

Seasonal study of Blood Parasites: *Dirofilaria immitis* and *Dipetalonema reconditum* in the Guard dogs of Tabriz city, Iran

Abstract:

Dirofilariasis or canine heartworm disease is one of the most dangerous invasive diseases in dogs. Invasion caused by *Dirofilaria immitis* is the most critical case. Filarial infection has been seen all over the world with different agents including different species of nematodes, but *D. immitis* is more important than other species in dogs.

In this study, one hundred guard dog collars were used that visited the small animal clinics of Tabriz city in Iran during the period of 3 months (May, June, and July) summer season year 2023, because blood parasites is more common in dogs in summer. A total of 100 blood samples of guard dogs, regardless of age, sex, and breed, were examined for blood parasites. Of these, 70 were adult dogs, 30 were puppies, 35 were females, and 65 were males. The prevalence of blood parasites in dogs was determined by wet blood smear, centrifuge hematocrit and modified Knott's technique.

The blood parasites found in this study were *D. immitis*, *Dipetalonema reconditum*. In this study, out of 100 guard dogs, 9 dogs (9%) were found infected with blood parasites. The prevalence of *D. immitis* and *D. Reconditum* recorded in our study was 7% and 2%. Two puppies out of 30 puppies (6.6%) were infected with blood parasites, and in the group of adult dogs, 7 collars out of 70 dog collars (10%) were infected with blood parasites. The highest recorded infection percentage was related to *D. immitis* and 7 collars (7%) out of 100 cases were positive. The findings showed the highest prevalence of blood parasites in May (10%), followed by June (9.37%) and July (8.33%). The results showed that the incidence of blood parasites in male dogs was higher than in female dogs. Males were infected (9.23%) and females (8.57%).

The results of this study showed that the prevalence of *D. immitis* and *D. Reconditum* recorded in guard dogs of Tabriz city in Iran was 7% and 2%. Guard dogs should be kept in a sanitary environment and should be regularly tested for the presence of any blood parasites and to prevent the growth of blood parasites and the dangerous consequences of these parasites inside the body, they should be regularly and timed treated with anti-parasitic drugs.

Keywords: *Dirofilaria immitis*, *Dipetalonema reconditum*, Guard dogs, Tabriz, Iran.

1. Introduction:

Dirofilariasis or canine heartworm disease is one of the most dangerous invasive diseases in dogs. Invasion caused by *D. immitis* is the most critical case (1, 2). Filarial infection has been seen all over the world with different agents including different species of nematodes, but

Dirofilaria immitis is more important than other species in dogs (3). The reason for its importance is a severe and deadly disease called heartworm disease and also its role as a zoonotic disease (4). This disease is a metazoonotic disease that has an indirect life cycle and is transmitted by specific species of mosquitoes, including Aedes, Anopheles and Culex, of which 70 types of mosquitoes are capable of Maintenance and development of Dirofilaria immitis larvae (5, 6). Dirofilaria adult worms are usually 15 to 35 cm long and 3 mm wide. Male worms are half the size of female worms. The life-span of each adult worm reaches five years. Sometimes there may be 300 heartworms in the body of an animal. Its reservoir is often canine and it has a global spread disease. The clinical symptoms of this disease in dogs are very variable, from the asymptomatic stage to mild symptoms such as emaciation and gradual weight loss, cough, inactivity and early fatigue during activity, to severe symptoms such as dyspnea, temperature increase, and membrane damage. Mucous (cyanosis), anemia, cardiac complications and death ends (7). Diagnosis of the disease is done by different methods such as parasitological tests (modified Knott method to observe microfilar) and findings of radiography, echocardiography, and electrocardiography (8, 9). But since the presence of microfilaria in the blood has a variable nature and in many cases (5 to 67%) they are not observed at all; therefore, the diagnosis of heart infection in dogs depends on a combination of methods such as hematology and serology (10). Nowadays, methods such as ELISA and immunochromatography are used for screening, which show the contamination by checking the somatic antigens of the heartworm in the serum. In addition to ease of use, serum screening tests are much more sensitive than parasitological methods (11). *Dipetalonema reconditum* lives in the peritoneal cavity, subcutaneous connective tissues of dogs and other carnivores, whose adult worm is not pathogenic, but it should be distinguished from Dirofilaria immitis microfilar in the microfilar blood test (12).

2. Materials and methods:

The method of this study is a cross-sectional epidemiological method. In this study, only dogs over 6 months of age were sampled (100 dogs).

2.1. Experimental animals and geographical area: In this study, one hundred guard dog collars were used that visited the small animal clinics of Tabriz city in Iran during the summer season year 2023. The gender and breed and the location of the dogs, clinically suspected cases of heart diseases that can be caused by dirofilariasis or anemia caused by dirofilaria were recorded.

2.2. Blood sampling and collection of blood samples:	٧٤
from all guard dogs from the available cephalic veins and the blood samples were collected in	٧٥
a sterile manner inside disposable test tubes containing EDTA blood anticoagulant and the	٧٦
characteristics of the dogs were written on the label of each test tube.	٧٧
2.3. Techniques used to Examination of blood samples:	٧٨
2.3.1. Wet blood film technique:	٧٩
A drop of dog's blood was placed on a clean glass slide	
and a coverslip was placed on it and then examined under a microscope (13).	٨٠
2.3.2. Microfiller concentration techniques include:	٨١
2.3.2.1. Hematocrit tube test and Buffy-coat layer:	٨٢
Micro hematocrit tubes were filled with blood up to two-thirds volume and one end of each	٨٣
tube was closed with micro hematocrit paste. Then the microhematocrit tubes were centrifuged	٨٤
at 3000 rpm for 5 minutes. Then, the formed layer of buffy-coat was poured on a clean glass	٨٥
slide and after placing a coverslip and checked for the presence of microfilariae under the	٨٦
microscope (14).	٨٧
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2.3.2.2. Modified Knott technique:	٨٩
One milliliter of dog blood was poured into a centrifuge tube containing 9 milliliters of 2%	٩٠
formalin. After mixing the contents of the test tube, we allowed 15 minutes for hemolysis to	٩١
take place. Then the mixture was centrifuged at 1500 rpm for 5 minutes. The supernatant was	٩٢
discarded and the sediment was stained with an equal volume of methylene blue (1:1000).	٩٣
The test tube mixture was placed on a glass slide and checked for the presence of microfilariae	٩٤
under the microscope (15).	٩٥
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3. Results:	٩٧
This study was conducted to record the prevalence of helminthic blood parasites in guard dogs	٩٨
in Tabriz, Iran. The blood parasites found in this study were <i>Dirofilaria immitis</i> , <i>Dipetalonema</i>	٩٩
<i>reconditum</i> . The average length of the microfilaria in <i>Dirofilaria immitis</i> is 313 microns, but it	١٠٠
is 270 microns in <i>Dipetalonema reconditum</i> (Figure 1 and 2). Dogs infected with blood	١٠١
parasites showed symptoms of fever, anemia, hemoglobin urea, thinness, darkness, appetite	١٠٢
disorder, edema, cough and hard breathing. The incidence of blood parasites was studied in a	١٠٣
period of 3 months (May, June, and July) summer season year 2023 in Tabriz city.	١٠٤
A total of 100 blood samples of guard dogs, regardless of age, sex, and breed, were examined	١٠٥
for blood parasites. Of these, 70 were adult dogs, 30 were puppies, 35 were females, and 65	١٠٦

were males. The prevalence of blood parasites in dogs was determined by wet blood smear, centrifuge hematocrit and modified Knott's technique. In this study, out of 100 guard dogs, 9 dogs (9%) were found infected with blood parasites (Table-1).

Two puppies out of 30 puppies (6.6%) were infected with blood parasites, and in the group of adult dogs, 7 collars out of 70 dog collars (10%) were infected with blood parasites (Table-2). The highest recorded infection percentage was related to *Dirofilaria immitis* and 7 collars (7%) out of 100 cases were positive (Table-3). The findings showed the highest prevalence of blood parasites in May (10%), followed by June (9.37%) and July (8.33%) (Table 4). The results showed that the incidence of blood parasites in male dogs was higher than in female dogs. Males were infected (9.23%) and females (8.57%) (Table 5).



Figure 1- Microfiler of *Dirofilaria immitis* in blood smear of guard dogs, 2 complete microfilaris (X40)

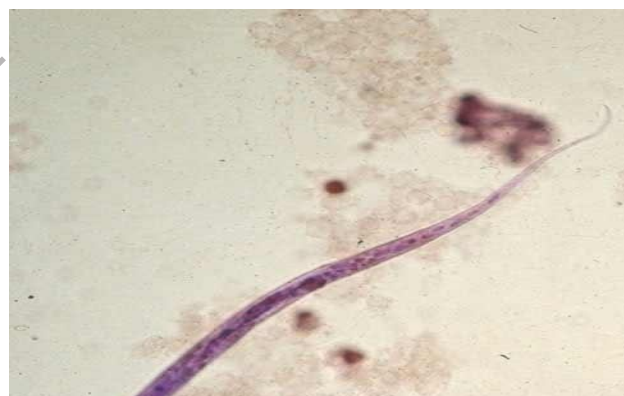


Figure 2- Microfiler of *Dipetalonema reconditum* in blood smear of guard dogs, the end of microfiler tail area (X40)

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Table 1: Prevalence of *Dirofilaria immitis*, *Dipetalonema reconditum* in guard dogs of Tabriz city

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Animal	Examined	Infected	infection %
Guard dog	100	9	9
Total	100	9	9

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Table 2: Prevalence of *Dirofilaria immitis*, *Dipetalonema reconditum* according to the age of guard dogs in Tabriz city

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Age	Examined	Infected	infection %
Pups	30	2	6.6
Adult	70	7	10
Total	100	9	9

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Table 3: Prevalence of *Dirofilaria immitis*, *Dipetalonema reconditum* according to parasite species in guard dogs of Tabriz city

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species	Examined	Infected	infection %
<i>Dirofilaria immitis</i>	100	7	7
<i>Dipetalonema reconditum</i>	100	2	2

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Table 4: Monthly prevalence of blood parasites in guard dogs in Tabriz city

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Month	Examined	Infected	infection %
May	20	2	10
June	32	3	9.37
July	48	4	8.33
Total	100	9	9

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Table 5: Prevalence of blood parasites according to gender in guard dogs of Tabriz city

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Sex	Examined	Infected	infection %
Male	65	6	9.23
Female	35	3	8.57

Total	100	9	9
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4. Discussion:

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Parasitic diseases caused by blood parasites such as *Dirofilaria immitis* and *Dipetalonema*

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Reconditum causes severe infection in dogs and is found worldwide (16, 17). The prevalence of *Dirofilaria immitis* and *Dipetalonema Reconditum* recorded in our study was 7% and 2% (Table-3).

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Respectively, whereas the respective prevalence of both of these species was reported as 2.7 and 0% by Durrani et al. , 3.06 and 1.3% by Chakrabarti and Chaudhury , 10.9 and 3.6% by Martin and Colin , 5.9 and 0% by Deidrick and Boyce , 3.54 and 4.16% by Bulman et al , 23.9 and 5.4% by Magi et al. , 12.3 and 2.1% by Perez-Sanchez et al. , 53.8 and 0.0% by Hatsushika et al. , and 10.7 and 5.5% by Petruschke et al. (18).

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Our findings in Tabriz city are almost consistent with the results obtained by various researchers mentioned above. Our study was conducted in the summer months (May to July). Although the difference in the prevalence of the mentioned blood parasites in different months of the year was not significant. However, in our study, the lowest prevalence of blood parasite infection was recorded in July (8.33%) and the highest prevalence was recorded in May (10%).

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In our study, in terms of age, the prevalence of blood parasites in different age groups of dogs does not show a statistically significant difference between them. However, there is a higher trend of the prevalence of parasitic infection in older dogs than in young dogs. Prevalence 6.6% was recorded in puppies and 10% in adult dogs. This difference is probably due to the low immunity in fighting blood parasite infections in old age.

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That our results are similar and consistent with the reports of Perez Sanchez et al. and Olmer et al. (18).

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Also, in the study of Bokai et al. in Meshkinshahr, the prevalence of *dirofilaria immitis* increased from 18.4% to 56.8% with increasing age of dogs (24).

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The results of our study showed that the incidence of blood parasites in male dogs was higher than in female dogs. Males were infected (9.23%) and females (8.57%) (Table 5). But in the

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study of Bokai et al. in Meshkinshahr, the prevalence of dirofilaria immitis has been reported, 1۷۰
34.3% in male dogs and 35% in female dogs (24). 1۷۱

In 2007, Sevimli and colleagues also reported the symptoms of anemia, including the clinical 1۷۲
symptoms caused by dirofilaria immitis in dogs (19). Sharma and colleagues also reported the 1۷۳
decrease of hemoglobin in this disease in 1981 (20). Also Meyer and colleagues reported an 1۷۴
increase in fibrinogen levels in dogs with heartworm. According to the studies of Rhee and 1۷۵
TADA in 1991 and 1998, the highest prevalence of dirofilaria parasite in Asia was in Japan 1۷۶
and Korea at the rate of 8.62% (21, 22) and the lowest was reported in India with 2.3% (23). 1۷۷
In 1998, Bokai and colleagues reported the infection rate of Meshkin shahr city dogs with 1۷۸
dirofilaria parasite of 26.7% in Iran (24). 1۷۹

In Tabriz city, there are many stray dogs wandering in the streets and roads. These stray dogs 1۸۰
usually contain various types of parasitic infections and since they have not received any anti- 1۸۱
parasitic drugs to treat parasites due to improper care and unsanitary conditions, such dogs act 1۸۲
as a reservoir of parasitic infections for Guard dogs. For this reason, these parasitic infections 1۸۳
usually remain in the environment for a long time. 1۸۴

Guard dogs should be kept in a sanitary environment and should be regularly tested for the 1۸۵
presence of any blood parasites and to prevent the growth of blood parasites and the dangerous 1۸۶
consequences of these parasites inside the body, they should be regularly and timed treated 1۸۷
with anti-parasitic drugs. 1۸۸

Ethical approval: 1۸۹
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In this study, ethical considerations have been fully observed. 1۹۱

Acknowledgments 1۹۲

This article has been written using the results of the Master's degree student thesis that has been 1۹۳
approved in the Islamic Azad University of Tabriz Medical Sciences Unit, With ID 1۹۴
(<https://ethics.research.ac.ir/IR.IAU.TABRIZ.REC.1402.383>) in the ethics committee. 1۹۵

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Authors' Contribution 1۹۸

Study concept and design: Y.G. Acquisition of data: R.G. Analysis and interpretation of data: ۱۹۹
Y.G. Drafting of the manuscript: Y.G. and B.A.T. Revision of the manuscript: Y.G. Statistical ۲۰۰
analysis: B.A.T. and Y.G. ۲۰۱

Conflict of interest statement

The authors declare that they have no conflicts of interest. ۲۰۲

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