Journal of Helminthology (2009) **83,** 255–260 doi:10.1017/S0022149X08198832

Prevalence of *Dirofilaria immitis* in dogs from Hatay province, Turkey

M. Yaman[1], M. Guzel[2]*, I.S. Koltas[3], M. Demirkazik[3] and H. Aktas[3]

[1]Department of Parasitology, Faculty of Veterinary Medicine, University of Mustafa Kemal, 31040 Hatay, Turkey: [2]Department of Internal Medicine, Faculty of Veterinary Medicine, University of Mustafa Kemal, 31040 Hatay, Turkey: [3]Department of Parasitology, Faculty of Medicine, University of Cukurova, 01330 Adana, Turkey

Abstract

The present study was conducted to determine the prevalence of canine dirofilariosis in Hatay province, south of Turkey. A total of 269 blood samples were collected from owned dogs in this multi-centre survey between March and July 2006. Blood samples were examined by modified Knott and enzyme-linked immunosorbent assay (ELISA) techniques to detect circulating microfilariae and antigens of *Dirofilaria immitis*, respectively. Seventy out of 269 dogs (26.0%) were positive for *D. immitis*. However, 61.4% of positive dogs had occult infection. The prevalence of canine dirofilariosis was heterogeneous in Hatay province, with higher values in shoreline (33.0%) and riverside (30.9%) areas followed by the lowland (25.5%) or mountainous (15.2%) areas. No statistically significant differences were observed in relation to sex (females, 33.3%; males, 24.4%, $P > 0.05$). When evaluating the prevalence of *D. immitis* by age, the highest prevalence was observed in dogs older than 4 years of age. Large breeds (29.6%) and the dogs living outdoors (30.2%) showed a higher prevalence in comparison to small breeds (21.4%) and the dogs living indoors (10.5%), respectively. In conclusion, according to the results of this study canine dirofilariosis had a high prevalence in Hatay province. Therefore, prophylaxis against heartworm is advisable to decrease the incidence of canine dirofilariosis.

Introduction

Canine heartworm disease (dirofilariosis) caused by the *Dirofilaria immitis* is a zoonotic parasitic disease inhabiting the right ventricle and pulmonary artery of infected dogs. Dirofilariosis is a disease with worldwide distribution, endemically seen in temperate, tropical and subtropical countries (Marquardt *et al.*, 2000). In recent years, many epidemiological studies have been performed in many countries, including Tanzania (Matola, 1991), Japan (Hatsushika *et al.*, 1992), USA (Theis *et al.*, 1999; Nelson *et al.*, 2005), Spain (Aranda *et al.*, 1998), Canada (Klotins *et al.*, 2000), Italy (Cringoli *et al.*, 2001), Argentina (Rosa *et al.*, 2002; Vezzani *et al.*, 2006), South Korea (Song *et al.*, 2003), Brazil (Reifur *et al.*, 2004), Europe (Genchi *et al.*, 2005) and South America (Labarthe & Guerrero, 2005).

It is also known that Turkey is an endemic region for dirofilariosis. Several studies have been reported regarding the prevalence of dirofilariosis in dogs from different regions of Turkey (table 1). It is of interest to determine the geographical distribution of the disease, which may be associated with ecological factors, such as existence of water, rich vegetation and mosquitoes

*Fax: + 90 3262455704
E-mail: muratguzel05@gmail.com

Table 1. Prevalence of *D. immitis* in dogs previously reported in different provinces of Turkey.

Cities – Years	Detection method	No. of dogs examined	Prevalence (%)	Authors
Elazig – 1984	BE, PE	120	5	Tasan (1984)
Bursa – 1992	BE	168	3	Coskun *et al.* (1992)
Sivas – 1997	PE	50	6	Atas *et al.* (1997)
Van – 2000	BE, AD	106	46.2	Agaoglu *et al.* (2000)
Aydin – 2001	BE	158	13.9	Voyvoda & Pasa (2004)
Izmir – 2001	BE	40	2.5	Voyvoda & Pasa (2004)
Ankara – 2002	BE, AD	280	9.3	Oge *et al.* (2003)
Ankara – 2003	BE	300	6.3	Yildirim (2004)
Istanbul – 2003	BE, AD	263	1.5	Oncel & Vural (2005)
Sanliurfa – 2003	BE	92	7.6	Sahin *et al.* (2004)
Elazig – 2004	BE, AD	120	9.1	Balıkci & Sevgili (2005)
Kayseri – 2006	BE, AD	280	9.6	Yildirim *et al.* (2007)
Kocaeli – 2005	AD	71	18.3	Simsek *et al.* (2008)
Ankara – 2005	AD	27	14.8	Simsek *et al.* (2008)
Sakarya – 2005	AD	65	12.3	Simsek *et al.* (2008)
Mersin – 2005	AD	19	10.5	Simsek *et al.* (2008)

AD, antigen detection of adult *D. immitis*; BE, blood examination for microfilariae detection; and PE, post-mortem examination for adult *D. immitis* detection.

all year round, that are important for the biological cycle of *D. immitis* (Rosa *et al.*, 2002). In this study, the purpose was to determine the prevalence of *D. immitis* and the risk factors of canine heartworm disease in Hatay province.

Materials and methods

Study areas and sampling

Hatay province is located in the south of Turkey on the east Mediterranean coast. The climate of this province is typical of the Mediterranean region, with warm, wet winters and hot summers with high humidity (higher than 69%). A total of 269 owned dogs (older than 6 months) were randomly selected from six districts of Hatay province in this multi-centre survey. Iskenderun (shoreline area), Antakya (riverside area), Kirikhan and Hassa (lowland area), and Yayladagi and Altinozu (mountainous area) were selected for sampling between March and July 2006 (fig. 1). A 4 ml blood sample was withdrawn from the cephalic vein of each dog, and transferred to an EDTA-containing tube during daytime. Whole blood samples were centrifuged at 3000 rpm at room temperature, and the plasma samples obtained were stored at −18°C until analysis. A questionnaire was recorded regarding the dog's age, sex, breed (large breed or small breed) and housing (indoor or outdoor), and prophylactic measures, if used for heartworm disease.

Questionnaire

A total of 269 owned dogs with ages ranging from 6 months to 14 years were examined in the present study: 221 dogs (82.2%) were male and 48 dogs (17.8%) female; 196 dogs (72.9%) were of larger breeds heavier than 20 kg (Kangal dog, pointer, collie, german shepherd, boxer, setter, dalmatian, husky, golden retriever, and mixed breed) and 73 dogs (27.1%) were of smaller breeds (terrier, poodle, cocker, Yorkshire terrier, pincher, and mixed breed). One hundred and five dogs (39.0%) were aged 0.5–2 years, 103 (38.3%) were 2–4 years old, 26 (9.7%) were 4–6 years old and 35 (13.0%) were older than 6 years; 212 dogs (78.8%) were living outdoors and 57 dogs (21.2%) were living indoors. None of the dogs had received specific heartworm prophylaxis.

Detection of microfilariae and D. immitis *adult antigen*

One millilitre of each blood sample was processed using a modified Knott method (Georgi *et al.*, 1990). The identification of filarial species was based on well-established morphological criteria (Watson *et al.*, 1973). Circulating *D. immitis* antigen was detected by use of a commercial antigen enzyme-linked immunosorbent assay (ELISA) kit (DiroCHEK, Synbiotics Corp., San Diego, California, USA) with spectrophotometry, according to the manufacturer's instructions. DiroCHEK canine heartworm antigen test kit is an enzyme immunoassay designed to detect the presence of circulating antigen from adult females of *D. immitis*.

Statistical analysis

The chi-square test was performed to compare the prevalence of *D. immitis* according to sex, age, district, housing and breed categories (large breed or small breed).

Results

Prevalence of infection

Seventy out of 269 tested dogs (26.0%) were positive for *D. immitis* infection with microfilariae and/or antigen detection tests. The individual results of the DiroCHEK heartworm antigen and microfilariae tests are shown in table 2. Sixty-three (23.4%) out of 269 samples tested positive with the antigen-detecting ELISA. In addition, 43 (61.4%) of the positive dogs were determined to have occult *D. immitis* infection; however, seven dogs (10.0%) were antigen negative but microfilariae positive. The regional distribution of canine heartworm disease in

https://doi.org/10.1017/S0022149X08198832 Published online by Cambridge University Press

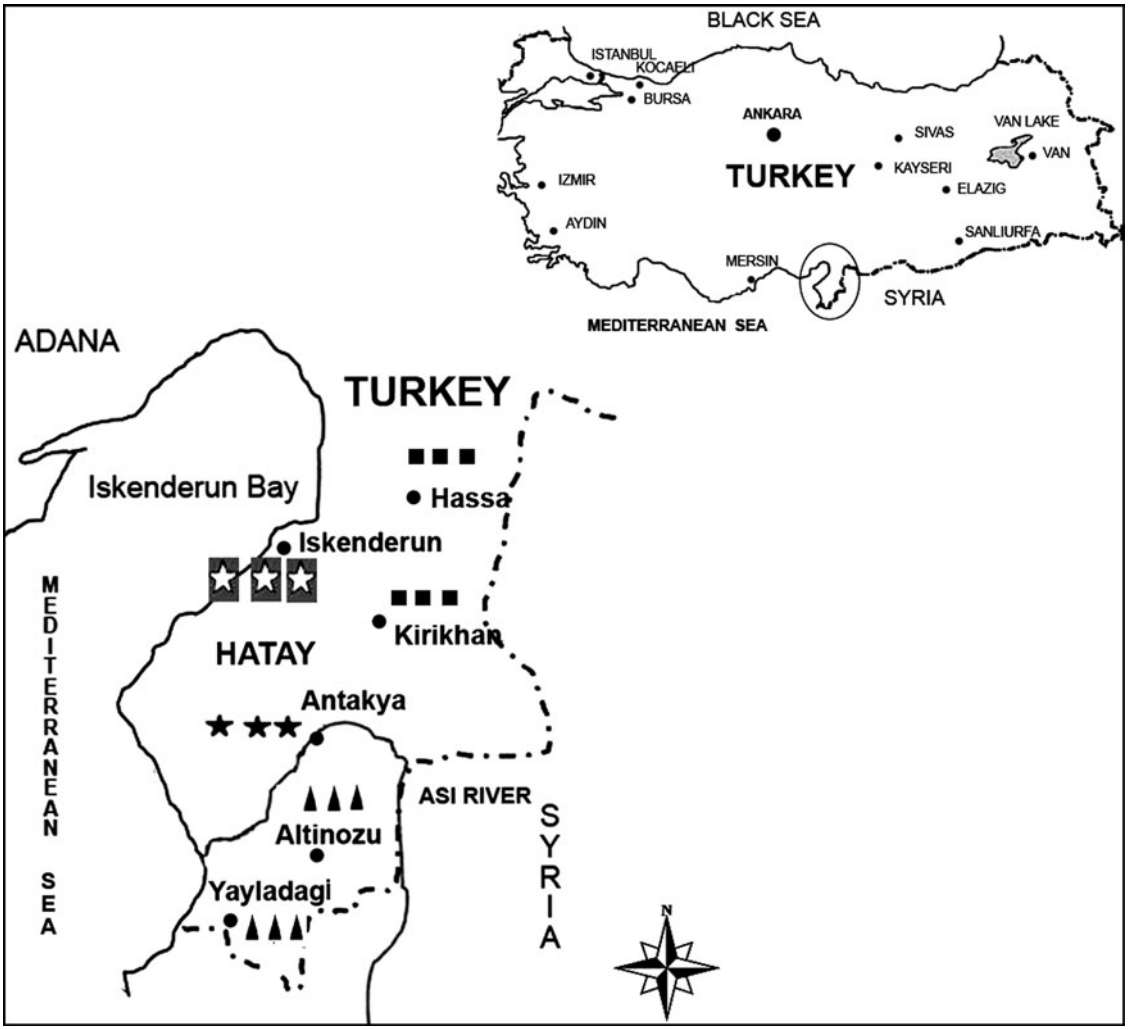

Fig. 1. Location of study areas in Hatay province and the different cities of Turkey shown in table 1: (black star) riverside; (white star in black rectangle) shoreline area; (black rectangle) lowland areas; and (black triangle) mountain areas of Hatay province.

Hatay province is also shown in table 2. Canine heartworm prevalence showed a heterogeneous pattern in different regions of Hatay province. The highest rates were detected in the shoreline area (33.0%) and riverside (30.9%), followed by lowland (25.5%) and mountainous (15.2%) areas.

The results of the association analysis of different factors with heartworm disease are presented in table 3. No differences in prevalence were observed between the sexes (females, 33.3%; males, 24.4%, $P > 0.05$). The mean overall positivity rates of *D. immitis* infection were 15.2% in the 0.5–2-year-old group, 29.1% in the 2–4-year-old group, 38.5% in the 4–6-year-old group, and 40.0% in the group older than 6 years. In the present study, the prevalence of dirofilariosis in heavier dogs (>20 kg) (29.6%) was found to be statistically higher compared to that of lighter dogs (16.4%, $P < 0.05$). The prevalence rates of dogs kept outdoors (30.2%) and indoor dogs (10.5%) were statistically different ($P < 0.05$).

Discussion

Dirofilariosis is a serious disease and has long been known to spread from tropical and subtropical provinces to temperate-zone countries such as Turkey. The geographical distribution of the disease is important for the biological cycle of *D. immitis* (Rosa *et al.*, 2002). The weather is a critical factor affecting the prevalence of this disease. Hot weather and suitable temperatures are necessary for the development of mosquitoes to produce third-stage larval development in the intermediate host; the worm needs temperatures higher than 18°C for nearly 1 month (Montoya *et al.*, 1998). Hatay is located in a temperate zone with a hot and humid environment from April to November (17.2–32.4°C) and the mean humidity is higher than 69% throughout the year (Turkish State Meteorological Service, 2008). This is conducive for growth and reproduction of mosquitoes. In Turkey, the prevalence of *D. immitis* ranges from 1.5 to 46.2% (table 1).

https://doi.org/10.1017/S0022149X08198832 Published online by Cambridge University Press

Table 2. Overall heartworm prevalence in Hatay province.

Research area	No. of dogs examined	No. of infected dogs	Rate (%)	Microfilariae (+) Antigen (+)	Microfilariae (+) Antigen (−)	Microfilariae (−) Antigen (+)
Shoreline area (Iskenderun)	88	29	33.0[a]	5	4	18
Riverside (Antakya)	55	17	30.9[a]	3	2	12
Lowland (Kirikhan and Hassa)	47	12	25.5[ab]	6	–	6
Mountainous (Yayladagi and Altinozu)	79	12	15.2[b]	4	1	7
Total	269	70	(26.0%)	20 (28.6%)	7 (10.0%)	43 (61.4%)[X]

[a,b] The different letters indicate a significant difference among groups by chi-square test ($P < 0.05$).
[X] Prevalence rate of occult *Dirofilaria immitis* infection.

These different prevalence rates may reflect different testing methodologies (post-mortem inspection, detection of microfilariae and serological testing) or regional differences. In the present study, it was found that *D. immitis* had a high prevalence (26.0%) in the dogs from Hatay province. This result showed that Hatay province was the second highest prevalence following the Van province, located in Van Lake (46.2%), and the value is close to that of shoreline areas like Kocaeli province (18.3%). However, the lowland areas of Turkey (Kayseri, Elazig and Ankara) presented a lower prevalence of heartworm infection compared to our result (nearly 10%).

The prevalence of canine heartworm disease varied regionally, with higher values in shoreline areas and riversides and lower values in the lowland and mountainous areas. The geographic distribution of *D. immitis* is mainly on riverside and shoreline areas with an extended habit over a large tropical part of the world (Theis *et al.*, 1999). A previous study of dirofilariosis demonstrated that prevalence was significantly higher in coastal areas than in urban and mountainous areas (Song *et al.*, 2003). Rosa *et al.* (2002) reported that an increase in prevalence may occur in dogs that live in riverside areas because of the higher probability of being exposed to additional risk factors (types of confinement and presence of intermediary hosts). The present data revealed higher values in shoreline (33.0%) and riverside areas (30.9%), followed by the lowland (25.5%) or mountainous (15.2%) area.

Diagnosis of dirofilariosis is usually based on concentration techniques for detecting microfilariae in the blood. Immunodiagnostic techniques have recently been developed, enabling the diagnosis of occult infections. Occult dirofilariosis is defined as the presence of parasite antigens, indicating the presence of adult worms, and the absence of microfilariae. Occult infections may occur in up to 30% of infected dogs (Rawlings *et al.*, 1982). In the present study, sixty-three (23.4%) out of 269 samples, tested positive with the antigen-detecting ELISA. In addition, 61.4% of positive dogs had occult *D. immitis* infection. This result is close to the previous report by Alves *et al.* (1999) (57.1%). The high percentage of occult infection is not uncommon and has also been reported by several other researchers (Labarthe *et al.*, 1997; Reifur *et al.*, 2004). Occult infections may be due to different causes, including treatment with macrocyclic lactones or immune-mediated reactions that are able to eliminate the microfilariae or to decrease the fecundity of adult worms. In the present study, none of the dogs had received specific heartworm prophylaxis; however, some of the dog may have received ivermectine injections as antiparasitic treatment against ectoparasites. Labarthe *et al.* (1997) suggested that the abusive use of

Table 3. The prevalence of *D. immitis* correlated with sex, age, breed (large breed or small breed) and housing.

	No. of dogs examined	No. of positive dogs	Mean (%)	χ^2	*P*
Sex					
Female	48	16	33.3	1.193	>0.05
Male	221	54	24.4		
Age (years)					
< 2	105	16	15.2[a]	10.519	<0.05
2–4	103	30	29.1[a]		
4–6	26	10	38.5[b]		
> 6	35	14	40.0[b]		
Breed					
Larger	196	58	29.6[a]	4.122	<0.01
Smaller	73	12	16.4[b]		
Housing					
Outdoors	212	64	30.2[a]	8.029	<0.01
Indoors	57	6	10.5[b]		
Total	269	70	26.0		

[a,b] The different letters indicate a significant difference among groups by chi-square test.

https://doi.org/10.1017/S0022149X08198832 Published online by Cambridge University Press

microfilaricidal agents contributed substantially to the elevated rate of occult dirofilariosis. Therefore, veterinarians should be aware of the risk of misdiagnosing heartworm patients when testing them only for microfilariae, especially if microfilaricidal drugs are used for the control of ectoparasites (Alves *et al.*, 1999). In the present study, seven dogs (10.0%) were antigen negative but microfilariae positive, as well. The status of being antigen negative but microfilariae positive may be explained by undetectably low levels of heartworm antigen. It has been reported that commercial ELISA kits may have low sensitivity when the parasite burdens are below five *D. immitis* adult parasites (Martini *et al.*, 1996; Klotins *et al.*, 2000; Atkins, 2003). Furthermore, immune clearance of antigen–antibody complexes (Menda, 1989) and the presence of microfilariae for 1–3 years after the death of adult females, would cause negativity against adult *D. immitis* while being microfilariae positive.

When evaluating the prevalence of *D. immitis* in terms of sex, there are contradictory results. According to some of the researchers, no significant differences have been detected between the sexes (Oge *et al.*, 2003; Song *et al.*, 2003; Simsek *et al.*, 2008). Whereas, some researchers (Selby *et al.*, 1980; Montoya *et al.*, 1998) have reported that male dogs had higher infection rates. The generally higher infection rates in male dogs may be due to their stronger attraction to mosquitoes. However, in the present study, there was no statistically significant difference in prevalence between males (24.4%) and females (33.3%).

Regarding the prevalence of *D. immitis* in terms of age, the results of the present study were similar to those of Yildirim *et al.* (2007), that older dogs have more time and more opportunities to become infected with heartworm. The likelihood of heartworm infection has been found to increase with an increased period of exposure to mosquitoes (Lee, 1993; Rosa *et al.*, 2002).

In the present study, the prevalence of *D. immitis* infection in the larger dogs was found to be higher compared to that of smaller dogs. This result was consistent with other research (Butts, 1979; Selby *et al.*, 1980). Selby *et al.* (1980) showed an increased prevalence of heartworm infections in hunting, sporting dogs. They speculated that this was because of their use for field training or hunting purposes, which likely contributes to an increase of the exposure risk to the infected mosquitoes (Theis *et al.*, 1999). Similarly, in the present study most of the infected dogs were sheepdogs, hunting dogs or guard dogs, which were living outdoors.

In conclusion, this survey demonstrated that canine dirofilariosis is prevalent in Hatay province (26.0%) of Turkey, especially in shoreline and riverside areas (more than 30% prevalence). Since dirofilariosis is a zoonosis and is frequently diagnosed in dogs in the Hatay province, preventive treatments for dogs should be considered, to decrease the incidence of canine dirofilariosis.

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(Accepted 12 November 2008)
First Published Online 28 January 2009