

Occurrence of *Dirofilaria immitis* in cats from the Aegean region in Turkey*

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Summary

Dirofilaria immitis (*D. immitis*) is a vector-mediated zoonotic parasite that can cause significant cardio-pulmonary problems in humans and animals. Although dogs are the main hosts of the parasite, in recent years, its importance has been increasing in cats, especially in endemic areas. This study aims to determine the occurrence of *D. immitis* infection in cats in the Aegean region, Turkey. The animal material of the study consisted of 200 cats of different breeds and ages (at least 1 year old) and both sexes. The cats were tested for *D. immitis* and Feline Immunodeficiency Virus (FIV) antibodies and Feline Leukaemia Virus (FeLV) antigen with commercial immuno-chromatographic test kits. In addition, blood samples were examined for microfilariae using a direct blood smear technique and the modified Knott's test. Out of 200 cats, one (0.5%) cat was seropositive for *D. immitis*, 15 (7.5%) cats were seropositive for FIV, and one (0.5%) cat was seropositive for FeLV. One cat seropositive for *D. immitis* was showing signs of active heartworm infection. All samples were found negative for microfilariae by direct blood examination and the modified Knott's method. This study is the first report of the occurrence of *D. immitis* in the Aegean Region, with a seroprevalence of 0.5%. Also, it provides evidence that cats in the Aegean region are at risk of becoming infected with *D. immitis*.

Keywords: cat, *D. immitis*, heartworm, seroprevalence, Turkey

Vector-mediated diseases are an important problem in veterinary medicine and are frequently encountered in veterinary clinics. Dirofilariasis is a vector-mediated zoonotic disease caused by *Dirofilaria immitis* (*D. immitis*), a filarial nematode known as heartworm disease (17, 29). The disease is seen mainly in dogs. However, cats, ferrets, wild carnivores (foxes, jackals, wolves, raccoons, wild felines, sea lions, black bears), and humans can also be infected (29, 34). In the biology of *D. immitis*, more than 60 mosquito species belonging to the genera *Aedes*, *Anopheles*, *Culex*, *Mansonia*, and *Psorophora* serve as vectors (3, 43). In carnivores, mature parasites settle in the right ventricle and pulmonary arteries of the heart. Their microfilariae are also found in peripheral blood, viscera, and cerebrospinal fluid (5, 17, 29, 35).

Although cats are susceptible hosts, they are resistant to infection with mature *D. immitis* (15, 25, 28). Heartworm disease in cats is different from what it is dogs in terms of host response, pathophysiology, and clinical presentation. Infected cats have 2-4 mature

heartworms per cat (1-8), and their lifespan ranges from 2 to 4 years (versus an average of 7.5 years for dogs) (10). Dirofilariasis in cats may present as sub-clinical infection or chronic respiratory findings and acute death. Also, acute-onset dyspnea and heartworm-associated respiratory disease (HARD) characterized by an interstitial pattern on chest radiography has been demonstrated (4, 17).

Although dirofilariasis is seen worldwide, it is endemic mostly in tropical and subtropical regions (7, 9, 17, 18, 23). Furthermore, dog and cat mobility from endemic to non-endemic areas could potentially spread this vector-borne infection. In cats, few studies have determined the true prevalence of *D. immitis* infection because of transient clinical manifestations, death before the disease is detected, or diagnostic difficulties. However, the prevalence of *D. immitis* infection in cats is determined by the prevalence in canine reservoir species. The prevalence in cats is generally estimated to range between 5% and 20% of the prevalence in the canine population in the same region (17, 32).

In Turkey, the disease is endemic in the canine population, and several studies have reported that the

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prevalence of *D. immitis* infection in dogs varied from 1.52% and 46.2% (1, 2, 26, 39-42). However, there is only one epidemiological study on feline dirofilariasis in Turkey. According to that study, conducted in the Kars region, the prevalence of dirofilariasis in cats was 20.7% (8). Therefore, the present study aimed to investigate the occurrence of *D. immitis* infection in cats in the Aegean region, Turkey, and contribute to the limited data on dirofilariasis in cats.

Material and methods

Ethical statement. The Animal Research Ethics Committee of the Aydın Adnan Menders University reviewed and approved all study procedures under protocol number 64583101/2019/047.

The animal material of the study consisted of 200 cats of different breeds and ages (at least 1-year old) and of both sexes brought to small animal clinics of the Faculty of Veterinary Medicine at Adnan Menderes University for examination, treatment, and general control between 11 July 2019 and 11 January 2021. The cats came from five different cities in the Aegean region of Turkey: Aydın, İzmir, Denizli, Muğla, and Manisa (Fig. 1). All cats' signalment, medical history, anamnesis information, and physical examination findings were recorded.

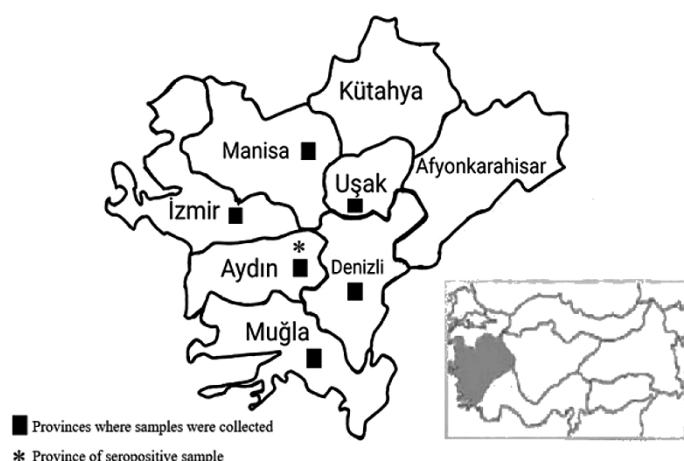


Fig. 1. A schematic map illustrating the geographic locations of the sampled cats

Blood samples were taken from the *vena cephalica antebrachii* into anticoagulant (K3-EDTA) and serum separator tubes. Complete blood counts were performed with an Abacus Junior Vet Hematology Device (Abacus Junior Vet, Diatron MI LTD, Hungary). Blood samples taken into serum tubes were centrifuged at 3000 g for 10 minutes, and their serums were separated. Serum samples from the cats were tested for *D. immitis* and Feline Immunodeficiency Virus (FIV) antibodies and Feline Leukaemia Virus (FeLV) antigen with commercial immuno-chromatographic test kits (FeliCheck-3, Anigen, Korea, sensitivity 96%, specificity 97.9%).

Direct blood examination and the modified Knott's technique were used to investigate microfilariae. In the direct blood examination, 1-2 drops of the blood sample were placed between a slide and a coverslip. The slides were examined under a microscope, and microfilariae were

searched for in the blood. In the case of high erythrocyte density, 2% saponin or 0.04% ammonium hydroxide was used to lyse the erythrocytes (14). In the modified Knott's technique, 0.5 ml of anticoagulated blood sample was completed to 5 ml with 4.5 ml of 2% formalin, then centrifuged at 1500 rpm for 5 minutes, and the supernatant was discarded. The sediment was mixed with an equal volume of 0.1% methylene blue. Samples of the sediment were examined under a microscope between a slide and a coverslip (14).

Results and discussion

Two hundred cats were sampled, including 102 females (51%) and 98 males (49%). The ages of the cats ranged from one to 14 years (median 3.22 years). Most samples were taken from Domestic short-haired cats, followed by British Shorthair, Scottish fold, and Persian cats. Forty-two (21%) of the cats lived indoors only, whereas 158 (79%) led indoor-outdoor lifestyles. In addition, 39% of the cats were regularly treated with antiparasitic drugs.

One (0.5%) of the 200 cats was positive for *D. immitis* antibodies. The *D. immitis* positive test result was confirmed by testing three times. All blood samples analyzed by direct microscopic examination and the modified Knott's method were negative for microfilaria. The *D. immitis* seropositive cat was a two-year-old male of the domestic shorthair breed and lived an indoor-outdoor lifestyle. The antiparasitic treatment program for the cat was irregular. The cat showed active heartworm signs, such as cough, intermittent vomiting, and shortness of breath, which had persisted for a long time and the cause of which could not be determined. Characteristic radiographic abnormalities seen in heartworm diseases, such as dilation of the pulmonary arteries and a mild diffuse interstitial pattern, were determined on thorax radiographs. In addition, fifteen (7.5%) of the 200 cats were positive for FIV, and one (0.5%) was positive for FeLV. There were no co-infections among the positive cats.

Pets, especially cats and dogs, play an important role in societies worldwide. Although pets have positive interactions with their owners, they are also a potential source of many human and animal diseases. Even if they appear healthy, they can carry disease subclinically and spread zoonotic agents (21).

Infectious diseases transmitted by vectors are an important health problem that negatively affects humans and animals. Vectors, such as ticks, fleas, sand flies, and mosquitoes, play an essential role in transmitting many rickettsial, spirochetal, parasitic, and bacterial diseases (21). *Dirofilaria immitis* is a vector-transmitted nematode responsible for canine and feline cardiopulmonary dirofilariasis. It also causes pulmonary dirofilariasis in humans (35). However, thanks to the increasing awareness of the disease and the development of diagnostic methods, it is an increasingly diagnosed disease in cats (19, 30). The presence and prevalence of the disease

in cats are reported in many countries in Asia, Africa, Europe, and America (10, 13, 20, 25, 30). In studies conducted in different parts of Europe, the prevalence of the disease was 9.4% (3/32) in Greece, 4.8% (32/62) in Italy, 3.5% (5/141) in Portugal, and 24.40-25.20% (61-63/250) in Spain (6, 10, 25, 38). The presence of *D. immitis* in cats has also been demonstrated in Romania (28). There is only one epidemiological study on feline dirofilariasis in the Eastern Anatolia Region in Turkey. In that study, conducted in the Kars region, the prevalence of dirofilariasis in cats was 20.7% (8). In our study, *D. immitis* seropositivity in cats from different cities in the Aegean region was 0.5% (1/200). The difference between the studies may be related to the geographical area, the diagnostic method, ambient temperature, vector population, vector control, housing conditions, and preventive treatment (20, 29). In addition, the sensitivity of different antibody tests used for serological testing varies according to larval developmental stages, so inconsistencies between results obtained by different test methods can be seen (36).

Due to climatic conditions and abundant intermediate hosts, Turkey is a suitable country for developing *D. immitis*. It is also known that Turkey is an endemic region for dirofilariasis, and the prevalence of heartworm infection in the dog population in different areas of Turkey varies between 1.52% and 46.2% (1, 11, 27, 39-42). In a study conducted on dogs in the Aegean region (Aydın and İzmir Provinces), the prevalence of the disease was reported as 10% (33). Some studies reported that the prevalence of infection with *D. immitis* in cats is 5-20% of the concurrent prevalence in the local dog population (6, 12, 24, 32). Consistent with these studies, cat seroprevalence (0.5%) in our study was approximately 20% of the dog prevalence previously detected in the same region (10%). The relatively low adult parasite burden, lack of microfilaremia, the short lifespan of the parasite, and mosquito feeding preferences may also have contributed to the lower prevalence of feline infection (17, 30).

Diagnosis is quite tricky, even if the suspicion of dirofilariasis in cats has increased. Unlike in dogs, microscopic detection of microfilariae in cats has low sensitivity because cats usually develop only a few parasites, and most of them are single-sex (12, 17, 29). Thus most cases are amicrofilaremic (negative Knott's test) (32). However, detection of microfilaremia provides a definitive diagnosis (14). This study used direct microscopic examination and the modified Knott's test in 200 cats. All cats were negative for microfilariae, including the antibody-positive cat. Similar to the studies above, this situation may be related to a low parasite count, single-sex infection, or the presence of only immature parasites.

Serological tests are typically the initial screening tool, and they can provide useful information (16). However, these tests have certain limitations, and an understanding of these limitations is necessary to

interpret the results correctly. Antigen tests detect the circulating female reproductive antigens of mature parasites. These tests are likely to give false-negative results due to low parasite amounts, low antigen concentrations, the possibility of male single-sex infections, and immature infections (17). A positive antigen result strongly indicates the presence of heartworms. However, a negative result does not rule out that the animal can be infected with only male and pre-adult worms. Antibody tests detect antibodies produced by the host in response to infection. An advantage of antibody tests is that they can detect infection two months after infection (22). However, these tests describe exposure to an infectious agent (and development of larvae to at least the L4 stage) and cannot distinguish between current and past infections (31). In the present study, cats were tested for *D. immitis* antibodies with a commercial immunochromatographic test. One cat (1/200) was determined positive. The positive test result was confirmed by testing three times. The result may indicate a mature infection, ongoing exposure to heartworm larvae, or previous exposure. However, the seropositive cat showed active heartworm signs, such as intermittent coughing, vomiting, dyspnea, loud breathing, and weight loss (37). Thorax radiograph findings and response to treatment were also consistent with active infection.

A limitation of this study was the lack of other additional diagnostic tests, such as antigen tests and echocardiography, for a better clinical characterization of the seropositive cat.

In conclusion, the study confirms the presence of *D. immitis* in cats in the Aegean region, Turkey, with a prevalence of 0.5% in the study area. It also provides evidence that cats in the Aegean region are at risk of becoming infected with *D. immitis*. Therefore, cats living in areas where the disease is endemic in dogs also require chemoprophylaxis. In addition, the prevalences of FIV and FeLV infections in the Aegean region were determined as 7.5% and 0.5%, respectively.

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