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Original Article

Seasonal study of Blood Parasites: *DirofilariaImmitis* and *Dipetalonema Reconditum* in the Guard Dogs of Tabriz city, Iran

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ABSTRACT

Dirofilariasis, also known as 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 observed worldwide, involving different agents and species of nematodes.However, *D. immitis* is more important than other species in dogs. For this study, one hundred guard dog collars were examined from dogs that visited small animal clinics of Tabriz , Iran during the summer of 2023 (May, June, and July), because blood parasites are more prevalent in dogs in summer. A total of 100 blood samples from guard dogsof various ages, sexes, and breeds 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 the dogs was determined using wet blood smear, a centrifuge hematocrit, and modified Knott's technique. The blood parasites found in this study were *D. immitis*, *Dipetalonema reconditum*.Out of 100 guard dogs, 9 dogs (9%) were found to be infected with blood parasites. The prevalence of *D. immitis and D. Reconditum* in our study was 7% and 2%, respectively. Two out of thirty 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 infection percentage recorded was related to *D. immitis* with 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 male dogs more likely to be infected than females. The incidence of blood parasites in was higher than in female dogs. Males were infected at a rate of 9.23%, while females were infected at a rate of 8.57%.The results of this study showed that the prevalence of *D. immitis and D. Reconditum* in guard dogs in Tabriz, Iran was 7% and 2%, respectively. Guard dogs should be kept in a sanitary environment and regularly tested for blood parasites. To prevent the growth of blood parasites and their dangerous consequences, they should be regularly treated with anti-parasitic drugs.

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1. Introduction

Dirofilariasis,also known as 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 oberved worldwide, involving different agents and species of nematodes.However, 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 its role as a zoonotic disease (4). This metazoonotic disease has an indirect life cycle and is transmitted by specific species of mosquitoes, including Aedes, Anopheles and Culex.Seventy otypes of mosquitoes are capable of Maintaining and developing 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, an animal may have up to 300 heartworms in its body. The disease is often found in canines and has a global spread. Clinical symptoms in dogs vary significantly, ranging from an asymptomatic stage to mild symptoms such as emaciation, gradual weight loss, cough, inactivity, and early fatigue during activity to severe symptoms such as dyspnea, increased temperature, and membrane damage. Other symptoms include mucous (cyanosis), anemia, cardiac complications, and death (7). Diagnosis of the disease is done using different methods such as parasitological tests (the modified Knott method to observe microfilariae) and radiography, echocardiography, and electrocardiography (8, 9).

However, since the presence of microfilariae in the blood has a variable nature and often go unobserved (in 5-67% of cases), 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. These methods check for contamination by examining 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 and 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

This study employed a cross-sectional epidemiological method. Only dogs over 6 months of age were sampled (100 dogs).

2.1. Experimental animals and Geographical Srea

One hundred guard dog collars were used that visited the small animal clinics in Tabriz , Iran, during the summer of 2023. The gender and breed and the location of the dogs, as well as clinically suspected cases of heart diseases caused by dirofilariasis or anemia caused by dirofilaria were recorded.

2.2. Blood Sampling and Collection of Blood Samples

5 ml of venous blood were collected from all guard dogs from the available cephalic veins. The blood samples were collected in sterile disposable test tubes containing EDTA blood anticoagulant and the dogs' characteristics e were written on each test tube's label.

2.3. TechniquesUsed to Examination of Blood Samples

2.3.1. Wet Blood Film Technique

A drop of dog's blood was placed on a clean glass, covered with s a coverslip ,and examined under a microscope (13).

2.3.2. Microfiller Concentration Techniques include

2.3.2.1. Hematocrit Tube Test and Buffy-coat layer

Micro hematocrit tubes were filled with blood up to two-thirds volume, and one end of each tube was closed with micro hematocrit paste. Then the micro hematocrit tubes were centrifuged at 3000 rpm for 5 minutes. The formed buffy-coat layer was poured onto a clean glass slide. After placing a coverslip, the presence of microfilariae was checked under a microscope (14).

2.3.2.2. Modified Knott technique

We poured one milliliter of dog blood into a centrifuge tube containing 9 milliliters of 2% formalin. After mixing the contents of the test tube, we waited for 15 minutes for hemolysis to occur. Then, we was centrifuged the mixture at 1500 rpm for 5 minutes. We discarded the supernatant and stained the sediment with an equal volume of methylene blue (1:1000). The test tube mixture was placed on a glass slide and checked under the microscope for the presence of microfilariae (15).

3. Results

The purpose of this study was to record the prevalence of helminthic blood parasites in guard dogs in Tabriz, Iran. The blood parasites found in this study were *Dirofilaria immitis*, *Dipetalonema reconditum* .The average length of the microfilaria in *Dirofilaria immitis* is 313 microns, whereas Dipetalonema reconditum microfilariae are 270 microns long (Figures 1 & 2). Dogs infected with blood parasites showed symptoms of fever, anemia, hemoglobin urea, thinness, darkness, appetite disorder, edema, cough and hard breathing. The incidence of blood parasites was studied over a 3 -months period (May, June, and July) during the summer of 2023 in Tabriz. A total of 100 blood samples from guard dogs of various ages, sexes, and breeds were examined for blood parasites. Of these samples, 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. Out

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

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

of 100 guard dogs in this study, 9 dogs (9%) were found to be infected with blood parasites (Table 1).Out of 30 puppies ,two puppies were infected with blood parasites. In the group of adult dogs, 7 collars out of 70 dog collars (10%) were infected with blood parasites (Table 2).

The highest infection percentage recorded 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 female dogs. Males were infected (9.23%) and females (8.57%) (Table 5).

4. Discussion

Parasitic diseases are caused by blood parasites such as *Dirofilaria immitis and Dipetalonema 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%, respectively (Table 3). The respective prevalence 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). Our findings in Tabriz are almost consistent with those of 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%). In our study, the prevalence of blood parasites in different age groups of dogs does not show a statistically significant difference. However, older dogs are more likely to be infected than young dogs. Prevalence was 6.6% in puppies and 10% in adult dogs. This difference is probably due to lower immunity to blood parasite infections in old age.

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

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

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

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

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

Species	Examined	Infected	Infection %
Dirofilaria immitis	100	7	7
Dipetalonema reconditum	100	2	2

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

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

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

Sex	Examined	Infected	Infection %
Male	65	6	9.23
Female	35	3	8.57
Total	100	9	9

That our results are similar to and consistent with the reports of Perez Sanchez et al. and Olmer et al. (18). Additionally, Bokai et al.'s study in Meshkinshahr found that the prevalence of Dirofilaria immitis increased from 18.4% to 56.8% with the dogs' age.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 study of Bokai et al. in Meshkinshahr, the prevalence of dirofilaria immitis has been reported, 34.3% in male dogs and 35% in female dogs (19). In 2007, Sevimli and colleagues also reported the symptoms of anemia, including the clinical symptoms caused by dirofilaria immitis in dogs (20). Sharma and colleagues also reported the decrease of hemoglobin in this disease in 1981 (21). Also Meyer and colleagues reported an increase in fibrinogen levels in dogs with heartworm. According to the studies of Rhee and TADA in 1991 and 1998, the highest prevalence of dirofilaria parasite in Asia was in Japan and Korea at the rate of 8.62% (22, 23) and the lowest was reported in India with 2.3% (24). In 1998, Bokai and colleagues reported the infection rate of Meshkin shahr city dogs with dirofilaria parasite of 26.7% in Iran (19). In Tabriz, there are many stray dogs wandering in the streets and roads. These stray dogs usually contain various types of parasitic infections and since they have not received any anti-parasitic drugs to treat parasites due to improper care and unsanitary conditions, such dogs act as a reservoir of parasitic infections for Guard dogs. For this reason, these parasitic infections usually remain in the environment for a long time. Guard dogs should be kept in a sanitary environment and 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 treated with anti-parasitic drugs.

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Authors' Contribution

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

Ethics

This study has fully observed ethical considerations.

Conflict of Interest

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

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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