Canine Filariosis Around Istanbul, Turkey Employing Naphtol AS-TR Phosphatase

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Abstract

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The aim of this study was to find the prevalence of filarial nematodes in dogs in Istanbul, Turkey and identify the species by using naphtol AS-TR phosphatase technique. A total of 286 blood samples were taken from the dogs of different areas of Istanbul, Turkey, between March 1999 and November 2002. The Knott technique was used to detect the microfilariae in blood. Two dogs were found to be positive for microfilariae. The positive blood samples were stained by naphtol AS-TR phosphatase for differentiation. The microfilariae were identified as *Dipetalonema reconditum*. In this study, differentiation of canine microfilaria based on somatic distribution of acid phosphatase activity was performed for the first time and according to these results *D. reconditum* was reported for the first time from Turkey.

Dog, Filaria, Dipetalonema reconditum, Knott-technique, Naphtol AS-TR Phosphatase

Canine filariosis is a nematode disease caused by *Dirofilaria* spp., *Dipetalonema* spp. and *Brugia* spp. in dogs. Among the species *D. immitis* has a specific importance based on heart localization of adults and it infects also a wide variety of mammals including humans. The differentiation of microfilariae based on morphological peculiarities is difficult and naphtol AS-TR phosphatase technique is the most reliable method for their differentiation. Prevalence of dog filariae has been studied in various countries, in Turkey, no survey on prevalence of filarial nematodes of dog has been encountered except case reports based on finding of adults and morphological identification of microfilariae.

There are many species of filariae in dogs (Table 1). Three of them, *Dirofilaria immitis*, *D. repens* (Table 2) and *Dipetalonema reconditum* (Erdil 1966) have been reported from dogs in various parts of Turkey.

Although the most important host is the dog, *D. immitis* infects also a wide variety of mammals including human beings (Cheng 1986; Soulsby 1982). In this respect, the species identification of microfilariae is gaining in importance. It is reported that the differentiation of microfilariae based on morphological peculiarities is difficult and naphtol AS-TR technique is the most reliable method for their differentiation. It is based on acid phosphatase enzyme activity in the different body parts of each species of microfilariae (Acevedo et al. 1981; Chalifoux and Hunt 1971; Ortega-Mora et al. 1989; Schrey 1996; Whitlock et al. 1978). In this study, identification of filariid species in dogs was performed and the prevalence was determined in Istanbul for the first time.

Materials and Methods

This survey was performed between March 1999 and November 2002. A total of 286 blood samples of stray dogs in the various parts of Istanbul were examined for filarial nematodes. All dogs were over 1 year-old and both sexes. About 5 ml blood was drawn from the cephalic vein of each dog (between 10:00 am-05:00 pm) and put into

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Table 1. Filarioid nematodes in dogs (Kelly 1979)

Species	Host Range	Vector	Tissue sites (adults)	Tissue sites (microfilariae)	Length of microfilariae (μm)	Geographical distribution
Dirofilaria immitis	Dog, cat, marine mammals, man	Mosquito	Heart and pulmonary artery	Blood	286-349 (mean:314)	America, Africa, Australia, Italy, Spain
Dirofilaria repens	Dog, cat, man	Mosquito	Subcutaneous tissues	Blood	200	USSR, Europe, India, Far East
Dirofilaria conjunctivae	Considered identical to *D. repens* and may also be confused with *D. tenuis*, which is found in the subcutaneous tissues of raccoons and man					
Dipetalonema reconditum	Dog	Fleas, ticks	Connective tissues	Blood	258-292 (mean:270)	America, Africa, Australia, Italy
Dipetalonema dracunculoides	Dog	Louse Fly (*Hippobosca longifennis*)	Peritoneal membranes	Blood	195-230	Africa
Dipetalonema grassi	Dog	Tick	Subcutaneous tissues	Skin, rarely in blood	570	Italy, Kenya
Brugia malayi	Man, cat (dog)	Mosquito	Lymphatic system	Blood	170-260 (mean:220)	India, Africa, Far East
Brugia pahangi	Dog, cat, felidae	Mosquito	Lymphatic system	Blood	280	Africa, Far East
Brugia patei	Dog, cat	Mosquito	Lymphatic system	Blood	Similar to B.malayi	Africa

Table 2. Filarial nematodes of dogs in Turkey

Cities -Years	No of examined dogs	No of infected dogs and species found	Reference
Elazg-1983	283 (Knott Technique)	20 (7.07%) with *D. repens*-microfilaria 5 (1.77%) with *D. repens*+nonspecific-microfilaria 28 (9.89%) with nonspecific-microfilaria	Tasan 1983
Ankara-1983	50 (Necropsy)	2 (4%) with *D. repens*-adult	Doganay 1983
Elazg-1984	120 (Knott Technique)	1 (0.83%) with *D. immitis*-microfilaria 3 (2.5%) with *D. repens*-microfilaria	Tasan 1984
	120 (Necropsy)	6 with *D. immitis*-adult 3 with *D. repens*-adult	
Eskisehir-1986	2 (Necropsy) 20 (Knott Technique)	2 with *D. immitis* adult 6 with *D. immitis* microfilaria	Sarnic 1986
Bursa-1989	100 (Necropsy)	2 (2%) with *D. immitis*-adult	Tinar et al. 1989
Ankara-1989	27 (Necropsy)	3 (11.1%) with *D. immitis*-adul	Zeybek 1989
Konya-1990	4 (Necropsy)	4 with *D. immitis*-adult	Cantoray et al. 1990
Gemlik-1992	168 (Knott Technique)	5 (2.98%) with *D. immitis*-microfilaria	Coskun S.Z. et al. 1982
Van-1992	9 (Knott Technique)	7 with *D. immitis*-microfilaria	
	10 (Necropsy)	8 with *D. immitis*-adult	Agaoglu and Sahin 1992
Ankara-1992	33 (Necropsy)	3 (9.09%) with *D. immitis*-adult	Zeybek et al. 1992
Kayseri-1993	50 (Necropsy)	8 (16%) with *D. immitis*-adult	Sahin et al. 1993
Sivas-1997	50 (Necropsy)	3 (6%) with *D. immitis*-adult	Atas et al. 1997

a tube coated with lithium-heparin to prevent coagulation. Knott technique (Sloss et al. 1994) was used for the detection of microfilariae. When microfilariae were seen, 2 ml of this positive sample was mixed with 12 ml of distillated water to lyse the red blood cells (Rossi and Abbate 1990). This mixture was centrifuged at 1500 rpm for 5 min. Supernatant was discarded and thin smears were prepared from the sediment. Smears were air dried, fixed in absolute acetone for 1-2 min at 4 °C and stained with naphtol AS-TR phosphatase (Sigma, St. Louis, USA, Catalog No. N6125) technique (Chalifoux and Hunt 1971). Identification of the microfilariae was done according to Acevedo et al. (1981), Chalifoux and Hunt (1971) and Schrey (1996).

Results

Two of the 286 (0.69%) examined dogs had microfilariae in their blood. Only one type of microfilariae were observed in smears stained with naphtol AS-TR phosphatase technique.

The entire body of each microfilaria was stained bright red and higher enzyme activity was noticed between the excretory and anal pore. These microfilariae were identified to be *Dipetalonema reconditum*.

The dimension of microfilariae varied between 260-290 × 4.5-6 μm (average: 275 × 5.25 μm). The districts and the numbers of examined and infected dogs are given in Table 3.

Table 3. The districts, the numbers of examined and infected dogs and the species found

Districts	No. of examined dogs	No. of infected dogs	Infection rate (%)	Species found
Büyükcekmece	51			-
Haramidere	20			-
Kadiköy	39			-
Ümraniye	44	1	2.27	*Dip. reconditum*
Kemerburgaz	50			-
Zeytinburnu	14			-
Alibeyköy	18			-
Sariyer	50	1	2	*Dip. reconditum*
Total	286	2	0.7	

Discussion

Among many filarial nematodes in dogs (Kelly 1979; Soulsby 1982) mainly 2 species, namely *Dirofilaria immitis* and *D. repens* occur in Turkey (Table 2). Although the presence of *D. reconditum* has been reported from one dog, results were based only on the lenght, width and tail morphology of the microfilariae and therefore suspected (Erdil 1966). As it is seen in Table 1, other species are distributed mostly Africa, India and Far East. Adults of canine filariae inhabit the subcutaneous tissue or lymphatic system, only *D. immitis* adults reside inside the heart. Because of the ability of this parasite to infect the human beings in the same pattern, it is the most dangerous species among the dog filariae and its possible public health importance has been discussed in the studies (Atas et al. 1997; Doganay 1983) reporting *D. immitis* and conducted in other parts of the Turkey. *D. repens* and *D. reconditum* are considered to be non-pathogenic (Acevedo et al. 1981; Kelly 1979; Lindsey 1965; Soulsby 1982). Therefore it is very important to make an accurate differential diagnosis of microfilariae of these non-pathogenic species from the pathogenic and zoonotic *D. immitis*. In the previous studies (Agaoglu and Sahin 1992; Coskun et al. 1992; Doganay 1983; Sarnic and Alkan 1986; Tasan 1983; Tasan 1984) performed in Turkey, morphological features and length-width measurements were used for the identification of microfilariae. No doubt that this procedure is time-consuming and difficult whatever technique is employed.

The present findings show that the only species found in dogs in Istanbul was *D.reconditum* and naphtol AS-TR phosphatase technique based on acid phosphatase activity given an accurate diagnostic criteria since the microfilariae of 3 species of dogs have been reported (Acevedo et al.1981; Balbo and Abate 1972; Chalifoux and Hunt 1971; Whitlock et al. 1978) to have completely different staining pattern. In this study the staining pattern of *D.reconditum* fits the original description by Chalifoux and Hunt (1971). According to the results of the present study, the major filaria of the dogs in Istanbul is *D. reconditum*. Although no pathogenic effects have been ascribed to this parasite and also it is not of public health importance compared to *D. immitis*, it is important to accurately diagnose and differentiate its microfilariae from those of *D. immitis*, in the diagnosis of heartworm infections.

Výzkum filariózy u psů v okolí Istanbulu pomocí naftol AS-TR fosfatázové techniky

Cílem studie bylo zjistit prevalenci filárií u psů v okolí Istanbulu a určit jejich druh pomocí naftol AS-TR fosfatázy. Od března1999 do listopadu 2002 bylo odebráno celkem 286 vzorků krve psů z různých oblastí Istanbulu. K detekci mikrofilarií v krvi byla použita

Knottova metoda. Test byl pozitivní u dvou psů. Pozitivní krevní vzorky byly k diferenciaci obarveny naftol AS-TR fosfatázou. Mikrofilárie byly identifikovány jako *Dipetalonema reconditum*. V této studii byly poprvé v Turecku mikrofilárie diferencovány na základě somatické distribuce aktivity kyselé fosfatázy, identifikovány jako *D. reconditum*.

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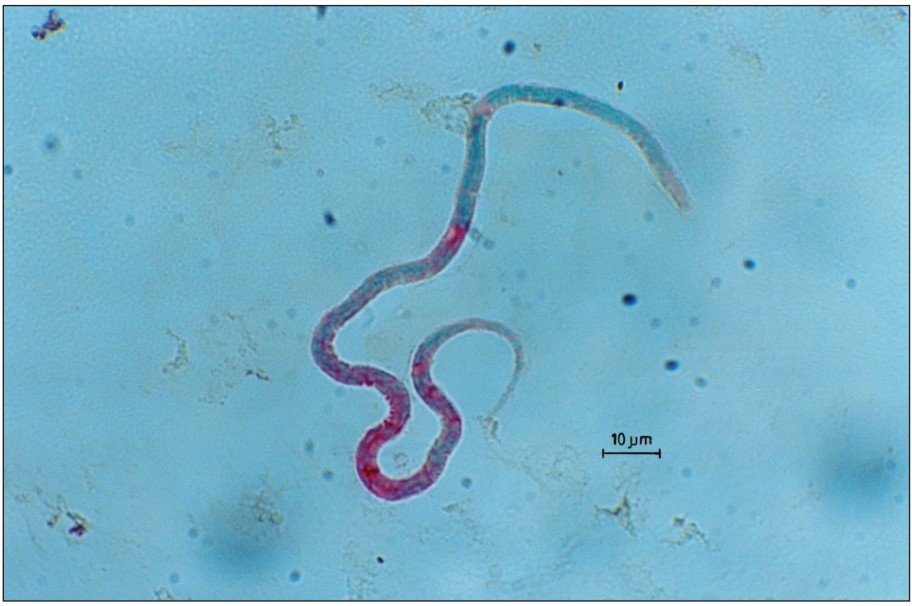

Fig. 1. Pink-stained microfilaria of *Dipetalonema reconditum* with naphtol AS-TR phosphatase technique