

## PREVALENCE OF INFECTION WITH *DIROFILARIA IMMITIS* AND *DIROFILARIA REPENS* IN DOGS FROM THE SOUTH-EASTERN PART OF ROMANIA

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As a result of global warming, the change in the biological cycle of vectors and the increase in intercontinental travels, we are now facing an increase in the number of cases of vector-borne diseases. Among these, heartworm disease has escalated in recent years in Romania, with cases increasing in many parts of our country. Our study was carried out in the South-Eastern part of Romania, with the aim of establishing the prevalence of heartworm disease in dogs, with the identification of the two species *Dirofilaria immitis* and *Dirofilaria repens*. Blood samples were collected during the period May - July 2022 and January - July 2023, from 220 dogs with and without an owner from Tulcea county, from places such as Tulcea, Murighiol, Somova, Mineri, Niculițel. The blood samples were tested through the Knott method, which is considered as the reference method in the diagnosis of dirofilariosis in dogs. The results show a prevalence of *Dirofilaria spp.* infection of 11.36% in dogs. Of the 220 samples examined by the Knott test, 25 were positive for circulating microfilariae, of which 21 showed infestations with *Dirofilaria immitis* and 4 with co-infection with *Dirofilaria immitis* and *Dirofilaria repens*. The study underlines the importance of introducing monitoring and control programs for heartworm disease in dogs in Romania.

**Key words:** dirofilariosis; dogs; Tulcea;

**Introduction.** Canine cardiopulmonary and subcutaneous dirofilariosis are caused by zoonotic filaroid worms, *Dirofilaria immitis* and *Dirofilaria repens*, respectively. Human dirofilariosis in the ancient world is primarily caused by *D. repens*, whereas *Dirofilaria immitis* is highly significant for veterinary purposes. Numerous mosquito species, including those of the genera *Culex*, *Aedes*, *Ochlerotatus*, *Anopheles*, *Coquillettidia*, *Armigers*, *Ansonia* and *Psorophora*, have been identified as competent vectors of these mosquito-borne filarial infections. These mosquito-borne filarial infections share the same definitive hosts, which are primarily canids (Younes *et al.*, 2021).

Historically, canine heartworm infection was restricted to southern Europe. Up until the mid-1980s, northern Italy was the most endemic region for the parasite. Birago discovered the parasite in a greyhound dog during an autopsy in northern Italy in 1626 (Genchi *et al.*, 2014).

Dogs that are infected are the primary source of *D. repens* because their peripheral blood typically contains microfilariae. *D. repens* infections have been documented as widespread in central and southern Italy as well as in other

southern European nations like France, Greece and other former Yugoslavia since the early 1900s (Genchi and Kramer, 2017).

The first known human observation of *D. repens* was probably made in 1566 by the Portuguese physician Amato Lusitano, who noticed the tip of a worm suddenly appearing in the big angle of the eye in a 3-year-old girl. The worm is sometimes located in the eye, causing opacity (Capelli *et al.*, 2018).

As for the present, both the quantity of epidemiological reports and the epidemiological state have altered. Climate change has resulted in new climatic conditions for Europe, which affects the presence of new competent vectors that broaden the risk zone (mosquitoes more resistant to low temperatures, overwintering eggs, etc.) and increase the duration of exposure to the parasite (daytime and crepuscular/nocturnal mosquitoes) (Morchón *et al.*, 2022).

Heartworm illness is linked to numerous pathophysiological pathways, such as right ventricular overload and cardiopulmonary abnormalities linked to parenchymal lung disease (Tudor *et al.*, 2014).

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Heartworm disease in dogs can present with a wide range of symptoms, from mild to severe and even fatal. Reduced exercise tolerance, a persistent cough that advances from moderate to severe dyspnea, prostration, ascites, cachexia, and post-exercise syncope or excitation are the most common early clinical symptoms (Bendas *et al.*, 2022).

The second species, *Dirofilaria repens* causes some common signs in pets, like itch (pruritus), papulae, erythema, alopecia, crusting, hyperkeratosis, lichenification and acantosis. Occasionally, subcutaneous nodules can be seen, formed by a cyst enclosing an adult nematode. In most cases, however, no pathogenic signs are observed in animals carrying *repens* microfilariae (Tarello, 2011).

A minimum of ten years ago, *Dirofilaria spp.* were identified in Romania. According to Tomazatos *et al.* (2018), there have been different investigations on the prevalence of parasites in dogs that have found local infection rates ranging from 3% to over 60%.

The aim of this study was to obtain a current prevalence of infection with *Dirofilaria immitis* and *Dirofilaria repens* in dogs from the south-eastern part of Romania.

## MATERIAL AND METHOD

This study included 220 dogs from Tulcea county, Romania. Their age and gender were taken into account. Based on this data, a statistic was made, resulting in 124 females and 96 males with the age between 7 months and 18 years. Regarding the medical history of the dogs, it was known in only 55 of the dogs, since the rest of the samples came from dogs without an owner which did not have any medical record. Based on the age, the dogs were grouped as follows: puppies (7 months – 1 year); young (1-3 years); adults (4-7 years); seniors (8-10 years); and geriatrics (11-18 years). Therefore, 220 blood samples were collected in tubes with anticoagulant (ethylenediaminetetraacetic acid- EDTA) from the

cephalic vein. The samples were analyzed in the Clinical Laboratory of Parasitology and Parasitic Diseases of the Faculty of Veterinary Medicine in Iasi.

### Laboratory techniques

Blood samples were tested for the identification of circulating microfilariae using the modified Knott method.

The modified Knott's test is an easy and inexpensive technique based on concentration, staining, detection and morphometric identification of circulating of different species. The technique foresees the dilution of 1 mL of EDTA venous blood with 9 mL of 2% formalin (Genchi *et al.*, 2021).

The technique consists in adding to a 15 ml tube, 1 ml of canine blood sample, which was mixed with 9 ml of 2% formalin. The tube was centrifuged for 5 minutes at 1500 rpm and the supernatant was removed, adding 1% methylene blue to the sediment. After mixing, from the resulting sediment, 1 drop was transferred to a slide, covered with a coverslip and examined under an optical microscope.

The modified Knott test (Knott, 1939) is preferred and is the recommended test for circulating microfilariae (Evans *et al.*, 2019).

## RESULTS

The 220 samples come from 124 females and 96 males.

Of the 198 samples collected in Tulcea, 55 samples come from dogs with owners and 143 from dogs without owners.

From the 143 blood samples collected from stray dogs, the vast majority of samples were collected from dogs from the Public Shelter and thus resulted in 128 negative samples, 11 positive, infected with *Dirofilaria immitis* and 4 positive with co-infection with *Dirofilaria immitis* and *Dirofilaria repens* (figure 1).



Figure 1 – *Dirofilaria spp.* – ob. X 10

And out of the total of 55 samples from dogs with owners, 48 were negative and 7 were infected with *Dirofilaria immitis*.

In the town of Niculițel, 19 blood samples were collected, of which 16 were negative and 3 were positive for *Dirofilaria immitis*.

The rest of the samples, in total of 3 collected from dogs from Murighiol and Somova, and turned out to be negative.

In terms of age, the vast majority of blood samples were collected from young dogs, aged between 1 and 3 years, as well as from adult dogs, aged between 4 and 7 years (76,8%) (table 2).

Table 1

**Prevalence of *Dirofilaria immitis* and *Dirofilaria repens* in the localities of Tulcea county**

Localities	Positive samples (Total positive/Total samples)	
Tulcea	22/198	(11.11%)
Niculițel	3/19	(15.78%)
Somova/Murighiol	0/3	(0%)

Of the 124 females tested, 112 were negative and 12 were positive (9.67%). Of the 12 samples, 11 were positive for *Dirofilaria immitis* and 1 for co-infection with *Dirofilaria immitis* and *Dirofilaria repens* (table 1).

Of the 96 males tested, 83 were negative and 13 were positive (13.54%). Of the 13 samples, 10 were positive for *Dirofilaria immitis* and 3 for *Dirofilaria immitis* plus *Dirofilaria repens* (table 2).

Table 2

**The results of the samples analyzed by gender and age**

Gender	Positive samples (Total positive/total tested)	
Female	12/124	(9.67%)
Male	13/96	(11.36%)
Age		
1-3 years	7/129	
4-7 years	13/64	
8-10 years	4/21	
11-18 years	1/6	

Thus, the modified Knott test showed a total of 25/220 samples positive for microfilariae (11.36%), of which 21/25 samples were positive for *Dirofilaria immitis* microfilariae and 4/25 for *Dirofilaria immitis* plus *Dirofilaria repens*.

The highest prevalence was detected in the age group 4-7 years, followed by the groups 1-3 years (young), 8-12 years and geriatrics dogs (13-18 years).

Table 3

**Prevalence of *Dirofilaria immitis* and *Dirofilaria repens***

Species	Positive samples (Total positive/Total samples)	
<i>Dirofilaria immitis</i>	21/220	(9.54%)
<i>Dirofilaria repens</i>	0/220	(0%)
<i>D. immitis</i> and <i>D. repens</i>	4/220	(1.81%)

## DISCUSSIONS

This study presents the results obtained from the examination of blood samples of 220 dogs from Tulcea county using parasitological

methods, in order to establish the prevalence of canine dirofilariosis in this county.

Analyzing various studies from previous years, we can observe an increased prevalence of heartworm disease in the South-East of Romania. As can be seen in an article written in 2015 in

which it is specified: Based on molecular detection, an overall prevalence of 6.92 % ( $n = 27$ ; 95 % confidence interval (CI) 4.70–10.03 %) for *D. repens*, 6.15 % ( $n = 24$ ; 95 % CI 4.07–9.14 %) for *D. immitis* and 2.05 % ( $n = 8$ ; 95 % CI 0.96–4.16 %) for *A. reconditum* was recorded, with significant variations according to sampling areas. Coinfections of *D. immitis* and *D. repens* were recorded in 23.91 % ( $n = 11$ ) positive dogs (Ionică et al., 2015). Comparing the number of blood samples analyzed and the positive results, we can see an increase in the prevalence of heartworm disease.

No study has been published on risk factor analyzes using a multivariate approach, which would be more suitable for highlighting confounding factors and biases (Capelli et al., 2018). Therefore, some of the associations found and often reported as risk factors are likely the results of the interaction of different factors related to the host (sex, age, breed and lifestyle), the vector (presence, density, vectorial capacity and attraction to dogs), the environment (rural, urban, climate) and the human intervention (use of specific chemoprophylaxis and/or physical or chemical protection against mosquitoes) (Capelli et al., 2018).

Comparing these factors with the data presented, it was found that the most increased prevalence was among males (13 positive samples), males classified as adults, with age between 4-7 years.

From the total number of 220 samples, 25 were positive for circulating microfilariae, of which 21 were infected with *Dirofilaria immitis* and 4 with co-infection with *Dirofilaria immitis* and *Dirofilaria repens*. In the specialized literature it is noted that the interspecific relationship between *D. immitis* and *D. repens* has been only partially studied, suggesting an inhibition of the development of *D. immitis* in dogs previously infected with *D. repens* (Ionică et al., 2017). However, it indicates that once the animal develops a patent co-infection, the microfilariae of the two species show a similar circadian periodicity, probably as a reaction to the same stimuli, without apparent influences between them (Ionică et al., 2017).

Referring to Table 3, where the prevalence of heartworm disease is presented in some of the localities in Tulcea county, we notice that it is higher in rural areas (Niculițel: 15.78%). This difference appears in general due the lack of information about external parasites treatments, but global warming should not be omitted, as it is mentioned in the article written by Martinescu et al., 2022. In this article it is specified that is

possible that Global warming is influencing the increase in cases of this disease, according to the abundance of etiological agents (Martinescu et al., 2022).

## CONCLUSIONS

The prevalence that we established in dogs (11.36%) of dirofilariosis in the South-East of Romania, respectively in Tulcea county, has an alarming increase, thus monitoring the disease, as well as prophylactic treatment is a main objective. Also, clinician veterinarians should include this disease in the differential diagnosis of various cardiac, respiratory and dermatological diseases.

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