Türkiye Parazitoloji Dergisi, 32 (3): 225 - 228, 2008
Türkiye Parazitol Derg.

The Prevalence of *Dirofilaria immitis* in Dogs in Kırıkkale

Kader YILDIZ[1], Sibel YASA DURU[2], Buğrahan B. YAĞCI[2], Naci ÖCAL[2], Aycan N. GAZYAĞCI[1]

Kırıkkale University Faculty of Veterinary Medicine,
[1]Department of Parasitology, [2]Department of Internal Medicine, Kırıkkale, Türkiye

SUMMARY: Heartworm infection is one of the most important parasitic diseases in dogs. The aim of the present study was to determine the prevalence of heartworm in dogs in Kırıkkale. Blood samples of 172 dogs were collected. Microfilariae were detected in 10 (5.8%). dogs with the Knott technique. The anal and excretory pores stained with acid phosphates showed that the microfilariae belonged to *Dirofilaria immitis*. Microfilariae were found in dogs aged between 2-10 years, and especially in Siberian huskies, setters, pointers and crossbred sheepdogs. The serum samples of 142 dogs were analyzed using the commercial ELISA test kit for detection of anti- *D. immitis* antibody. The occult infection rate was found to be 27.46% in dogs. According to these results, Kırıkkale region has a high risk for heartworm infection.

Key Words: *Dirofilaria immitis*, dog, prevalence, seropositivity, Kırıkkale

Kırıkkale'de Köpeklerde *Dirofilaria immitis*'nin Yayılışı

ÖZET: Kalp kurdu enfeksiyonu köpeklerin önemli paraziter hastalıkları arasında yer almaktadır. Bu çalışmada Kırıkkale'de köpeklerde bu enfeksiyonun yaygınlığını belirlemek amaçlanmıştır. Yöre köpeklerinden toplam 172 kan örneği toplanmıştır. Modifiye Knott tekniği kullanılarak 10 köpekte (%5,8) mikrofiler tespit edilmiştir. Mikrofiler saptanan kan örnekleri asit fosfataz boya ile boyanmıştır. Boşaltım ve anal delikleri boyanan bu mikrofilerlerin *Dirofilaria immitis*'e ait olduğu belirlenmiştir. Mikrofilerler 2-10 yaş arası köpeklerde ve özellikle Siberian husky, setter, pointer ve melez köpek ırklarında saptanmıştır. 142 köpek serumu ticari ELISA kiti kullanılarak anti-*Dirofilaria immitis* antikorları yönünden incelenmiştir. Yöre köpeklerinde occult enfeksiyon oranı %27,46 olarak saptanmıştır. Bu sonuçlarına göre Kırıkkale, kalp kurdu enfeksiyonu yönünden yüksek risk taşımaktadır.

Anahtar Sözcükler: *Dirofilaria immitis*, köpek, yayılış, seropozitivite, Kırıkkale

INTRODUCTION

Dirofilaria immitis are found in the dog, fox, wolf, dingo and cat as definitive hosts (7). Adult parasites localized primarily in *Arteria pulmonalis* of definitive hosts and also this nematode may be found in right ventricle, right atrium and sometimes in the vena cava (5, 7). Female parasite is ovoviviparous. Microfilariae are circulated in blood of definitive hosts. They are taken up by intermediate hosts which are mosquito species (7). Microfilariae develop into infective L3 stages approximately 2 weeks in mosquito host (7, 11). The prepatent period of heartworm infection is approximately 6-7 month (5).

Adult worms causes endocarditis, defeat of heart valves, endarteritis, circulation defeat and hypertension in definitive hosts. Hypertrophy of heart, liver congestion, cirrhosis and ascites are commonly symptoms of heartworm infection in dogs (7, 11, 17).

Heartworm infection is recognized in dogs in the worldwide (8, 11). Especially, it is spread from regions of subtropical climate to temperate areas. Turkey is suitable country for development of this parasite due to climatic conditions and abundant intermediate hosts. Heartworm infections were reported different region of Turkey in previous studies (3, 4, 14-16, 18-22). The first heartworm infection in Kırıkkale region was detected in Kırıkkale University Faculty of Veterinary Medicine Clinic at December, 2004. Thereafter, we aimed to determine the prevalence of heartworm infection in dogs in Kırıkkale region.

Makale türü/Article type: **Araştırma / Original Research**
Geliş tarihi/Submission date: 05 Aralık/05 December 2007
Düzeltme tarihi/Revision date: 19 Mart/19 March 2008
Kabul tarihi/Accepted date: 17 Nisan/17 April 2008
Yazışma /Correspoding Author: Kader Yıldız
Tel: (+90) (318) 357 33 01 Fax: (+90) (318) 357 33 04
E-mail: kaderyildiz@hotmail.com
15. Ulusal Parazitoloji Kongresi'nde (18-23 Kasım 2007, Kayseri and Ürgüp) sunulmuştur.

MATERIAL AND METHODS

Area of survey: Kırıkkale (615 km^2) is located Central Anatolia. The largest river of Turkey, Kızılırmak, flow inside this province. Kırıkkale possess both suitable climatic conditions and mosquitoes for development of this parasite.

Table 1.The prevalence of *Dirofilaria immitis* in dogs in Kırıkkale

Region	Exam. dog	Microfilaria positive dog			Infection rate
	n	n	Sex		%
			Female	Male	
Kırıkkale city center	32	-	-	-	-
Yahsihan	62	5	1	4	8
Baliseyh	24	3	-	3	12,5
Delice	16	1	1	-	6,2
Keskin	10	1	-	1	10
Bahsili	7	-	-	-	-
Sulakyurt	21	-	-	-	-
Total	172	10	2	8	5,8

Blood samples and microfilaria examination

Study was performed on total of 172 dogs (121 female and 51 male) from various villages in Kırıkkale from May 2005 to April 2007. The dogs examined were randomly selected. None of the dogs had received protective medication for heartworm infection during the last year. After the clinical examination, blood samples were taken in *vena cephalica* of dogs in tube with heparin. The blood samples analysed with modified Knott technique in laboratory. Microscopy examination of six slides from every Knott technique was carried out.

Filter test was performed blood samples after detected of microfilaria with Knott test. Blood samples infected with microfilariae were pass into polycarbonate filter which has 8 micron diameter pores. Then, these filters fixed by acetone and stained with acid phosphates stain and viewed on light microscope (BX50 light microscopy, Olympus Optical Co., Ltd., Tokyo, Japan).

Antigen testing

The blood samples were collected into centrifuge tubes without anticoagulant. The sera stored in deepfreeze -20º C. The sera were analysed by commercial ELISA tests (DiroCHEK®, Synbiotics Corporation, San Diego, CA) with manufacturer instructions.

RESULTS

Microfilariae were detect of 5,8% dogs with Knott technique (10/172). Samples of microfilariae were measured as 300-310 x 7,5 micron using micrometric ocular. These microfilariae were belonging to *D.immitis* because of observing acid phosphates activity on anal and excretory pores (Figure 1).

Table 1 shows the prevalence of heartworm infection in Kırıkkale region. Heartworm infections tended to be more prevalent in male dogs than in female dogs (8/10 v. 2/10). Microfilaria were detected in dogs between 2-10 ages (Table 2) and observed especially Siberian husky, setter, pointer and crossbred sheepdog species (Table 3).

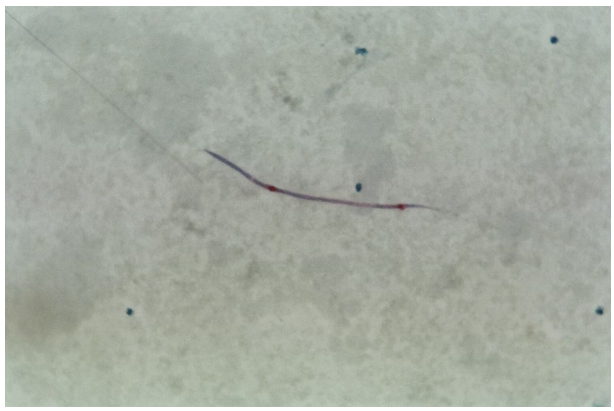

Figure 1. Microfilaria stained with acid phosphates stain

Table 2. The prevalence of *D.immitis* in correlated with age of dogs

Dogs Examined		Microfilaria positive	
Age	**n**	**n**	**%**
1 >	8	-	-
1	10	-	-
2	39	1	2.5
3	35	3	8.5
4	25	2	8
5	21	1	4.7
6	8	-	-
7	6	1	16.6
8	5	-	-
9	3	1	33.3
10	5	1	20
11-14	7	-	-
	172	10	5.8

All dog examined sheltered in outdoor. None of them was detected ectoparasite infection. 139 of 172 dogs were seemed healthy according to clinical examination. The other dogs (33/172) have different symptoms such as itch, hair loss, cough, sneeze, voice alteration, eczema, lack of appetite, weakness, exercise intolerance and cataract.

In serological analysis of serum samples in present study, seropositivity rate was detected as 34.5% of dogs (49/142). Occult infection rate was seemed as 27.46% of dogs examined (39/142). The microfilaria positive dogs (n: 10) were observed as seropositive. 11 of seropositive dogs (but no microfilariae) have some symptoms including cough, voice alteration and dermatitis**.** The other seropositive dogs were appeared to healthy.

Table 3. The prevalence of *D.immitis* in correlated with gender of dogs

Species	Exam. dog	Microfilaria positive	
	n	n	%
Anatolian shepherd	66	1	1.5
Crossbreed sheepdog	65	6	9.2
German shepherd	14	-	-
Setter	7	1	14.2
Pointer	8	1	12.5
Terrier	2	-	-
Siberian Husky	3	1	33.3
Collie	2	-	-
	172	10	5.8

DISCUSSION

The first detection of *D.immitis* in Turkey was in 1951 (9). After the first report, the prevalence of *D.immitis* was reported as 0.15-46.22% in Turkey using microfilariae examination (1, 6, 10, 14, 18, 21, 22), serological (4, 14, 15, 22) and necropsy procedures (3, 16, 19, 20). Figure 2 was seen the detected heartworm infection areas in Turkey. In present study, the patent infection rate was detected as 5.8% in Kırıkkale.

Figure 2. Heartworm infection areas in Turkey

The highest prevalence of *D.immitis* was detected in Yahşihan and Balışeyh district in this study. These areas have different small branch of river which are probably serve as ideal habitats for the development of mosquitoes.

Climate is critical factor in the prevalence of heartworm infection. Especially, the environmental temperature is important factor for *D.immitis* maturation to infective third-stage larvae (L3) in the mosquito (11). The population of mosquito species was increased from July to September in Turkey (2). Kırıkkale which the climate allows the development of a large population of mosquitoes is localised in temperate region of Turkey (Table 4).

Table 4. Climate characteristics of Kırıkkale (annual)

Mean temperature (°C)	-22,4 - 39,7 (mean 12,3)
Rainfall (mm)	368,9
Mean relative humidity (%)	61
Altitude	700 m

The breed of dog may be important for dirofilariosis. The prevalence of heartworm infection is usually higher in larger dog species than that of small ones (12, 22). In present study, heartworm infection was most prevalent found in Siberian husky, setter, pointer and crossbreed sheepdog species. Crossbreed sheepdog species is frequently used as sheep dogs in Kırıkkale, but the other infected dog species is generally found in home garden. All dogs examined lived outdoor, for this reason, these dogs could be more contact with the intermediate mosquitoes.

Montoya *et al.* (12) were suggested that age of dog was important risk factor of heartworm infection. The infection was more prevalent in old dog than that of younger one because of long exposure period in endemic areas, (13, 14). In present study, heartworm infection was increased with the age of dog examined. This situation may be related to mosquito exposure with dog. Also, patent infection was not detected under 2 year dogs in this study.

The circulating microfilariae were not found in peripheral blood in some dogs with adult heartworm. This type of infection is known occult infection (5). Serological techniques are used to detect of occult infection in dog (11). In previous study, the occult infection was reported in dog in Turkey as 1.52 – 29.6% (4, 15, 22). In present study, the occult infection rate was detected in dog as 27.46% in Kırıkkale. All microfilaria positive dogs were found seropositive with ELISA procedure in this study.

In conclusion, mosquito population is abundant in Kırıkkale region. Patent and occult infection rate were detected as 5,8% and 27.46%, respectively. According to these results, this region has highly risk for heartworm infection. The protective measures should be used to control and eradication of heartworm infection in Kırıkkale province. The periodical screening using microfilaria detection or antigen testing and chemoprophylaxis in dog population are priority preference in Kırıkkale.

ACKNOWLEDGEMENTS

This project was financially supported by Research Fund (Kırıkkale University Research Fund, project no: 2005/17). We would like to thank Assis. Prof. Dr. A. Yıldırım for valuable aid.

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