

Investigation of infection with *Dirofilaria immitis* parasite in stray dogs in Tabriz city of Iran

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Abstract

Dirofilaria immitis is one of the important parasites in bloodstream of dogs, causing severe cardiopulmonary complications in this animal. This nematode is common between human and dog and it has been reported from different parts of the world and some parts of Iran. The objective of this study was to investigate the *Dirofilaria immitis* parasite microfilar in stray dogs . The study was conducted on blood of 100 stray dogs suspected to heartworm disease (*Dirofilaria immitis*) in the Tabriz city using Knott method modified. Out of 100 blood samples taken, 14 samples were positive in terms of *Dirofilaria immitis* microfilar, which 11 blood samples belonged to male dogs and 3 blood samples belonged to female dogs. According to findings of this study and *Dirofilaria immitis* infection in dogs of this area, the need for more comprehensive investigation and control of the infection in Tabriz city is emphasized.

Key words: *Dirofilaria immitis*; stray dogs; Tabriz city; Iran

Introduction

Dog heartworm disease or *Dirofilaria immitis* is metazoosis disease that has indirect evolution, and it is transmitted by certain species of mosquitoes such as *Aedes*, *Culex*, *Anopheles*, *Armigeres*, *Myzorrhynchus*, and *Taeniorhynchus* (Anderson and Davis, 2014, Manrique-Saide *et al.*, 2010, Vezzani *et al.*, 2006). Its source is often canines and this disease has global widespread. The evolution of parasite is indirect and female gender of this worm releases its microfilaria into bloodstream, and microfilaria are spread throughout the body via bloodstream (Vezzani *et al.*, 2006).

The main hosts of *Dirofilaria immitis* are dogs, wolves, foxes and rarely humans and cats. Horse, California seal, porpoise, and black bear might be infected with this parasite (Ettinger and Feldman, 2009). Infection with heartworm or Dirofilariosis is caused by *Dirofilaria immitis* that mainly affects canines. Recent investigation of veterinarians showed that almost 240 thousand cases of this disease were diagnosed in the United States in 2001 (Ettinger and Feldman, 2009). Heartworm disease may range from asymptomatic form (there are only radiographic lesions) to severe and life threatening form, including chronic disease of the lungs arteries and heart. In the chronic form of infection with heartworm, glomerulonephritis, anemia, and thrombocytopenia might be diagnosed (Ettinger and Feldman, 2009). Heartworm disease in dogs does not depend on age or strain. Most of infected dogs are 4 to 8 years old, but the disease in dogs under one year (over 6 months) and older dogs is also common (Nelson and Couto, 2014). Male dogs are involved about 2 to 4 times more than females dogs (Nelson and Couto, 2014). Clinical signs of the disease vary highly in dogs, beginning from asymptomatic stage to mild symptoms such as weight loss and gradual weight loss, cough, physical inactivity, and premature fatigue during activity and ending in severe symptoms such as dyspnea, increased temperature, and damage to mucous membranes (cyanosis), anemia, cardiac complications, and death (Nelson and Couto, 2014).

Considering the increasing Earth's temperature by 0.74 ° c, some areas of the world that were free of *Dirofilaria immitis* were infected again in warm months (Genchi *et al.*, 2005, Kronefeld *et al.*, 2014, Morchón *et al.*, 2012, Vezzani *et al.*, 2006). Human infection with adult worms has been also reported in heart and the inferior vena cava (Takeuchi *et al.*, 1981).

Common symptoms are generally caused by the somatic migration of third-stage larvae, causing fever, cough, and pneumonia (Theis, 2005). It also creates calcified granuloma and coin lesions that are misdiagnosed with some cancers (Miliaras *et al.*, 2010).

Considering the high importance of this disease in terms of health of dogs and risk of this parasite to human health, the objective of the study was to investigate the infection of stray dogs of Tabriz city to *Dirofilaria immitis*.

Materials and Methods

This study was conducted on 100 stray dogs in Tabriz city to determine infection with *Dirofilaria immitis* parasite. The samples were taken from north, south, east and west of Tabriz and its villages, for better evaluation of infectious rate. For this purpose, Knott modified method that is 15 times accurate than direct method was used. In general, 5 ml blood sample was taken from veins of dogs through heparinized syringe. Then, blood was mixed in the syringe and 1 ml was poured into centrifuge tube and 9 ml 2% formalin was added to it and it was mixed. After hemolysis of red blood cells, it was centrifuged at 1500 rpm for 5 min. Then, supernatant was evacuated. Then, a drop of Methylene blue was added to precipitate. To observe microfilaria, after mixing it, slide was prepared and stained with Hematoxylin (Bowman, 2014).

Results

The results of the study showed that 14 dogs of 100 dogs evaluated were positive in terms of *Dirofilaria immitis* microfilaria (14%). Based on the results of the study, 11 positive samples belonged to male dogs and 3 positive samples belonged to the female dogs (Table 1).

Table 1. The prevalence of *Dirofilaria immitis* in dogs in Tabriz, Iran

Region	Exam. dog	Microfilaria positive dog		Infection rate	
	n	n	Sex		%
			Female	Male	
Northern	28	5	1	4	5
Southern	22	2	0	2	2
Western	24	4	1	3	4
Eastern	26	3	1	2	3
Total	100	14	3	11	14

Discussion

In this investigation conducted in Tabriz city, 14 dogs had heartworm disease among the 100 dogs that were suspected to heartworm. Microscopic investigation of blood samples showed that all cases of infection caused by the *Dirofilaria immitis* parasite and there was no infection caused by *Dipetalonema reconditum* microfilar and *Dirofilaria repens*.

Based on the results, *Dirofilaria immitis* infection in endemic areas is spreading. Iran is one of the endemic areas of heartworm disease in the world (Miliaras *et al.*, 2010, Ranjbar-Bahadori *et al.*, 2009, Takeuchi *et al.*, 1981, Theis, 2005). Additionally, dog is considered as source of *Dirofilaria immitis* infection in Iran (Ranjbar-bahadori and Eslami, 2007) and it is necessary to determine the prevalence of this parasite to control the infection. Due to the large population of dogs in Iran, infection rate in dogs is high and more importantly, microfilar parasite is concentrated in the blood and it is available readily for mosquitoes (Eslami, 1995). In areas where dogs are straying, ponds and collected rainwaters are appropriate place to breed the infant of adult mosquito (Vezzani *et al.*, 2006). Meanwhile, nightly temperature during the summer is suitable for mosquito growth (Eslami, 1995). Due to the long lifetime of heartworms, prolonged presence of microfilar in the blood, and the lack of sufficient safety against adult parasites, high contact with the infected environment will increase the infection, undoubtedly (Salahi-Moghadam *et al.*, 2000).

Separation of adult worm from a hydrosol in five years old child showed that the infection caused by *Dirofilaria immitis* can be passed on to humans from dogs (Salahi-Moghadam *et al.*, 2000). The results of the study conducted by researchers showed that the infection rate of stray dogs in Khuzestan Province is 12.6% (Ranjbar-Bahadori *et al.*, 2009).

Some researchers have reported that there is no relationship between the infection with *Dirofilaria* and gender (Ranjbar-Bahadori *et al.*, 2009), while other researchers have reported that infection rate of male dogs (74.4 percent) is more than female dogs (Hatsushika *et al.*, 1992). The infection rate of the parasites was reported 18% in the Dominican Republic (Duran-Struuck *et al.*, 2005), 54% in Brazil (Garcez *et al.*, 2006), and the infection with *Dirofilaria* in the investigated dogs in Grenada, West Indies was reported 17% (Coomansingh *et al.*, 2015). The results of the studied conducted by researchers in Turkey showed that the infection rate of dogs is 9.6% (Yildirim *et al.*, 2007), while in previous studies conducted in Turkey the infection rate has been reported 3% to 46.2% (Ağaoğlu *et al.*, 2000, Coskun *et al.*, 1992, Oge *et al.*, 2003, Tasan, 1984).

The results of present study also showed that the infection rate is higher in male dogs, that this result is consistent with some previous studies. High infection of male dogs is due to high absorption of mosquitoes compared to female dogs (Montoya *et al.*, 1998). When stray dogs tested in were treated with appropriate adult and microfilar killer drugs, infection was reduced. According to reports of the possibility of human infection with the parasite in Iran, the common aspect of infection and its health importance should be taken into consideration (Salahi-Moghadam *et al.*, 2000). Therefore, in order to control the *Dirofilaria immitis* in endemic areas, it is recommended that control programs to be implemented strictly on infected dogs using screened serological tests, and in the cases that these tests are positive, necessary steps should be taken in order to treat them. Finally, treatment of infected dogs, controlling the carrier mosquitos, if they do not cause environmental degradation and health risks, and developing control programs will be effective measures in preventing *Dirofilaria immitis*.

Ethical issues: The authors have no ethical issues to declare.

Conflict of interests: The authors declare that we have no conflict of interests.

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References

- 1) Ağaoglu Z, Akgül Y, Ceylan E, Akkan H, 2000. Van yöresi köpeklerinde *Dirofilaria immitis*' in yaygınlığı. *YYÜ Vet Fak Derg* 11: 41-43.
- 2) Anderson EM, Davis JA, 2014. First Records of *Armigeres malayi* and *Armigeres milnensis* in Timor-Leste. *Journal of the American Mosquito Control Association* 30: 51-53.
- 3) Bowman DD, 2014. *Georgis' parasitology for veterinarians*: Elsevier Health Sciences, 340-352.
- 4) Coomansingh C-m, Yabsley M, Wagner N, Pinckney R, Bhaiyat MI, Chikweto A, *et al.*, 2015. Meta-Analysis of the Prevalence of *Dirofilaria Immitis* in Dogs from Grenada, West Indies.
- 5) Coskun S, Tinar R, Akyol C, Aydin L, Demir S, 1992. Dogal enfekte köpeklerde *Dirofilaria immitis* mikrofilerlerine ivermektinin etkisi. *Uludag Univ Vet Fak Derg* 11: 121-128.
- 6) Duran-Struuck R, Jost C, Hernandez A, 2005. *Dirofilaria immitis* prevalence in canine population in the Samana Peninsula (Dominican Republic)-June 2001. *Veterinary Parasitology* 133: 323-327.
- 7) Eslami A, 1995. *Veterinary Helminthology: Nematodes and Acanthocephala*. Tehran-Iran: University of Tehran, 98-605.
- 8) Ettinger SJ, Feldman EC, 2009. *Textbook of veterinary internal medicine*. London, UK: WB Saunders Co, 138-177 and 213-220
- 9) Garcez LM, de Souza NF, Mota EF, Dickson LA, Abreu WU, Cavalcanti Vde F, Gomes P 2006. Focus on canine heartworm disease in Majoró Island, North of Brazil: A risk factor for human health. *Revista Da Sociedade Brasileira De Medicina Tropical* 39: 333-336.
- 10) Genchi C, Rinaldi L, Cascone C, Mortarino M, Cringoli G, 2005. Is heartworm disease really spreading in Europe? *Veterinary parasitology* 133: 137-148.
- 11) Hatsushika R, Okino T, Shimizu M, 1992. The Prevalence of Dog Heartworm (*Dirofilaria immitis*) Infection in Stray Dogs in Okayama, Japan. *Kawasaki medicine* 18: 75-83.
- 12) Kronefeld M, Kampen H, Sassnau R, Werner D, 2014. Molecular detection of *Dirofilaria immitis*, *Dirofilaria repens* and *Setaria tundra* in mosquitoes from Germany. *Parasites & vectors* 7: 1.
- 13) Manrique-Saide P, Escobedo-Ortegón J, Bolio-González M, Sauri-Arceo C, Dzib-Florez S, Guillermo-May G, *et al.*, 2010. Incrimination of the mosquito, *Aedes taeniorhynchus*, as the primary vector of heartworm, *Dirofilaria immitis*, in coastal Yucatan, Mexico. *Medical and veterinary entomology* 24: 456-460.
- 14) Miliaras D, Meditskou S, Kelekis A, Papachristos I, 2010. Human pulmonary *Dirofilaria* infection: one more case in Greece suggests that *Dirofilaria* is a rather common cause of coin lesions in the lungs in endemic areas of Europe. *International Journal of Immunopathology and pharmacology* 23: 345-348.
- 15) Montoya J, Morales M, Ferrer O, Molina J, Corbera J, 1998. The prevalence of *Dirofilaria immitis* in Gran Canaria, Canary Islands, Spain (1994–1996). *Veterinary parasitology* 75: 221-226.
- 16) Morchón R, Carretón E, González-Miguel J, Mellado-Hernández I, 2012. Heartworm disease (*Dirofilaria immitis*) and their vectors in Europe—new distribution trends. *Frontiers in physiology* 3: 75-85.
- 17) Nelson RW, Couto CG, 2014. *Small animal internal medicine*: Elsevier Health Sciences,
- 18) Oge H, Doğanay A, Oge S, Yildirim A, 2003. Prevalence and distribution of *Dirofilaria immitis* in domestic dogs from Ankara and vicinity in Turkey. *DTW Deutsche tierärztliche Wochenschrift* 110: 69-72.
- 19) Ranjbar-Bahadori S, Eidi Delvarzadeh M, Shemshadi B, 2009. *Dirofilaria immitis* infection in stray dogs of Khuzestan, a province in South-Western Iran. *International Journal of Veterinary Research* 3: 133-136.

- 20) Ranjbar-bahadori S, Eslami A, 2007. Prevalence of blood filaria in dogs in Golestan province (north of Iran) using modified Knott method and determination of its periodicity. *Journal of Veterinary Research* 62: 11-14.
- 21) Salahi-Moghadam A, Moobedi A, Bani Hashemi S, editors. Case report of *Dirofilaria* in Hydrocoel of a child with 5 years old age. 3 rd National Congress of Parasitology, sari, Iran Mazandaran medical sciences university Publication; 2000.
- 22) Takeuchi T, Asami K, Kobayashi S, Masuda M, Tanabe M, Miura S, *et al.*, 1981. *Dirofilaria immitis* infection in man: report of a case of the infection in heart and inferior vena cava from Japan. *The American journal of tropical medicine and hygiene* 30: 966-969.
- 23) Tasan E, 1984. Elazig kirsal yore kopeklerinde helmintlerin yayilisi ve insan sagligi yonunden onemi. *Doga Bilim Dergisi* 3: 160-167.
- 24) Theis J, 2005. Public health aspects of dirofilariasis in the United States. *Veterinary Parasitology* 133: 157-180.
- 25) Vezzani D, Eiras DF, Wisnivesky C, 2006. Dirofilariasis in Argentina: historical review and first report of *Dirofilaria immitis* in a natural mosquito population. *Veterinary Parasitology* 136: 259-273.
- 26) Yildirim A, Ica A, Atalay O, Duzlu O, Inci A, 2007. Prevalence and epidemiological aspects of *Dirofilaria immitis* in dogs from Kayseri Province, Turkey. *Research in Veterinary Science* 82: 358-363.