

## Seroprevalence of *Dirofilaria immitis* in Stray Dogs in İstanbul and İzmir

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**Abstract:** Three hundred eighty stray dogs (232 female and 148 male) from İstanbul and İzmir were tested with antigen detecting ELISA for *Dirofilaria immitis* infection. Blood samples were collected between November 2002 and April 2003. *D. immitis* infection was found in 4 dogs tested. Seroprevalence among these dogs in İstanbul was 1.52%. Seroprevalence was the highest (2.22%) in the 3-6-year-old age group, followed by 1.02% in the 1-3-year-old age group. However, there was no significant difference between these age groups ( $P > 0.05$ ). No significant difference between male and female dog groups was observed in İstanbul ( $P > 0.05$ ). There was no seropositivity among stray dogs in İzmir.

**Key Words:** *Dirofilaria immitis*, ELISA, dog, seroprevalence

### İstanbul ve İzmir Sokak Köpeklerinde *Dirofilaria immitis*'in Seroprevalansı

**Özet:** İstanbul ve İzmir'den toplam 380 sokak köpeği (232 dişi, 148 erkek) antijen tespit eden ELISA ile *Dirofilaria immitis* enfeksiyonu yönünden incelendi. Kan örnekleri Kasım 2002 ile Nisan 2003 tarihleri arasında toplandı. Test edilen 4 köpekte *D. immitis* enfeksiyonu tespit edildi. Seroprevalans İstanbul'daki köpeklerde %1,52 olarak bulundu. Seroprevalans 3-6 yaş arasındaki köpeklerde en yüksekti (% 2,22). Bunu % 1,02 seroprevalans oranı ile 1-3 yaş arasındaki köpekler izledi. Bununla birlikte, bu yaş grupları arasında önemli bir fark görülmedi ( $P > 0,05$ ). İstanbul'dan alınan örneklerde, erkek ve dişi köpeklerin seroprevalans değerleri arasında istatistiksel bir fark bulunmadı ( $P > 0,05$ ). İzmir'den temin edilen köpek serumlarında pozitifliklik saptanmadı.

**Anahtar Sözcükler:** *Dirofilaria immitis*, ELISA, köpek, seroprevalans

### Introduction

Dirofilariasis, caused by *Dirofilaria immitis*, is a common and important disease of dogs in tropical, subtropical and temperate regions of the world (1). *D. immitis* is one of the most pathogenic nematode parasites of dogs. Adults of *D. immitis* are found mainly in the chambers of the right side of the heart and pulmonary artery. The females are approximately 30 cm long, while the males are 23 cm long with a spirally coiled tail. After reproduction, the female produce small, vermiform embryos called microfilaria. They can cross the capillary beds and so are found throughout the vascular circulation. There is a wide spectrum of clinical signs with heartworm disease and many dogs are asymptomatic. Cough exercise intolerance is one of the common signs and it can lead to more serious signs, including core pulmonale, allergic pneumonitis and vena caval syndrome (2).

The geographical distribution of heartworm infection is associated with availability of mosquitoes, the intermediate host. Mosquito population dynamics are influenced by environmental factors such as suitable components of still water and warm temperatures (1). The highest prevalence occurs in river valleys and humid areas where the environmental conditions are more favorable for the breeding of vectors (3). Frequency of infection is related to life style, with male dogs being more frequently infected than female dogs (4).

In recent years, several epidemiological studies have been performed in different countries. The parasite is widely distributed in Africa, Asia, Australia, Latin America and Mediterranean countries (4-9).

A few studies have been published regarding the distribution and prevalence of dirofilariasis in dogs in Turkey. *D. immitis* was first reported in one dog in Turkey in 1951 (10). Taşan (11) found that among 283

stray dogs in Elazığ, 18.7% had microfilarinemia. Coşkun et al. (12) showed that five of 168 dogs (2.98%) at the Army Veterinary Research and Training at Gemlik, Bursa, were infected with *D. immitis*. Then many cases of *D. immitis* have been reported in dogs in Turkey (13-18). A survey in 2003 revealed that 9.3% of 280 domestic dogs were infected with *D. immitis* in Ankara and vicinity (19).

The purpose of the current study was to investigate the prevalence of canine dirofilariosis by antigen detecting ELISA among stray dogs in İstanbul and İzmir. Prevalence of *D. immitis* in stray dogs in İstanbul and İzmir has remained unknown to date.

**Materials and Methods**

A total of 380 stray dogs, including 148 male and 232 female dogs, were examined for heartworm infection from November 2002 to April 2003. These dogs were sampled from various dog rehabilitation centers (DRC) and kennels in İzmir and İstanbul. Of these, 117 dogs, including 51 males and 66 females, were from İzmir, and 263 dogs, including 97 males and 166 females, were from İstanbul. The serum samples collected during the study are listed in Table 1.

The age range was from one to eight years. Blood was drawn from the cephalic vein of each dog, and the serum was separated by centrifugation and stored at -20 °C in a freezer for analysis. A record form was completed for each dog, giving pertinent identification, history and demographic data.

The circulating antigen of *D. immitis* was detected using PetCHEK HTWM PF (IDEXX) ELISA kit, according

to the manufacturer’s instructions. PetCHEK Canine Heartworm Antigen Test is an enzyme immunoassay designed to detect the presence of circulating antigen from adult *D. immitis* in serum or plasma. Statistical significance of the difference in seropositive rate of sex and age groups was examined by X<sup>2</sup> test.

**Results**

Four (1.05%) of the 380 samples tested with antigen detecting ELISA kits showed a positive reaction for *D. immitis* in this study. The regional distribution and prevalence values are presented in Table 1.

All positive reactions were obtained in samples taken from stray dogs in İstanbul. Therefore seroprevalence among stray dogs in İstanbul was 1.52%. There was no seropositivity among stray dogs in İzmir (Table 2).

The seroprevalence rates in males and females in İstanbul were 2.06% and 1.02%, respectively (Table 2). There was no significant difference between these groups (P > 0.05).

Seroprevalence was the highest (2.22%) in the 3-6 year-old age group in İstanbul, followed by 1.02% in the 1-3-year-old age group (Table 3). No significant difference was observed between these two age groups (P > 0.05).

**Discussion**

This study, carried out in İstanbul and İzmir, has shown low values for the prevalence of *D. immitis* (1.05%) in the dog population examined using antigen-detecting ELISA kit.

Table 1. *Dirofilaria immitis* infected dogs according to the district in İzmir and İstanbul.\*

| Districts    | Province | No. dogs   | No. infected dogs | P. rate (%) |
|--------------|----------|------------|-------------------|-------------|
| İzmir DRC    | İzmir    | 117        | -                 | -           |
| Tuzla        | İstanbul | 48         | 1                 | 2.08        |
| Bakırköy     | İstanbul | 52         | -                 | -           |
| Sarıyer      | İstanbul | 60         | 1                 | 1.66        |
| Kemerburgaz  | İstanbul | 59         | 2                 | 3.59        |
| Büyüçekmece  | İstanbul | 44         | -                 | -           |
| <b>Total</b> |          | <b>380</b> | <b>4</b>          | <b>1.05</b> |

Table 2. Sex distribution of dogs with *D. immitis* infection.\*

| Sex     | İstanbul |         |             | İzmir    |         |             |
|---------|----------|---------|-------------|----------|---------|-------------|
|         | No. exam | No. Pos | P. rate (%) | No. exam | No. Pos | P. rate (%) |
| Males   | 97       | 2       | 2.06        | 51       | -       | -           |
| Females | 166      | 2       | 1.02        | 66       | -       | -           |
| Total   | 263      | 4       | 1.52        | 117      | -       | -           |

Table 3. Age distribution of dogs with *D. immitis* infection.\*

| Age | İstanbul |         |             | İzmir    |         |             |
|-----|----------|---------|-------------|----------|---------|-------------|
|     | No. exam | No. Pos | P. rate (%) | No. exam | No. Pos | P. rate (%) |
| 1-3 | 98       | 1       | 1.02        | 75       | -       | -           |
| 3-6 | 135      | 3       | 2.22        | 37       | -       | -           |
| ≥ 6 | 30       | -       | -           | 5        | -       | -           |

\* No. dogs: number of dogs, No. infected dogs: Number of infected dogs, P. rate: Positive rate, No exam: number of examined, No. pos: Number of positives.

*D. immitis* has been reported by many researchers in dogs in Turkey. In most of the surveys canine dirofilariosis among dogs was determined generally by necropsy (10-17). By comparing the results of our survey with those of other studies, it seems that it is higher than the 0.15% reported by Pamukçu and Ertürk (18) for Ankara, but is lower than the 5% reported by Taşan (17) for Elazığ and 9.3% reported by Öge et al. (19) for Ankara and is similar to the 2% reported by Tınar et al. (16), and 2.98% reported by Coşkun et al. (12) for Bursa.

Serological tests were developed to identify antigens of heartworms in the blood stream. False-negative test results occur most commonly when infections are light, female worms are still immature or only male worms are present (20). Antigen tests are currently recommended by the American Heartworm Society for primary screening and confirmation of heartworm infection in dogs (21,22). The diagnosis of canine heartworm infection can be made on the basis of finding *D. immitis* microfilariae in the blood or positive serologic tests with compatible findings on thoracic radiographs. However, dogs with prepatent or occult heartworm infections can be amicrofilaremic (20).

Hoover et al. (20) compared the PetChek test and seven other diagnostic blood tests for detection of antigens in *D. immitis* infection. They found that the PetChek test gave no false positive and had 9 false negatives or 27.3%. Öge et al. (19) reported that the false negative rate for the PetChek test in Ankara and vicinity was 7.7%. Courtney and Zeng (23) compared the PetCheck test and five other heartworm antigen test kits. The PetCheck was significantly more sensitive than all other tests. The dogs examined in this study were not available for necropsy. Although there are no necropsy data to confirm the sensitivity of the PetChek, the percentage of positive results in this study is closer to that recorded by Hoover et al. (20).

Montoya et al. (4) indicated that age is an important risk factor, determined by time of exposure in the endemic area. Therefore, older dogs have a higher prevalence of dirofilariosis than younger dogs (7,19,24). However in some surveys age did not appear to affect prevalence (25,26). The increase in prevalence in older dogs can be the result of two features in this host-parasite relationship. First, the lack of any age resistance related to the dog and secondly the longer exposure to the risk factor, the mosquito (19,27). A large proportion

of dogs examined were less than 3 years old in our study. Our results indicated that there was no significant difference between age groups ( $P > 0.05$ ).

Selbey et al. (28) found that male dogs had the highest relative risk for heartworm infection. They are more likely to be bitten by mosquitoes. In the present study, no significant differences in seroprevalence were observed between male and female dogs ( $P > 0.05$ ).

Weather is a critical factor in the prevalence of the disease. Transmission depends on the intermediate host, which have certain climate requirements. Hot weather and suitable temperatures are necessary for development of mosquitoes (4). In this study the lower prevalence of dirofilariasis in dogs that live in such areas can be attributed to less opportunity for exposure to the mosquitoes, due to mosquito control programs employed

by municipalities. Another factor could be that the dogs tested in this study may have been treated for heartworm infection.

In conclusion, the findings in this survey indicate that *D. immitis* is 1.52% in İstanbul and there is no seropositivity among stray dogs in İzmir. Free-ranging dogs should be kept under control in local DRCs in order to protect housed animals from the critical disease, which threatens animal and public health. Studies on the seroprevalence of canine dirofilariasis in the other regions of Turkey should be carried out.

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