



# Prevalence of Heartworm Disease and Associated Polyorganic Lesions in Dogs With Sudden Death, Necropsied During 2022-2023 in Timisoara, Romania

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## Abstract

**Introduction:** Heartworm disease is increasingly diagnosed among the canine population from Romania.

**Methods:** In this study, 34 dogs were necropsied between 2022 and 2023.

**Results:** The prevalence of heartworm disease was 17.64% (6/34). Multiple organs were affected, including the pancreas, heart, lungs, kidneys, spleen, and liver. Generalized circulatory changes, with thrombosis and congestive-hemorrhagic foci, were observed, along with inflammatory processes. Acute pancreatitis has been very rarely reported by scientists, highlighting the importance of the present study.

**Conclusion:** By knowing all the lesions that can evolve and the organs that can be affected, veterinarians can improve laboratory diagnostic methods. Thus, specific biomarkers indicating degenerative processes in different organs can be supplemented to provide a more accurate diagnosis.

**Keywords:** *Dirofilaria immitis*, Necropsy, Histopathology

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## Introduction

Heartworm disease is a vector-borne disease, with zoonotic potential, frequently diagnosed among the canine population around the globe, including Romania, being caused by *Dirofilaria immitis*. Nationally, a prevalence of up to 60% has been reported for this disease (1,2). Regionally, in western Romania (Timisoara), the current prevalence is approximately 9.74% for heartworm disease (3). Responsible owners often notice a health problem in the dog, with clinical signs of recurrent cough, dyspnea, dark urine, and intolerance to sustained efforts. These clinical signs can help diagnose the disease. Necropsy examination is enlightening and highly helpful in clarifying the etiological agent. Multiple organs can be affected depending on the evolutionary period of the disease. The most advanced lesions are found in the heart and large vessels at the base of the heart being the predilection site of the causative nematode, followed by the lungs, liver, and kidneys (4). Most studies have described pathological changes in the aforementioned

organs (5). Few studies have reported pancreatic damage. Acute pancreatitis may be associated with chronic mitral valvular insufficiency, which may result from heartworm disease (6). In the present study, in one of the six dogs positively identified with *D. immitis* this lesion was observed.

The main objectives of the study were to find the prevalence of heartworm disease in dogs that have not been diagnosed during their lifetime and to update the lesional aspects of the disease. Thus, the veterinarian can improve laboratory testing methods.

## Materials and Methods

Overall, 34 dogs were necropsied at the Forensic Medicine Clinic (Faculty of Veterinary Medicine, Timisoara) between March 2022 and July 2023. Most of the owners reported a sudden death of the animals, which is why they requested a necropsy examination.

The dogs ranged in age from 3 days to 13 years with a vast majority mixed breeds (21/34) and other breeds,



including German Shepherd (5/34), Bucovina Shepherd (2/34), Caucasian Shepherd (1/34), Vizsla (1/34), Wirehaired Vizsla (1/34), Amstaff (1/34), Bull Terrier (1/34), and Shih Tzu (1/34).

Tissue samples were taken from all organs that showed macroscopic changes, which belonged to the cadavers with *D. immitis* adults in the heart and lungs. The tissue samples were prepared for histopathological analyses following standard procedures as described by Suvarna et al (7).

## Results

The prevalence of heartworm disease in dog cadavers was 17.64% (6/34). Of these, four were females and two were males. Only three belonged to a breed, namely, a German Shepherd, a Wirehaired Vizsla, and a French Bulldog.

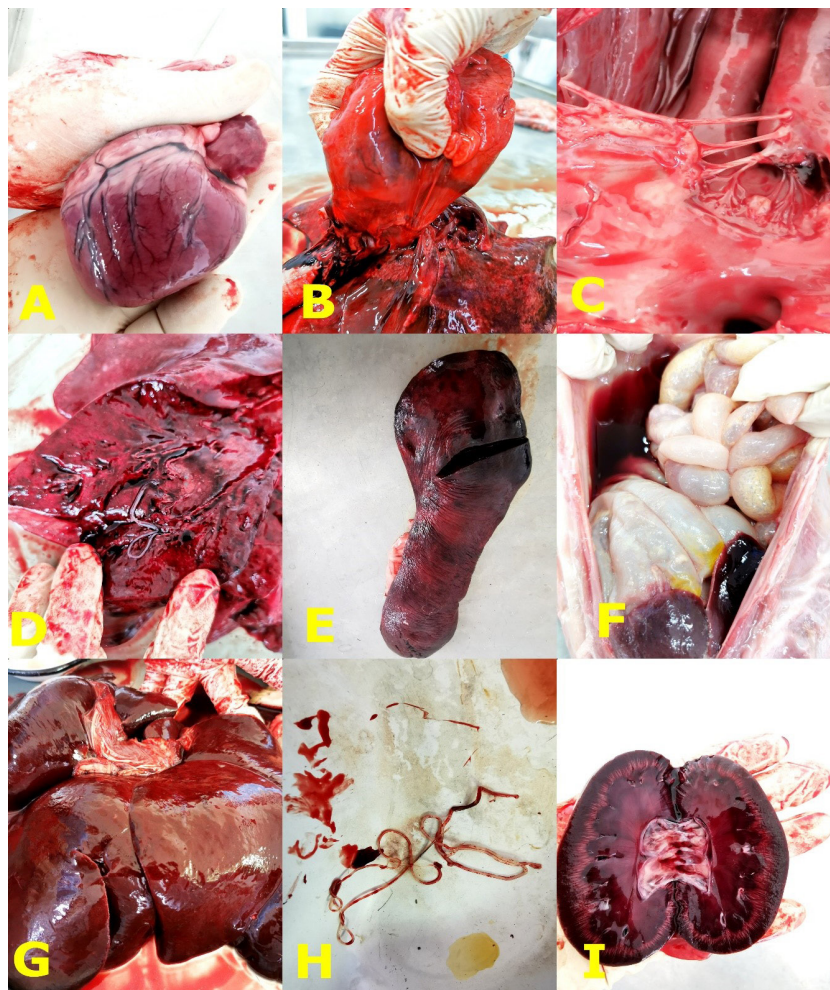
On macroscopic examination of the heart, the branches of coronary arteries occupied a large part of the organ due to ectasia and hyperemia (Figure 1A). The overall consistency of the heart was flaccid, more pronounced on the right side of the heart, which was dilated frequently.

A change in elasticity and consistency was also caused by pericardial effusion, detected in several dogs (Figure 1B). When sectioning the pericardial sac, a serosanguineous fluid was noted in variable quantities (20-100 mL).

Moreover, in the section, in four out of six dogs, there were varying degrees of mitral valvular endocardium opacification. This indicates an evolution of endocarditis (Figure 1-C), resulting from mechanical irritation of the parenchyma, following movements performed by viable adult parasites (Figure 1H).

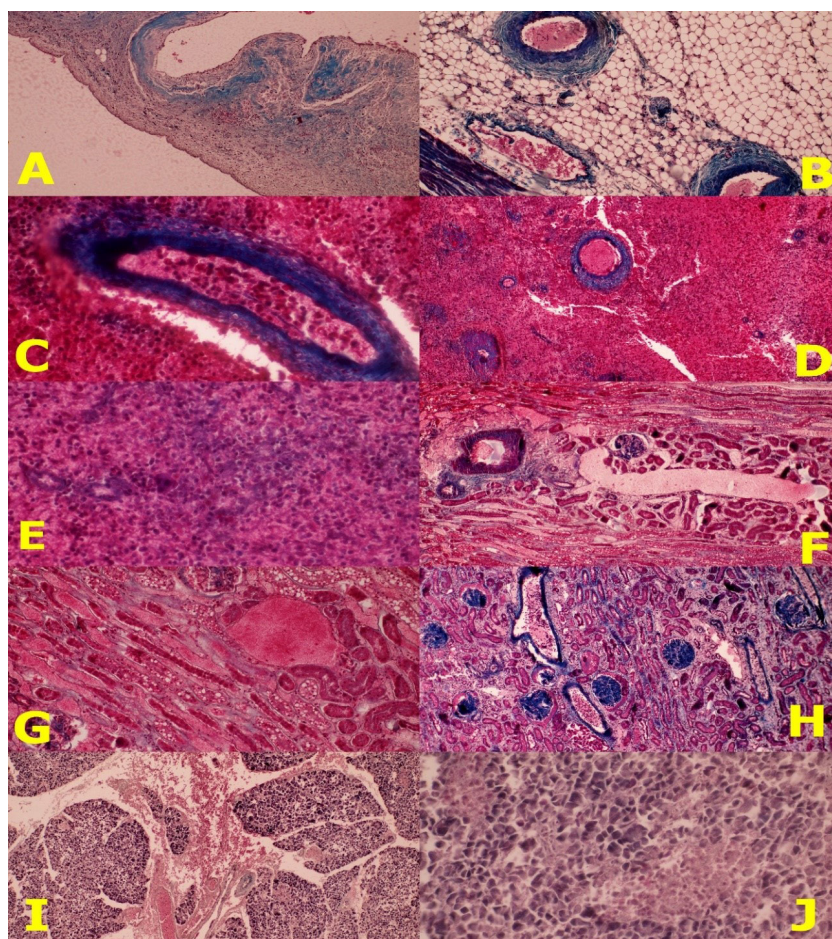
Microscopically, across the mitral valvular endocardium, there was a leukocyte infiltrate and fibroconjunctival proliferation, denoting an inflammatory onset and parenchymal degeneration (Figure 2A). Abundant adipose tissue, hyperemia, and coronary arteries ectasias were observed within the epicardium (Figure 2B).

The adults of *D. immitis* have also been identified in the pulmonary arteries, causing thrombosis and perivascular or interstitial inflammation, with extensive congestive-hemorrhagic areas (Figure 1D).



**Figure 1.** Gross Pathology. (A) Coronary arteries ectasia and right cardiac dilation, (B) Pericardial effusions (serosanguineous fluid), (C) Serous endocarditis, (D) Lungs - thrombosis, extensive congestive-hemorrhagic foci, and adult parasites, (E) Spleen - thrombosis and hemorrhagic congestive foci, (F) Serosanguineous peritoneal fluid, (G) Liver - passive hepatic congestion, (H) Some Adults of *D. immitis* identified, and (I) kidneys - interstitial nephritis and extensive congestive-hemorrhagic foci





**Figure 2.** Histopathological aspects. (A) Mitral valve – leukocyte infiltrates, edema, and fibroconjunctival proliferation, (B) Epicard - congested vessels with thickened wall and slight intramural leukocyte infiltration; intraluminal microfilariae, (C, D, E) Generalized ectasia of splenic vessels (presence of microfilariae (C), diffuse erythrocyte infiltrate in the white pulp with follicular degeneration, (F) Kidney – thrombosis, partially obstructed and fibrous-walled vessels, perivascular fibroconjunctival proliferation, subcapsular glomerular oedema, interstitial sero-hemorrhagic exudate, tubular vacuolar degeneration, (G) Kidney - sero-hemorrhagic interstitial exudate, granular, and vacuolar dystrophies of renal tubules, (H) Kidney - microfilariae present in the vascular lumen and predominantly eosinophilic intravascular and interstitial infiltrate, (I) Pancreas - intense serous-hemorrhagic exudate, congested vessels, and partial lysis of some pancreatic acins, and (J) Pancreas - degenerated pancreatic acins and lymphocytic infiltration

At the opening of the abdominal cavity, a serosanguineous peritoneal fluid was present in different quantities (300 mL and 1.5 L, [Figure 1F](#)). In all dogs, the liver was enlarged due to passive congestion ([Figure 1G](#)).

The spleen showed extensive congestive-hemorrhagic foci, the consistency being pasty and even friable in certain portions ([Figure 1E](#)). Microscopically, there was a generalized ectasia of splenic vessels (some with intraluminal microfilariae), along with a massive erythrocyte infiltrate and follicular degenerations in the white pulp ([Figure 2C, 2D, 2E](#)).

In the kidneys, the same congestive-hemorrhagic foci predominated, along with interstitial nephritis ([Figure 1I](#)). Microscopically, there was an abundant sero-hemorrhagic interstitial exudate, with granular and vacuolar dystrophies of the renal tubules ([Figure 1G](#)). A subcapsular and periglomerular edema was present, along with capillary ectasias.

Microscopically, thrombosis with vascular wall fibrosis was noted ([Figure 1F](#)), followed by edema and marginal

fibroconjunctival proliferation. A massive leukocyte infiltration was located perivascular and interstitial where intraluminal microfilariae were identified ([Figure 1H](#)).

In one out of six dogs, acute pancreatitis was also noted, microscopically characterized by the presence of abundant sero-hemorrhagic exudate with pancreatic acini degeneration and generalized vascular congestion ([Figure 2I, 2J](#)).

### Discussion

Globally, studies report the prevalence of heartworm disease in dogs diagnosed during their lifetime. Thus, according to a large study conducted in Romania (8), a lower prevalence of *D. immitis* was reported in Dolj (7.84%), and similar values were identified in Brasov (15.38%), Teleorman (13.73%) and Tulcea (15.94%).

Regarding the lesions, the changes at the organic level confirmed those previously presented by other researchers. In addition to right ventricular dilation, many researchers have identified large thrombi (9). Pasca

et al reported lung and pulmonary artery lesions as the most advanced ones. They described the erosions of the vascular endothelium with mesenchymal proliferation that can favor thrombus formation and cause associated hemorrhages. A bronchial hemorrhagic infiltration prevailed in the lung, with fibroconjunctival hyperplasia and even pleural fibrosis (5). Rafailov et al observed no obvious macroscopic changes in the lung parenchyma, but an inflammatory process was described microscopically (10). A generalized vasculitis with vessel wall damage and sclerosis has been observed by others in multiple organs such as the heart, lung, liver, kidney, spleen, and brain (4). The same authors also explained the presence of chronic endocarditis or pericarditis (4). Ranjbar-Bahadori et al also found membranoproliferative glomerulonephritis with predominantly monocytic infiltrates, atrophy, and even periglomerular fibrosis (11). In some situations, parasites have migrated to the abdominal aorta or femoral artery, leading to necrotizing vasculitis (12). There may also be splenic infarctions, renal infarctions, and hemorrhagic foci in the bladder and pancreas, as well as a generalized hemorrhagic-necrotic inflammation of the gastrointestinal system (13,14).

#### Authors' Contribution

**Conceptualization:** Iasmina Luca, Yagoob Garedaghi, Iniobong Chukwuebuka Ikenna Ugochukwu, Adrian Olariu-Jurca.

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**Funding acquisition:** Yagoob Garedaghi.

**Investigation:** Iasmina Luca, Yagoob Garedaghi.

**Methodology:** Iasmina Luca, Yagoob Garedaghi, Adrian Stancu.

**Resources:** Iasmina Luca, Yagoob Garedaghi, Iniobong Chukwuebuka Ikenna Ugochukwu.

**Software:** Iniobong Chukwuebuka Ikenna Ugochukwu.

**Supervision:** Iasmina Luca, Yagoob Garedaghi, Iniobong Chukwuebuka Ikenna Ugochukwu.

**Validation:** Iasmina Luca, Yagoob Garedaghi.

**Visualization:** Iasmina Luca, Adrian Olariu-Jurca.

**Writing—original draft:** Iasmina Luca

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#### Competing Interests

The authors declare that they have no conflict of interests.

#### Ethical Approval

Not applicable.

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