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Epidemiological Study on *Dirofilaria immitis* Infection in Dogs from Dolj County, Romania

Alexandra Pocinoc, Mirela Imre*, Cristian Dreghiciu , Anamaria Plesko , Diana Hoffman, Alexandru Mladin, Elena Toma, Gheorghe Dărăbuș

Department of Parasitology, University of Life Sciences "King Mihai I" from Timisoara, 300645 Timișoara, Romania
E-mail: alexandra.pocinoc@usvt.ro

Abstract: *Dirofilariasis* caused by *Dirofilaria immitis* represents an emerging vector-borne parasitic disease of major veterinary and zoonotic importance. Its distribution is closely linked to climatic conditions favorable for mosquito vectors, particularly in warm and humid regions. The present study aimed to assess the prevalence and epidemiological profile of *D. immitis* infection in dogs from Dolj County, southern Romania, an area characterized by a temperate-continental climate with high mosquito density. Between January 2024 and April 2025, blood samples from 260 dogs of various breeds, ages, and living conditions were collected and examined using three diagnostic methods: direct microscopic examination, the modified Knott's test, and a rapid antigen test (Canine Heartworm Ag Senspert CHW). The microscopic examination identified microfilariae in 11.5% of samples, while the modified Knott test and rapid antigen test revealed infection rates of 15.4% and 16.5%, respectively. *D. repens* was detected in 2.3% of the dogs tested by the modified Knott method. The slight differences between methods are attributable to variations in diagnostic sensitivity and the stage of infection. Epidemiological analysis indicated a higher prevalence among dogs aged 2–6 years, predominantly in urban environments, and with no significant sex predisposition. The results confirm that *D. immitis* is circulating among the canine population in Dolj County and emphasize the importance of using complementary diagnostic techniques to achieve accurate detection. This study contributes to updating the epidemiological map of *dirofilariasis* in Romania and highlights the need for continuous surveillance, preventive measures, and public awareness to control this parasitic zoonosis.

Keywords: *Dirofilaria immitis*, heartworm disease, epidemiology, dogs, Romania, mosquito vectors

Introduction

Dirofilariasis is a vector-borne zoonotic disease with a heterogeneous global distribution, predominantly affecting tropical, subtropical, and temperate regions. In Europe, it is mainly confined to southern countries, with well-established endemic areas in northern Italy and a rising prevalence across Romania. The infection is caused by *Dirofilaria immitis*, responsible for cardiopulmonary dirofilariasis and *Dirofilaria repens*, which inhabits subcutaneous tissues; both species are transmitted by mosquitoes of the *Culicidae* family. Transmission intensity is influenced by climatic conditions, environmental characteristics, and vector abundance. Dolj County, located in southern Romania, has a warm and humid continental climate and a high density of owned and free-roaming dogs, factors that favor mosquito proliferation. The aim of this study was to determine the prevalence of *D. immitis* infection in dogs from this region and to evaluate the agreement among commonly used diagnostic methods, contributing to improved surveillance and control of canine dirofilariasis in endemic areas.

Material and method

The study was conducted over a 16-month period (January 2024–April 2025) in Dolj County, southern Romania, a region with a temperate-continental climate characterized by hot, humid summers and mild winters conditions favorable for mosquito proliferation. A total of 260 domestic dogs were examined at a private veterinary clinic in Craiova and in public shelters or households from urban and peri-urban areas. Only dogs with complete clinical data and without heartworm prophylaxis in the previous six months were included. The population encompassed various breeds, sexes, and ages (1–16 years). Clinical examination focused on cardiovascular and respiratory parameters, with signs ranging from nonspecific (fever, lethargy, weight loss) to specific for dirofilariasis (dyspnea, coughing, hemoglobinuria, jaundice, and subcutaneous nodules).



Peripheral blood was collected aseptically from the cephalic vein into EDTA tubes and analyzed within 24 h using three diagnostic methods:

- Direct wet mount – microscopic examination of fresh blood for motile microfilariae.
- Modified Knott's test – concentration and morphological differentiation of *D. immitis* and *D. repens* microfilariae using formalin fixation, centrifugation, and methylene blue staining.
- Rapid antigen test – detection of *D. immitis* antigens via a commercial immunochromatographic assay (Senspert Canine Heartworm Ag, VetAll Laboratories, Korea).

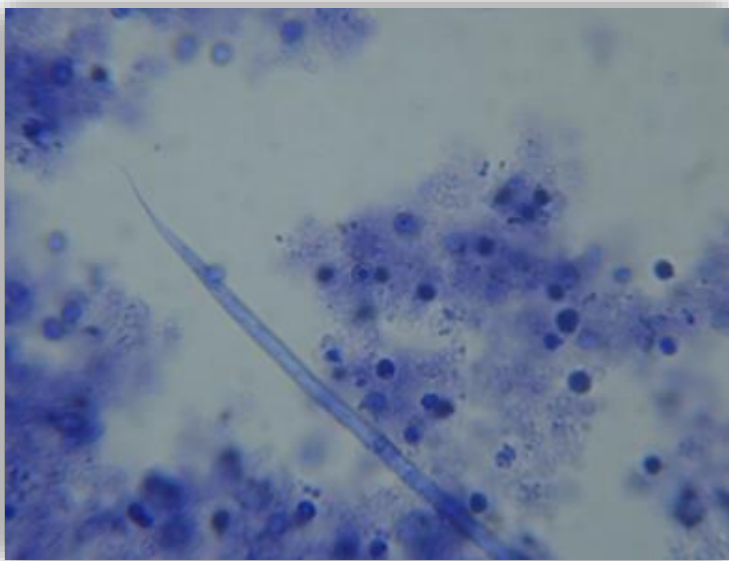
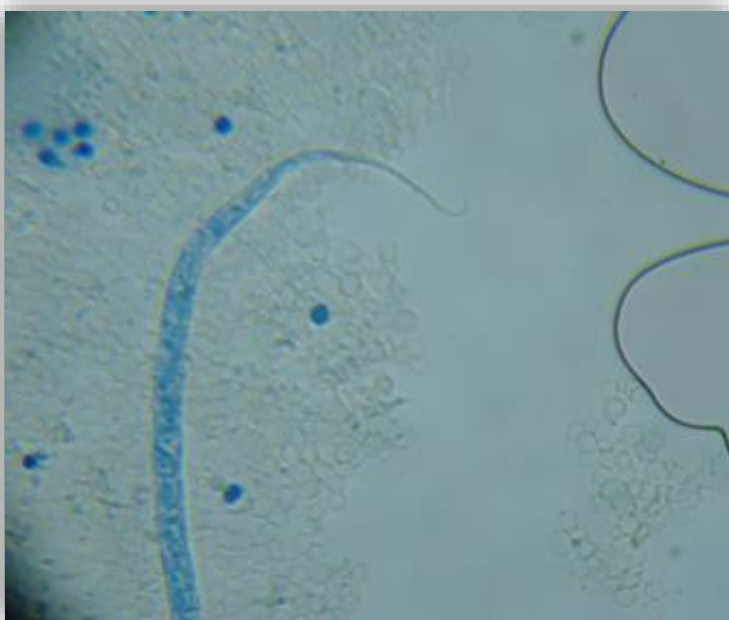
All analyses were performed under standardized biosafety conditions with internal and external quality controls to ensure accuracy and reproducibility.

Results and discussions

A total of 260 canine blood samples from Dolj County were examined using three complementary diagnostic methods. The direct wet mount detected motile microfilariae in 11.5% of samples, providing rapid but low-sensitivity results. The modified Knott's test identified microfilariae in 17.7% of samples, allowing species differentiation: 15.4% were positive for *Dirofilaria immitis* and 2.3% for *D. repens*. The rapid antigen test confirmed *D. immitis* infection in 16.5% of dogs, including several occult cases. Infections were more frequently detected in dogs aged 2–6 years, particularly among animals from urban areas, while no significant sex-related differences were observed. Mixed-breed dogs showed slightly higher prevalence than purebreds. The detection of both *D. immitis* and *D. repens* indicates their coexistence and simultaneous transmission within the same area, confirming the endemic status of dirofilariasis in Dolj County. Depending on the diagnostic method, the overall prevalence of *D. immitis* ranged between 15.4% and 16.5%, emphasizing the need for continuous epidemiological monitoring and routine testing in the local dog population.

Table 1. *Dirofilaria* species identified by different diagnostic methods

<i>Dirofilaria</i> species	Test results	D.	
		<i>immitis</i>	<i>repens</i>
Direct examination	positive	nr. 30	30
		% 11.5	11.5
	negative	nr. 230	230
		% 88.5	88.5
Modified Knott's test	positive	nr. 40	6
		% 15.4	2.3
	negative	nr. 220	254
		% 84.6	97.7
Rapid test	positive	nr. 43	0
		% 16.5	0.0
	negative	nr. 217	0
		% 83.5	0.0



The prevalence of *Dirofilaria immitis* infection detected in dogs from Dolj County (15.4–16.5%) is consistent with values reported in other Romanian regions and several southern European countries. Similar infection rates have been recorded in southern and western Romania (12–21%) [Giubega et al., 2021; Sandu et al., 2025], as well as in Serbia and Greece (14–24%) [Tasić-Otašević et al., 2022]. In contrast, markedly higher prevalences have been described in northern Italy, particularly in the Po River Valley, where up to 80% of untreated dogs were found positive [Genchi et al., 2009]. Earlier Romanian studies reported highly variable results, ranging from 6% to 65% depending on geographic area, diagnostic method, and use of prophylaxis [Olteanu, 1996; Tudor et al., 2008; Coman et al., 2007]. The occurrence of *D. repens* infection (2.3%) in the present study agrees with findings from Bulgaria (1.5–3%), Hungary (2–5%), and northern Greece (3–6%) [Dumitrache et al., 2021; Genchi et al., 2005], confirming the co-endemic distribution of both filarial species across southeastern Europe. These comparative data highlight the regional variability of canine dirofilariasis prevalence, strongly influenced by climate, vector density, and preventive coverage, and align with the broader European trend of northward parasite expansion facilitated by changing environmental conditions.

Conclusions

- Both *Dirofilaria immitis* and *Dirofilaria repens* are actively circulating among dogs in Dolj County, confirming the endemic status of dirofilariasis in southern Romania.
- The prevalence of *D. immitis* ranged from 15.4% to 16.5%, while *D. repens* was detected in 2.3% of examined dogs.
- Climatic conditions and high mosquito density favor sustained transmission in the region.
- The combined use of the modified Knott test and antigen detection provided the most accurate diagnostic results, enabling identification of both patent and occult infections.
- Regular testing and year-round chemoprophylaxis with macrocyclic lactones remain essential for effective control.

