Short Communication

Serological study of *Dirofilaria immitis* in urban dogs of Urmia using modified knot and rapid antigen tests

Maryam Naghipoor[1], Shahram Javadi[2*], Mousa Tavassoli[3], Shokoofeh Shamsi[4]

1- Graduated of Faculty of Veterinary Medicine, Urmia University, Iran
2- Department of Internal Medicine and Clinical pathology, Faculty of Veterinary Medicine, Urmia University, Iran
3- Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Iran
4- School of Animal & Veterinary Sciences, Charles Sturt University, Wagga Wagga, Australia

***Corresponding author:** *S.Javadi@urmia.ac.ir*

(Received 12 October 2017, Accepted 2 February 2018)

Summary

Dirofilariasis is a zoonotic infection caused *by Dirofilaria immitis* (*D. immitis*) the causative agent of canine and feline cardiopulmonary dirofilariasis (heartworm disease) in temperate and tropical areas throughout the world and Iran. The parasite is transmitted by different species of culicid mosquitoes. In order to study on *D. immitis* in healthy urban dogs in Urmia region, and comparison of two diagnostic methods i.e. modified knot test and rapid antigen test kit, the number of 100 dogs (63 males and 37 females) were undertaken to this study in a period of April till July (2014). After physical restriction, blood samples were collected from cephalic or saphenous vein in the appropriate tubes which transferred to the laboratory in an ice pack. Modified knot test was used to recognize *D. immitis*. Then, all samples were tested by Antigen rapid test kit. There was a difference between the result of modified knot test and rapid test kit. The results indicated that out of 100 samples, 3 samples were infected by *D. immitis*. There were no significant differences in dirofilariasis infection among different gender and ages. It is concluded the antigen rapid test should be accompanied by another diagnostic method in order to avoid false negative results.

Keywords: *Dirofilaria immitis;* dogs; modified knot test; rapid antigen test; Urmia.

Introduction

D. immitis is the most important zoonotic species of the genus *Dirofilaria* in the world (Navarrete et al., 1968; Schlotthauer et al., 1969; Toomes et al., 1983). Dirofilariasis can be an infectious zoonotic disease when humans are bitten by culicid mosquitoes harboring infective third-stage larvae (microfilariae), due to *D. immitis.* This nematode is a common filarial worm of dogs and cats in the world, accounts for the most number of human infection with over 400 cases recorded (Pampiglione et al., 1995; Vakalis et al.,

1997; Brown et al., 1993). Dirofilariasis has been reported as an emerging zoonosis in many countries world-wide such as Iran (Azari-Hamidian, 2007).

D. immitis or heartworm is more important than other filaria in dogs because it can cause some clinical signs (Brown et al., 1993). This worm lives in right ventricle, pulmonary artery and posterior vena cava and its microfilaria is found in the peripheral circulation (Eslami, 1998).

Infection to *D. immitis* in dogs was reported from different regions of the world (Hatsushika et al., 1992). The first infectious was recorded by Travassol in 1921 (Ettinger, 2010). Dirofilariasis is common in dogs in Iran with infection rates 16/1% (Ranjbar et al., 2011).

D. immitis was first reported from a dog in Iran in 1969 (Sadighian, 1969). Then infection to heartworm was reported from different areas of Iran including: Ardabil (Bokai et al., 1998), Shiraz (Jafari et al., 1996), Tehran (Meshgi et al., 2001), Tabriz (Meshgi et al., 2002), Tonekabon (Ranjbar et al., 2005), Golestan (Ranjbar et al., 2006) and Khozestan provinces (Razmi, 1999).

There is a lack of information about filariasia in the urban area of Urmia in North West of Iran. In this study, filariosis was indicated in urban dogs of Urmia with two diagnostic methods of the modified knot and rapid antigen tests.

Materials and Methods

One hundred dogs from different parts of Urmia were entered to this study. After the recording of necessary information including age, sex, and breed of the dogs, the blood samples (5 ml) were taken from cephalic or lateral saphenous veins for modified knot test and rapid antigen test.

Modified knot method

The blood samples were mixed with 9 ml formaldehyde 2% and were shacked well for RBC hemolysis. In the laboratory, the samples were centrifuged for 5 minutes in 1500 rpm. Methylene blue stain was added and the mixture was examined for the presence of microfilaria with photomicroscope. The differential diagnosis between microfilaria of *D. immitis* and *Dipetalonem recaonditum* was done regarding the morphological criteria (Eslami et al., 2004).

Rapid test

Two drops of not coagulated blood, serum or plasma of dogs were added to a concerned place of Heart worm disease kit (manufactured by Bionote, Australia). The result was read after 5-10 minutes.

Results

The results showed that 3 (3 %) of the total 100 urban dogs were infected with *D immitis*. Blood microfilaria (*D. immitis*) was

observed in 3 samples by modified knot method (Fig. 1). None of the dogs showed positive results based on rapid antigen test kit and all samples were negative with this test (Fig. 2).

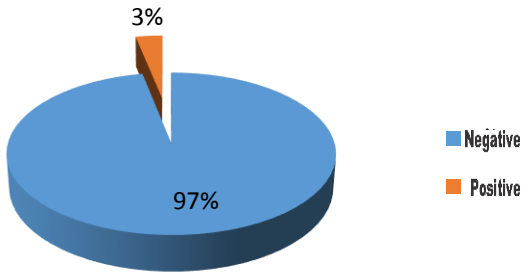

Fig 1. Percentage of healthy and *D. immitis*-infected dogs in Urmia distinct.

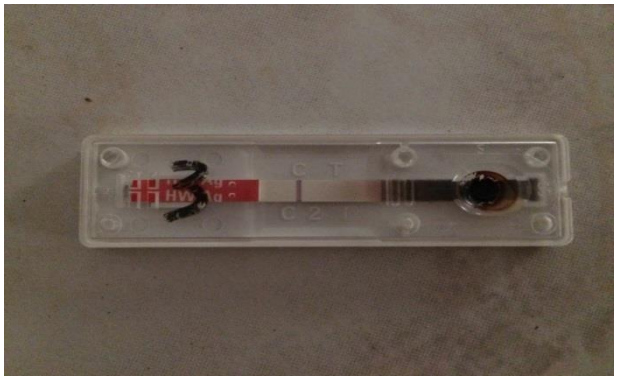

Fig. 2. Rapid antigen test for detection of *D. immitis* infection in dogs in Urmia distinct.

The dogs were from different breeds including Sarabi (Persian mastiff), German shepherd, Terrier and Boxer (Table1). Regarding epidemiology of the *D. immitis*, dogs with age of above 1 year old entered to the study. Three age groups of the dogs including 1-3, 3-5 and >5 years old were compared by both modified knot method and rapid ELISA kit. There was one infected dog to *D. Immitis* in each age group. No significant difference in dirofilaria infection was found among different age groups (P> 0.05). Concerning the gender of the dogs, two males and 1 female dog were infected by *D. Immitis.* There was no significant difference in dirofilaria infection between male and female dogs (P> 0.05). (Table 2).

Table1. Frequency of different breeds in urban dogs of Urmia

Breed	Percentage	Frequency
Sarabi	46%	46
German Shepherd	19%	19
Rottweiler	2%	2
Terrier	32%	32

Table 2. The seroprevalence of dirofilariasis in urban dogs of Urmia with different ages and sexes.

Characteristics		Positive No. (%)	Negative No. (%)	Total No. (%)
Age (year)	1-3	1 (1)	75 (75)	76 (76)
	3-5	1 (1)	17 (17)	18 (18)
	>5	1 (1)	5 (5)	6 (6)
Sex	Male	2 (2)	61 (61)	63 (63)
	Female	1 (1)	36 (36)	37 (37)

Discussion

Studies in the different regions of Iran showed that the infection to *D. immitis* or heart worm is distributed in many areas of the country. The extensive climatic distribution of the infection to the above-mentioned nematode is as follows: Ardabil (34.6 %) (Bokai et al., 1998), Shiraz (9.5 %) (Jafari et al., 1996), Tabriz (4.8%) (Meshgi

et al., 2002), Tehran (1.4 %) (Meshgi et al., 2001). This nematode was also reported in wild carnivores in Khozestan province (Eslami, 1998). Accordingly, different distribution of *D. immitis* infection has been world-widely reported including Taiwan (13.8 %) (Fon et al., 2001), Mexico (7.3 %-7.5 %) (Reifur et al., 2004), Canada (0.04%-0.19%) (Klotins et al., 2000).

The present study showed that the infection rate of *D. immitis* in urban dogs of Urmia is 3%. This is accordance with the results of some other studies in different regions of Iran and it is almost in the ranges reported from Tabriz (4.8%) (Meshgi et al., 2002) and Tehran (1.4 %) (Meshgi et al., 2001).

According the present study, the rapid antigen test is less sensitive than the modified knot test. As the modified knot test was able to identify three positive cases, the rapid antigen test was not able to detect any antibody. It can happen probably due to little number of *D. immitis* in the primary phases of the disease. This finding is not compatible with a study in South America which has been shown that the rapid antigen test is as sensitive as the modified knot test (Vezzani et al., 2007).

The statistical analysis did not show any significant relationship between the infection to heart worm and sex, age and breed of animals. These finding were also in line with those of other studies (Ranjbar et al., 2005), but the study in Brazil showing that the infection in the male dogs (89.7%) was higher than in the females (Souza et al., 1997). A study in Spain reported that the highest rate of the infection to *D. immitis* was observed in the age range of 6 years upward (Montoya et al., 2006).

In comparison with a study in shepherd dogs of Urmia (Javadi et al., 2011), which showed the infection rate of 24/8%, a remarkable difference is noticed. The reason may be related to the different living style in dogs living in urban area, which has lesser contact with intermediated hosts.

In conclusion, although the rapid antigen test is rapid, easy and more feasible in practice, it should be accompanied by another test such as the modified knot test.

References

Azari-Hamidian S.H., Yaghoobi-Ershadi M.R., Javadian E., Mobedi I. and Abai M.R. (2007). Review of dirofilariasis in Iran. *Journal of Guilan University of Medical Sciences*, 15(60), pp. 102–14.

Bokai S., Moobedi A., Mohebali M., Hoseini H. and Nadim A. (1998). Study on prevalence of dirofilariosis in Meshkinshahr-Northwest of Iran. *Journal of Faculty of Veterinary Medicine University of Tehran,* 53(1, 2): pp. 53-23.

Brown H. W. and Neva F. A. (1993). *Basic clinical Parasitology*. 5th edition, Printed in the republic of Singapore, pp. 158-159.

Eslami A. (1998). Veterinary Helminthology. *Tehran University*

Publications, pp. 584 - 603 and 642- 645. [Persian]

Eslami A. and Ranjbar-Bahadori Sh. (2004). Diagnostic methods of helminth infection. *Islamic azad university, Garmsar Branch Publications,* 296. [Persian]

Ettinger S.J. and Feldman E.C. (2010). *Textbook of Veterinary Internal Medicine,* Elsevier Saunders, St. Louis, pp. 1353-1373.

Fon C.K., Su K.H., Liao C.W., Du W.Y. and Chiou H.Y. (2001). Seroepidemiologic survey of *dirofilaria immitis* infection among domestic dogs in taipei city and mountain. Aboriginal district in Taiwan. *Veterinary Parasitology Journal,* 102 (1-2), pp. 113-120.

Hatsushika R., Okino T., Shimiza M. and Ohyama F. (1992). The prevalence of dog heart worm (*Dirofilaria immitis*) infection in stray dogs in Okayama, Kawasaki, Japan. *Medicine journal,* 3, pp.75-83.

Jafari S., Gaur N.S. and Khaksar Z. (1996). Prevalence of *Dirofilaria immitis* on dog of Fars province of Iran. *Journal of Applied Animal Research,* 9, pp. 27-31.

Javadi S., Hanifeh M., Tavassoli M., Dalir-Naghadeh B., Khezri A. and Hadian M. (2011). Dirofilariasis in Shepherd dogs of high altitudes areas in west Azerbaijan-Iran. *Journal of Veterinary Research Forum,* 1, pp. 53-57.

Klotins D.C., Martin S.W., Bonnett B.N. and peregrine A.S. (2000). Canine heart worm testing in Canada are being effective? *Canadian Veterinary Journal,* 41, pp. 929-937.

Meshgi B. and Eslami A. (2001). Study on filariosis of sheepdogs around of Tehran. *Journal of Faculty of Veterinary Medicine of Tehran,* 55, pp. 53-56.

Meshgi B., Eslami A. and Ashrafi Helan J. (2002). Epidemiological survey of blood filariae in rural and urban dogs of Tabriz. *Journal of Faculty of Veterinary Medicine of Tehran,* 57, pp. 59-63.

Montoya J.A., Morales M., Jeste M.C., Banares A., Simon F. and Genchi C. (2006). Seroprevalence of canine heartworm disease (*Dirofilaria immitis*) on Tenerife Island and epidemiological update. *Journal of Parasitology research,* 41(2), pp. 53-62.

Navarrete – Reyna A. and Noon G. (1968). Pulmonary dirofilariasis manifested as a coin lesion. Report of a case and review of the literature. *Archives of Pathology & Laboratory Medicine Journal,* 85, pp. 266-271.

Pampig lione S. (1995). UN cas probable de dirofilariose humaine sous conjonctivale observe par Amatus Lusitanicus dans le Midi de la France au XVle siècle. *Parasitie Journal,* 2, pp. 92.

Ranjbar – Bahadori Sh. and Eslami A. (2006). Study on blood filarial of dogs in Golestan province and determining of its periodicity. *Journal of Faculty of Veterinary Medicine University of Tehran,* 61, pp. 55-58.

Ranjbar – Bahadori Sh., Eslami A., Meshgi B. and Mohammad Mohtasham R. (2005). Study on blood filaria of dogs in Tonekaban. *Journal of Faculty of Veterinary Medicine of Tehran,* 60, pp. 353-356.

Ranjbar-Bahadori Sh., Veshgini A., Shirani D., Eslami A., Mohieddin H., Shemshadi B. and Masooleh R. (2011). Epidemiological aspects of canine dirofilariosis in the North of Iran. *Iranian Journal of Parasitology*, 6(1), pp. 85-90.

Razmi Gh. (1999). Study on situation of infection to dogs of Mashhad to types of filaria. *Journal of Faculty of Veterinary Medicine of Tehran,* 54, pp. 5-7.

Reifur L., Thomaz Soccol V. and Montiani-Ferreira F. (2004). Epidemiological aspect of filariosis in dogs on the cost of pavana state, Brazil with emphasis on *dirofilaria immitis*. *Veterinary Parasitology Journal,* 122(4), pp. 273-286.

Sadighian A. (1969). Helminth parasites of stray dogs and jackals in Shahsavar area, Caspian region, Iran. *Journal of Helminthology*, 2, pp. 372-374.

Souza N.F., Benigno R.N.M., Figueriedo M., Salim S. K., Silva D., Gancalves R., Peixoto P.C. and Serra F.M.N. (1997). Prevalence of *Dirofilaria immitis* in dogs in the city of Blem, Para, assessed on the basic of microfilaraemia Rev. Brasil. *Veterinary Parasitology Journal,* 6(1), pp. 83-86.

Vakalis N.C. and Himonas C.A. (1997). Human and canine dirofilariasis in Greece. *Parassitologia.* 39(4), pp. 389-91.

Vezzani D., Fontanarrosa M.F. aqnd Eiras D.F. (2008). Are antigen test kits efficient for detecting heartworm-infected dogs at the southern distribution limit of the parasite in South America? Preliminary results. *Research in Veterinary Science Journal,* 85(1), pp.113-115.