulation, lungs were examined pathomorphologically. The main histopathological finding, diffuse interstitial lesions, was comparable with that described by Kramer et al. (2011) and Boreham (2018). It is believed that the changes in lung tissue are not a direct consequence of the adult nematodes located in the pulmonary arteries, but are the result of immune reactions that destruction of parasite microfilariae in the pulmonary vascular network (Calvert & Losonsky, 1985).

As organs that are highly sensitive to abnormalities of the systemic blood circulation, the kidneys were pathomorphologically examined. The main change found by us, the accumulation of eosinophilic, protein structures, is also described by Nakagaki et al. (1990). Studies have shown that glomerulopathy in infection with *D. immitis* has an immunological pathogenesis (Paes-de-Almeida et al., 2003). Circulating or locally formed immune complexes, in antigen-antibody-complement interaction, are deposited in the glomerular capillary walls and mesangium. These complexes are usually water-soluble and low molecular weight. However, it is believed that the main reason for their accumulation in the glomerules, in High-grades Heartworm disease, is the excessive amount of antigens, leading to the formation of intermediate complexes and their binding to large amounts of complement (Boreham, 2018).

## Conclusion

Heartworm disease in it's Fourth-grade is a severe parasitic infestation in dogs associated with affection of different essential organs and few body systems. The main pathomorphological lesions that are observed affect the heart, lungs and kidneys. Our research and results confirm previous research established by other authors so far.

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