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Short Paper

A study on *Dirofilaria immitis* in healthy urban dogs from Ahvaz, Iran

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Summary

Heartworm infection is one of the most important parasitic diseases in dogs and an increasing number of cases are reported each year by necropsy in Ahvaz. This study was conducted to determine the prevalence of *Dirofilaria immitis* infection and to investigate the risk factors related to heartworm disease in urban dogs in this area. Blood samples were collected from 100 dogs from Feb 2007 to Feb 2008. All samples were examined by modified Knott test, direct smear and antigen detection (Immunochromatographic antigen rapid canine *D. immitis* Ag Test Kit) technique to detect circulating microfilaria and adult antigen of *D. immitis*, respectively. Of the total 100 dogs, 1% were positive by direct smear, 5% were positive by modified Knott test and 6% were positive by antigen detection. In addition, 1% of positive dogs were determined to have occult *D. immitis* infections. *Dirofilaria immitis* was the only canine filarial parasite present in this study. From these three methods, modified Knott test, due to microfilarial identification on concentrated blood had the highest validity. The difference between outdoor and indoor dogs was found significant (P = 0.033), whereas no statistically significant differences were observed between different age groups, sexes and breeds (P>0.05). The highest heartworm prevalence by modified Knott test was observed in older than 6-year-old dogs (8.3%) followed by 3–6 years (2.7%) and the 6-month to 3 years (3.5%) age groups. The infection was more prevalent in males and short haired breeds. Our results suggest that heartworm treatment and preventive care should be considered in urban dogs of Ahvaz, Iran.

Key words: *Dirofilaria immitis*, Microfilaria, Dog, Ahvaz

Introduction

Canine heartworm disease due to *Dirofilaria immitis* is the most common cause of pulmonary hypertension and pulmonary arteritis in adult dogs. Several mosquito species are responsible for the transmission of the disease (Atwell, 1988). Geographic distribution of *D. immitis* is worldwide, mainly on riverside and shoreline areas, with an extended habitat over a large part of the tropical world (Theis *et al*., 1999). It is widespread in tropical, sub-tropical and temperate zones (Cringoli *et al*., 2001). Regional information may help veterinarians assess the risk of *D. immitis* infection and assist in the development of diagnostic plans in suspected cases or recommendations for chemoprophylaxis. The prevalence of canine heartworm disease was reported 1.4% in Tehran (Meshgi and Eslami, 2001), 8.4% in Tabriz (Meshgi *et al*., 2002) and 15.45% in Golestan (Ranjbar-Bahadori and Eslami, 2007). However, information regarding the status of dirofilariasis infection in Ahvaz is scarce, despite the rapid increase of animals diagnosed by necropsy and treated in the veterinary hospital of Shahid Chamran University. Rapid diagnosis of heartworm infection is especially important in order to isolate infected dogs and prevent

transmission to susceptible animals. The high sensitivity and specifity of immunochromatography assay and its simplicity has led veterinarians to use this practical method for the evaluation of dirofilariasis (Courtney and Zeng, 2001).

As a whole, human and animal dirofilariasis has been recorded in eleven provinces of Iran. The species *D. immitis* has been reported in East Azerbaijan, West Azerbaijan, Ardebil, Tehran, Khorasan, Khuzestan, Fars, Golestan, Mazandaran, and Hormozgan provinces and *D. repens* in Tehran, Khorasan, Guilan, and Mazandaran provinces. Different investigators have reported *D. immitis* in dogs (with a frequency of 0.95 to 36.8%), jackals (7.5-57.1%), foxes (5.7-50%), wolves (20-50%), and cats (0.8%) and *D. repens* in dogs (1.4-60.8%) and jackals (10%) in the different areas of the country (Azari Hamidian *et al*., 2006).

This study was performed to investigate the prevalence of *D. immitis* infection by antigen-detecting immunochromatography assay, modified Knott test and direct smear techniques among dogs in the Ahvaz region and to assess the risk factors associated with the presence of heartworm disease.

Materials and Methods

Study area

The study was conducted in Ahvaz city, Iran, located at an elevation of 12 meters above sea level and the climate is hot-humid. Summers are hot and semi dry, while winters are semi cold and rainy.

Animals

One hundred adult dogs owned by native people were randomly selected by simple random sampling method from animals referred to the Veterinary Teaching Hospital of Shahid Chamran University from Feb 2007 to Feb 2008. A questionnaire for owners included questions about the dog's age, sex, breed (long or short haired), and type of housing (indoor or outdoor). Dogs younger than 6-month-old were excluded from the study considering the life cycle of *D. immitis*.

Blood sampling

2-3 ml of whole blood was drawn from the cephalic vein of each dog; half of the sample was stored with heparin and the other half was allowed to clot. Serum was harvested following centrifugation of clotted blood and was stored at -20°C until analysis in the parasitology laboratory of the Faculty of Veterinary Medicine in Shahid Chamran University. All samples were obtained during the day.

Direct smear

The presence of microfilaria was examined by microscopic examination of a fresh direct thin smear of 0.05 ml of blood, fixed in methanol, and stained with 1% Giemsa for 30 min.

Modified Knott test

The actual count of microfilaria present in the sediment of 1 ml of blood processed by modified Knott method (Jackson and Otto, 1975) with dilution was used as necessary to facilitate counting when a high microfilaremia was detected.

Detection of adult *D. immitis* antigen

Rapid heartworm antigen test kits (Anigen, Animal Genetic, Inc. Korea) for detection of antigen worms were used to examine serum samples. The test is based on immunochromatography.

Statistical analysis

Dogs were grouped by age, sex, breed and type of maintenance to determine whether these factors were associated with Dirofilaria infection, using Fisher's exact test. Statistical comparisons were carried out using SPSS 16.0 statistical software. The statistical significance level was set at $P>0.05$.

Results

Of a total of 100 dogs tested, 5 were positive for *D. immitis* infection with microfilaria in the Knott test and 1 was positive in direct smear, and by antigen detection 6 were positive. The length of microfilaria was 303.5 ± 5 µm, width 7.3 ± 0.5 µm, tapered cranial end and the tail was

straight. One case was antigen positive but the Knott test was negative. Four cases were positive in the Knott test but negative in the direct smear. The mean number of microfilaria in infected dogs was 2730 (min = 5, max= 4700) per ml of blood.

The results of the association analysis of different factors with *D. immitis* infection are presented in Table 1. No significant differences were found among dogs regarding sex, age, and breed (P>0.05). The difference between outdoor and indoor dogs was found significant (P=0.033). All of the infected dogs were designated as being always outdoors by their owners. The results of the association analysis of sex, age and breed of the dogs with microfilariae concentration per ml of blood are presented in Table 2.

Discussion

The prevalence of *D. immitis* in dogs in different regions is variable depending on the environmental and climatic conditions, vector population, diagnostic method and situation of infection (patent or occult). Different prevalence rates (1.4-15.45%) reported in previous studies in Iran (Meshgi and Eslami, 2001; Meshgi *et al*., 2002; Ranjbar-Bahadori and Eslami, 2007) should be related to these factors. Factors affecting the transmission of *D. immitis* include mosquito species, mosquito population density, mosquito fecundity and environmental temperature (Atkins, 2005). The lower prevalence of *D. immitis* in the present survey versus Golestan (Ranjbar-Bahadori and Eslami, 2007) and Tabriz (Meshgi *et al*., 2002) provinces was most likely due to the fact that all of these animals live in the city, a habitat that is less than ideal for mosquito populations to thrive.

It was determined in this study that only one filarial parasite species (*D. immitis*) is present from dogs in Ahvaz city. However, *Dirofilaria repens* and *Dipetalonema reconditum* were also reported in Iran (Sadighian, 1969; Mirzayans *et al*., 1972; Meshgi *et al*., 2002). Also, Maraghi *et al*. (2006) reported subcutaneous and subconjunctival human dirofilariasis due to *Dirofilaria repens* from Ahvaz.

More male (4%) than female (2%) dogs were affected in this study, although there was no significant difference between either group. Several researchers indicated that the heartworm infection was more common in

Table 2: Microfilaria concentration (mf/ml) correlated with sex, age and hair length of infected dogs by modified Knott test

		Min	Max	Mean
Sex	Female	5	3530	1767/5
	Male	140	4700	2420
Age (Year)	0.5-3	5	2420	1212/5
	3-6	85	3700	1892/5
	>6	220	4700	2460
Hair length	Short haired	5	4700	2352/5
	Long haired	18	4100	2059

Table 1: Distribution of *D. immitis* infection based on sex, age, hair length and type of maintenance by modified Knott test, direct smear and antigen detection

No. of examined dogs			No. of positive dogs		
			Modified Knott test	Direct smear	Antigen detection
Sex	Female	40	2	-	2
	Male	60	3	1	4
Age (Year)	0.5-3 (1.73 ± 0.62)	28	1	-	1
	3-6 (4.33 ± 0.78)	36	1	1	2
	>6 (8.42 ± 1.49)	36	3	-	3
Hair length	Short haired	48	4	1	5
	Long haired	52	1	-	1
Type of maintenance	Outdoor	56	5	1	6
	Indoor	44	-	-	-

male dogs than female dogs, and the generally higher infection rate in male dogs had been postulated to be due to their stronger attraction to mosquitoes (Selby *et al*., 1980; Montoya *et al*., 1998; Cringoli *et al*., 2001). Higher prevalence rates in males can also be attributed to the fact that more males dogs are kept outdoors for their use to defend safety and property (Song *et al*., 2003). But it is more difficult to explain why males are more subject to infection than females. However, when evaluating the prevalence of heartworm by sex, no significant difference was observed between males and females in several studies done in Japan (Ryo *et al*., 1992), Turkey (Oge *et al*., 2003), the Dominican Republic (Duran-Struck *et al*., 2005), Surinam (Panday *et al*., 1981) and Taipei (Fan *et al*., 2001).

Moreover, the present study indicated that mean seroprevalence of *D. immitis* infection in dogs increased with age. The older the age, the higher the seroprevalence for the older than 6-year-old age group versus their younger counterparts. Similar findings were reported previously by several researchers (Montoya *et al*., 1998; Fan *et al*., 2001; Song *et al*., 2003; Duran-Struck *et al*., 2005). A possible explanation for the higher seroprevalence of *D. immitis* infection in older dogs might be due to their longer exposure to the risk factor, mosquito (Rhee *et al*., 1998). Atkins (2005) also indicated that the age of the dogs was an important risk factor and determined by time of exposure in the endemic area.

The present study points out that long haired breeds represent a higher relative risk of dirofilariasis against short haired dogs. Hair length did not significantly influence the results of other studies (Jafari *et al*., 1996; Cringoli *et al*., 2001; Araujo *et al*., 2003; Reifur *et al*., 2004; Scaramozzino *et al*., 2005). It was originally thought that long haired dogs were less susceptible to heartworm infection because of the improbability that mosquitoes would be able to penetrate through the hair for a blood meal (Miterpakova *et al*., 2008). It has since been shown that this line of thought is erroneous. Mosquitoes usually have difficulty penetrating even short hair (Atkins, 2005). For this reason most feeding takes place on the under part of the abdominal region. Since even long-haired dogs have relatively little hair on this area, mosquitoes have no difficulty feeding upon them.

All of the infected dogs were designated as being always outdoors by their owners. Thus, a statistically significant difference was found between outdoor and indoor dogs in this survey. This finding is in accordance with the results obtained in other countries (Montoya *et al*., 1998; Song *et al*., 2003; Yildirim *et al*., 2007). The outdoors can attribute to more favorable environmental conditions for the intermediate host. Management of the indoor-outdoor time situation of a dog in heartworm endemic areas does have an effect on the risk of infection (Miterpakova *et al*., 2008). This influence is presumably due to vector exposure rates. Dogs that are outdoors all the time have a greater chance of being bit by mosquitoes and dogs that are not on prevention have a higher risk of getting infection.

Atkins (2005) pointed to an increased prevalence of heartworm infection in hunting and sporting dogs. They thought it was due to their use for field training or hunting purposes, which increases the exposure risk to the infected mosquitoes. Similarly, infected dogs in this study were generally used as guard dogs for defense and safety, which increases the infection risk.

Panday *et al*. (1981) and Oge *et al*. (2003) determined the microfilariae concentration as 9689 mf/ml and 6307.5 mf/ml, respectively. Ranjbar-Bahadori and Hekmatkhah (2007) determined the average of microfilaria in each ml of blood in infected dogs from Garmsar equivalent to 4470.6 ± 1243.54. In the present study, min = 5 and max = 4700 mf/ml were found in the peripheral blood of infected dogs and no significant difference was found according to the sex. Pappas and Lunzman (1985) found 4835 mf/ml in male dogs and 6029 mf/ml in female dogs and recorded no significant difference in the microfilariae concentration according to sex. In addition, no statistically significant differences were found in the microfilariae concentration according to age groups and breed (long or short haired dogs).

The immunochromatography assay is

the most rapid field diagnostic method used in clinical practice because the test procedure is simple and can be performed by veterinarians as well as by owners. Evaluation of the diagnostic kits (immunochromatography) showed an overall relative sensitivity and specifity of 98.5 and 100%, respectively (Courtney and Zeng, 2001).

Antigen detection techniques are recognized as more sensitive than direct smear and modified Knott test for the detection of microfilariae in peripheral blood (Oge *et al*., 2003). With regards to the diagnostic method, *D. immitis* infection should be confirmed by an antigen detection test, as these tests are more sensitive and because in the present work 1% of the cases were occult infections. The presence of occult infections is not uncommon and has been reported by several researchers (Labarthe *et al*., 1997; Oge *et al*., 2003; Reifur *et al*., 2004).

Human dirofilariasis is now classified as an emerging zoonosis. Man is an accidental host in which further development of the parasite is not possible. Consequently, there are no microfilariae in humans. Two species of the genus Dirofilaria, *D. immitis* and *D. repens*, are found in Iran. By now, nine human cases have been formally reported including four subcutaneous and two ocular cases of *D. repens*, a rare case of *D. immitis* in testicular hydrocele, and two pulmonary cases (Athari, 2003; Azari Hamidian *et al*., 2006). To date, this is the first report on the status of *D. immitis* infection in house dogs in Ahvaz City, Iran. Therefore, heartworm treatment and/or prevention are needed in this area. These initial findings also serve as baseline data for further studies related to heartworm management in the region.

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