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Short Communication

Feline Dirofilariosis Due to *Dirofilaria immitis* in Meshkin Shahr District, Northwestern Iran

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Abstract

Background: *Dirofilaria immitis* is a common nematode of the cardiovascular system, which infects carnivores all over the world. The infection is prevalent in dogs, but in cats and human is rare. Dirofilariosis is transmitted by mosquitoes. Cats are accidental hosts and are naturally resistant to the infection, compared to the dogs. Mild infection can cause severe illness in cats and may lead to death, so it is clinically important to diagnose the disease.

Methods: In the present study, 103 stray cats were collected from Meshkin Shahr district, Aradabil Province, northwestern Iran that is an endemic area for canine dirofilariosis. Blood samples were prepared from the saphenous vein of each cat and were examined for the presence of microfilariae by the modified Knott test.

Results: A 2 yr old male cat (0.97%) was microfilaremic. The cat was subjected to necropsy and 4 adult *D. immitis* (2 male and 2 female worms) were found in the right ventricle of the heart. PCR was then carried out and *D. immitis* was confirmed.

Conclusion: Cats and other carnivores in Meshkin Shahr district are at risk of infection due to the high activity of vectors in this climate and it is important to follow up the infection in this area.

Introduction

Dirofilariosis is a parasitic disease, caused by the filarial nematode *Dirofilaria immitis*, which is a com-

mon parasite of the cardiovascular system of the carnivores all over the world. The disease has been reported in dogs, cats, coyotes, oce-

lots, ferrets, wolves, red foxes and humans. Transmission occurs by mosquitoes (*Culex*, *Aedes* and *Anopheles*). Adult heartworms usually live in the right ventricle and large pulmonary arteries. Dirofilariosis can be detected by identification of microfilariae through modified Knott test (1).

Heartworms have been found in cats worldwide. However, cats are naturally resistant to dirofilariosis compared to dogs, which causes relatively low worm burden and few reproductively active female worms. The number of the microfilariae in the blood of cats is much less than that of the dogs. Prevalence of feline dirofilariosis in the endemic areas seems to parallel to that in dogs, but at a lower level (1/10). The most important diagnostic methods for heartworm infection are PCR, ELISA commercial kits, modified Knott test and necropsy (2). Radiography, echocardiography and electrocardiography are also helpful for diagnosis.

Feline dirofilariosis was first reported in 1921 by Travassos in Brazil. (3). In Iran *D. immitis* was first detected in a dog (4). Feline dirofilariosis has been reported in Tabriz (5) and Ahvaz (6) in Iran. However, there are reports showing high prevalence of dirofilariosis among dogs. As Meshkin Shahr is an endemic area for canine dirofilariosis (7), we decided to estimate the prevalence of dirofilariosis in stray cats of Meshkin Shahr in Ardabil Province, which is an endemic area for heartworm, using modified Knott test, PCR and necropsy.

Materials and Methods

Sample population

A population of one hundred and three asymptomatic stray cats from Meshkin Shahr district in Ardabil Province, northwestern Iran was examined for dirofilariosis during a period of one year (2014-2015). They were collected from four different villages, named Kojanagh, Urkandi, Aghbolagh and Sarikhanlou. The age range of the cats was between 1 and 9 yr old, estimated by the teeth. The cats were kept at

Meshkin Shahr Health Research Station until the end of the study.

Blood collections

A blood sample (5 ml) was collected from saphenous or cephalic vein of each cat after sedation by the intramuscular injection of ketamine (7.5 mg/kg) and acepromazine (0.05 mg/kg). The collected blood was divided into two tubes either containing EDTA as an anticoagulant for direct smear or without anticoagulant for modified Knott test.

Direct smear

One drop of the blood was placed on the slide and under light microscopy was checked for the presence of the circulating microfilariae.

Modified Knott test

This method was carried out for detection of the microfilariae in the sediments. One ml of complete blood was added to 9 ml of 2% formalin solution and then centrifuged for 10 min. One drop of methylene blue was added to the sediments and was checked using a light microscope to find microfilariae.

Necropsy

Those cats, which had microfilariae in their blood, were subjected to necropsy and hearts and pulmonary arteries were looked for the presence of adult heartworms. The collected worms were conserved in 70% alcohol and 10% buffered formalin.

PCR

DNA was extracted from the adult heartworm using Genomic DNA Extraction Kit (Bioneer) and stored at 4 °C for later use. The primers used in this study were: 5'-TGATGGTGGTTTGGTAA-3' (forward), and 5'-ATAAGTACGAGTATCAATATC-3' (reverse), for the mitochondrial Cox1 gene segments. PCR was performed in 25 reaction mixtures, containing 3 µl template DNA, 1 µl 20 pmol of each primer, 12.5 µl Taq 2X Master mix Red and 7.5 µl DW. The thermal cy-

cler was set at 94 °C for 5 min to activate the polymerase, and then 35 cycles, each of denaturation for 30 s at 94 °C, annealing for 45 s at 52 °C and extension for 60 s at 72 °C. Final extension was set for 7 min at 72 °C. A 4- μ l sample of the PCR product was mixed with 2 μ l of FluoroDye Fluorescent DNA Loading Dye on a piece of parafilm by pipeting and then analysed by electrophoresis in 1-1.5%-agarose gel followed by photography using transilluminator. PCR products were then sent for detecting the sequences.

Results

One hundred and three stray cats, consisting of 55 females (53.4%) and 48 males (46.6%) were examined. Microfilariae of *D. immitis*

were found in the direct blood smear and also modified Knott test of just one cat (0.97%) (Fig. 1). There were 16 microfilariae present in 100 μ l of the complete blood. All cats were asymptomatic. The infected cat was 2 yr old, collected from the village named Kojanagh, which previously showed high prevalence of canine dirofilariosis. Necropsy then showed that 4 adult heartworms (2 male and 2 female worms) were present in the right ventricle of the infected cat (Fig. 2). Molecular detection confirmed *D. immitis* showing a 689 bp band in the 1-1.5% agarose gel (Fig. 3). The sequences were the same as the *D. immitis* sequences previously recorded in the gene bank with 100% homology (Gene bank accession number KT282097).

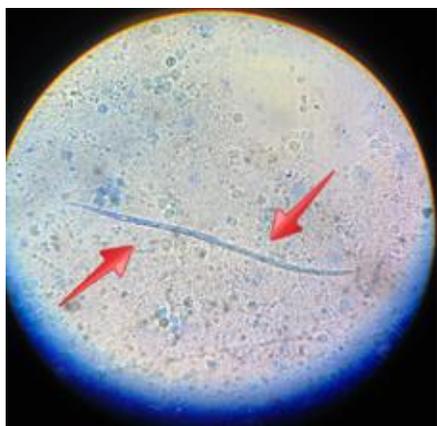


Fig. 1: Microfilariae in the modified Knott test smear



Fig. 2: Adult heartworms in the right ventricle

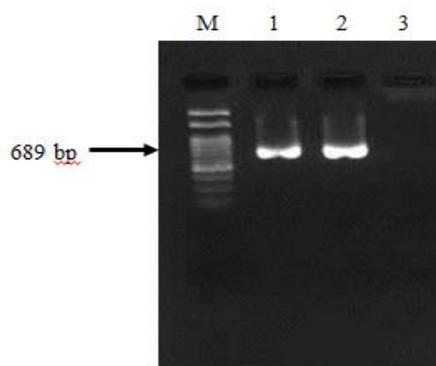


Fig. 3: The PCR-based detection of feline heartworm DNA in stray cats of Meshkin Shahr. The amplified 689-bp product from the positive test sample (lane 1) and positive sample (lane 2) were subjected to electrophoresis in 1-1.5% agarose gel. A 100-bp ladder (M) and a negative control free of DNA template (lane 3) were run in parallel

Discussion

Feline dirofilariosis is currently known as a potential cause of serious disease. It can present both a clinical and diagnostic challenge to practicing veterinarians. The infection can be occult and asymptomatic in some cats, which may lead to death within an hour because of the heartworm disease (8). However, it is possible that the presence of only two adult worms in the heart and/or lungs will cause severe illness (9). However, detection of feline dirofilariosis is so hard (8), because cats are accidental hosts for *D. immitis*, which are resistant to infection. Therefore, the availability of a specific and sensitive test for diagnosis of the infection would be so helpful for clinical management of infected cats.

In the present study, one out of 103 cats was microfilaremic and adult heartworms were present in the right ventricle. Eventually PCR confirmed feline dirofilariosis in this case.

The first study on dirofilariosis in cats in Iran was carried out by Ashrafi Halan et al in Tabriz. They examined 234 cats referred to a veterinary clinic. 25 out of 234 cats had some clinical signs such as lethargy, anorexia, cachexy dyspnea, chronic coughs and vomiting. The modified Knott test was used to detect the heartworm infection. microfilariae were present in blood of two cats (0.85%) (5).

Alborzi et al. also have reported *D. immitis* in Ahvaz with the prevalence of 0.83%. Overall, 120 cats were examined by the modified Knott test and Ag detection immunochromatographic kits. The heartworm infection was confirmed in only one male, 2.5 yr old cat (6).

These two reports suggest that the infection among felids in Iran is relatively rare. According to the report of the canine dirofilariosis in Tabriz (more than 30%), the low prevalence of feline dirofilariosis in this area (less than 1%) shows that cats are resistant to the infection. Study on canine dirofilariosis in the north of Iran also represented that 25.5% of dogs are infected to heartworm (10).

Carleton et al. examined 184 cats for detection of adult heartworms in northwest Georgia. Four cats (2.1%) were infected to *D. immitis* (11).

Feline dirofilariosis was also reported by Nogami et al. in Japan. Heartworms were present in 15 out of 1840 cats (0.8%). Microfilariae were detected only in one infected cat (12).

Canine dirofilariosis in Meshkin Shahr was studied previously (7). The prevalence of the disease among dogs was reported 34.6%, using modified Knott test, which is obviously higher than the prevalence of feline dirofilariosis. This can indicate that cats are naturally more resistant than dogs against the infection.

The result of the present study confirms that cats are accidental hosts for *D. immitis* and can rarely be infected. According to the possibility of occult infection in cats, it is recommended to use more specific and sensitive methods and investigations that are more extensive are needed to define the prevalence of feline dirofilariosis continuously.

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