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PREVALENCE OF CERTAIN VIRAL DISEASES AND *DIROFILARIA IMMITIS* INFECTION IN STRAY DOGS IN BANGLADESH

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ABSTRACT

Background: Stray and pet dogs constitute the dog population in Bangladesh without any established dog statistics. Diseases of dogs are not only associated with morbidity and mortality in dogs but also associated with human health problems as zoonotic diseases. Inland reports on the prevalence of dog diseases are limited in Bangladesh and there is need to investigate diseases in both stray and pet dog populations.

Objectives: The main objective is to determine the prevalence of Canine distemper (CD), Canine adenovirus- 1 (CAV-1), CAV-2, Canine influenza (CI) and *Dirofilaria immitis* (Heart worm infection = HWI) infection in stray dogs in Bangladesh.

Materials and Methods: Blood, ocular and nasal samples were collected from each of 30 randomly caught stray dogs (11 male and 19 female) and of different ages, including growing (n = 3) and adult (n = 27) dogs in the district of Mymensingh in Bangladesh from January to June 2010. These samples were tested by using Antigen Test Kits (RapiGEN Inc, Korea).

Results: All the 30 stray dogs showed negative results to CD, CAV-1 and CAV-2, whereas an overall high prevalence of CI (4/30; 13.33%) and HWI (15/30; 50.0%) were recorded. The higher prevalence of CI was recorded in growing (n = 2/3; 66.67%) than adult (n = 2/27; 7.41%) but it did not differ significantly ($p > 0.05$) between male (n = 2/11; 18.18%) and female (n = 2/19; 10.53%) dogs. Significantly ($p < 0.05$) higher prevalence of *D. immitis* infection was recorded in adult (n = 15/27; 55.56%) than growing (0/3; 0.0%), male (n = 8/11; 72.73%) than female (n = 7/19; 36.84%) dogs.

Conclusions: Further studies on CI and *D. immitis* are necessary on a large population of stray and pet small animals along with humans to ascertain their importance on health and zoonotic significance in Bangladesh.

Key words: Viral diseases, Dirofilariasis, Stray dogs, Antigen test kits

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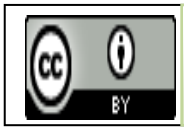

INTRODUCTION

There is no statistics of dog population in Bangladesh, however, the higher population of stray dogs @ 52 dogs / km^2 in Dhaka city and 14 dogs / km^2 in rural Bangladesh with a human to dog ratio 828:1 and 120:1 have been reported, respectively.[1,2] In rural Bangladesh very few people keep dog as a pet animal, but in urban areas pet dog keeping is getting popularity day by day. But still there is a large number of stray dog population compared to lower number of pet dogs in Bangladesh. The Dhaka City Corporation alone kills up to 20,000 stray dogs a year and this stray dogs culling program is mainly limited to the city corporation in the country. Recently, the prevalence of clinical diseases and conditions of pet animals especially dogs have been reported from Bangladesh.[3-10] In addition, sero-prevalence of brucellosis[11]and viral infection[12] in dogs also have been reported from Bangladesh. Review of literature revealed that at least three dozen of zoonotic diseases are acquired from dogs worldwide, but the reports on the prevalence of zoonotic diseases acquired from dogs are very limited from Bangladesh.[6,13,14] Moreover, the sero-prevalence of some important canine diseases has been reported elsewhere [15,16] but such reports are limited in Bangladesh.[11,12] This paper describes the prevalence of some viral diseases and heartworm infection in stray dogs in Bangladesh.

MATERIALS AND METHODS

Recently, RapiGEN Inc. (Korea) has developed a number of immuno-chromatographic assay kits for the qualitative detection of antigens of some important diseases of canine species, especially Canine distemper (CD), Canine Adenovirus-1 (CAV-1), CAV-2, Canine influenza (CI), Heartworm disease (HWD) test kits for commercial purposes. Accordingly, the kit manufacturer has provided some free test kits for trial study, which were utilized in this study.

These RapiGEN test kits were evaluated in the samples collected from 30 street dogs which were caught with the help of Municipal Corporation of Mymensingh district, Bangladesh during the period from January to June 2010. These stray dogs are usually caught at certain interval for euthanasia in the national rabies control program. Accordingly, 30 such stray dogs were controlled by the locally prepared mechanical iron device and randomly selected for collection of samples. Before collection of samples, the age, sex and any abnormalities were recorded from each of the selected dogs.

Collection of blood

Blood was collected directly from the left ventricle of the heart of each of the 30 selected dogs with the help of 10 ml sterile disposable syringe and needle. The collected blood was processed as conventional methods and used as whole blood and plasma.

Collection of ocular and nasal samples

The kit manufacturer provided sticks was inserted in the nose and eye mucosa (conjunctiva) individually in each of 30 selected controlled dogs for collection of swabs. Sample (fluid) was collected with swab, which then inserted into the assay diluents (extraction buffer bottle). Snap off the handling portion of swab stick was done, and then the cap was tightly closed. The extraction buffer bottle containing swab agitation was done for 10 seconds to assure good sample extraction.

The specimens were tested immediately after collection of the samples.

The whole blood was used for detection of Canine heartworm antigen, eyes and nasal swab samples for CAV-1 and CAV-2, plasma for CD and nasal fluid for Canine influenza antigens.

Principle of the tests

These antigen detection test device is an immuno-chromatographic assay for the qualitative detection of antigen in canine ocular, respiratory secretion and blood. The test device has a letter of 'T' and 'C' as test line and control line on the surface of the card. Both the test line and control line in result window is not visible applying any samples. The specially selected disease antibodies are used in test band as both capture and detector materials. These enable the device to identify specific disease antigen in canine ocular, nasal secretions and blood samples.

Test procedure

Test procedure of each of the disease tested was followed as manufacturer instruction. In brief, all the kit components and specimen was allowed to reach at room temperature prior to testing. Then the test device was recovered from the foil pouch prior to use. The test device was then placed horizontally and then three drops of test specimen were added into 'S' hole and then the test results was interpreted in 5 to 10 minutes.

Interpretation of the result

The presence of only one band within the result window indicates a negative result. The presence of two color bands ('T' and 'C') within the result window, no matter which band appears first indicates a positive result. If the control band is not visible within the result window, the result is considered invalid.

Statistical analysis

Statistical significance of the difference in prevalence of sex and age groups was examined by χ^2 test.

RESULTS AND DISCUSSION

The results on the prevalence of viral diseases and *Dirofilaria immitis* infection detected by using antigen test kits in 30 stray dogs are presented in Table 1. The use of CD antigen test kit and CAV types 1 and 2 antigen test kits failed to detect any CD and CAV infections in the tested dog samples, whereas an overall 13.33% and 50.0% dogs had canine influenza and *Dirofilaria immitis* infection (Table 1).

Canine adenoviruses

Adenoviruses which are infect a wide variety of mammals and birds. Two types of adenoviruses have been identified in the dogs: Canine adenovirus type 1 (CAV-1) which infects mainly liver causing Infectious canine hepatitis (ICH), and CAV-2 which causes respiratory and enteric diseases. All the oculo-nasal swabs collected from 30 stray dogs showed negative results with CAV-1 and CAV-2 antigen test kits (Table 1). Thus, the CAV infections remain unreported in dog population from Bangladesh.

Table 1. Age and sex-wise prevalence of certain viral diseases and *Dirofilaria immitis* infection in stray dogs detected by commercial antigen test kits

SNDiseases	Gender	Growing (n = 3)		Adult (n = 27)		Total (n = 30)	
		No. tested	Positive No. (%)	No. tested	Positive No. (%)	No. tested	Positive No. (%)
1. Dirofilariasis (Heartworm)	Male	1	0	10	08 (80.00)	11	08 (72.73)*
	Female	2	0	17	07 (41.18)	19	07 (36.84)
	Total	3	0	27	15 (55.56)*	30	15 (50.00)
2. Canine influenza	Male	1	1 (100)	10	01 (10.00)	11	02 (18.18)
	Female	2	1 (50.00)	17	01 (05.88)	19	02 (10.53)
	Total	3	2 (66.67)*	27	02 (07.41)	30	04 (13.33)
3. Canine adenovirus Type 1 (ICH)	Male	1	0	10	00	11	00
	Female	2	0	17	00	19	00
	Total	3	0	27	00	30	00
4. Canine adenovirus Type 2 (CAV-2)	Male	1	0	10	00	11	00
	Female	2	0	17	00	19	00
	Total	3	0	27	00	30	00
5. Canine distemper (CD)	Male	1	0	10	00	11	00
	Female	2	0	17	00	19	00
	Total	3	0	19	00	30	00
6. CD + CAV-1	Male	1	0	10	00	11	00
	Female	2	0	17	00	19	00
	Total	3	0	27	00	30	00

n = No. of dogs *Significantly ($p < 0.05$)

Canine distemper

Canine distemper (CD), also known as the 'hard-pad disease' is a viral disease of dogs that attacks the respiratory, gastro-intestinal and the nervous systems. Canine distemper virus (CDV), is a member of the genus *Morbillivirus* in the family *Paramyxoviridae* and is closely related to viruses that cause measles, rinderpest and distemper in other animals.[17] This disease is now recognized as a worldwide problem in carnivores and has the second highest fatality rate of any infectious disease, after rabies in dogs.[18] All the plasma samples collected from 30 stray dogs showed negative results with CD virus antigen test kit (**Table 1**). However, 6.52% prevalence of CD in dogs of Dhaka city,[6] 5.67% in dogs of Sylhet district,[3] 1.61% clinical prevalence of CD in pet dogs in Dhaka city[14] and 40% (n=2/5) in golden jackals in Mymensingh[19] have been reported from Bangladesh. Moreover, the 17.52% sero-prevalence of CD in stray dogs has been reported from Iran[20] and 9.03% in Turkey.[21] Negative results obtained on the prevalence of CD may be due to small population of stray dogs tested.

Canine influenza

Canine influenza (CI), commonly referred to as 'dog flu', is caused by varieties of the Influenza A virus capable of creating influenza in dogs and other carnivores. Among these varieties, the equine influenza virus (EIV) H3N8 is generally considered to be the most important one. It is strongly suggested that CI originated from H3N8 EIV, the predominant EIV strain in horses in North America.[22,23] Outbreaks of CI were reported at racetracks in several American states in 2004 and 2005.[24] The virus is believed to have spread from horses to Greyhounds, since both animals completed on the same tracks. This was the first time in history that influenza A could be scientifically proven as causing influenza in dogs. Then an outbreak of disease in quarry hounds in the UK in 2002 was subsequently identified as being caused by CIV,[25] followed by 37.5% prevalence of EIV in foxhounds.[26] The 0.4% sero-prevalence of CIV in dogs in Ontario showed that the only seropositive dog (1/125) was a greyhound that originated in Florida.[27] By analyzing the genome of the CIV, scientists have confirmed that H3N8 was transmitted from horse to dogs and then adapted to dogs through point mutations in the genes. Of the 30 stray dogs tested, 4(13.33%) had CIV infection (**Table 1**). This result suggests that CIV infection is currently prevalent in stray dog population in Bangladesh. However, the horse population is very limited in Bangladesh, duration of persistence of canine influenza in the host is unknown, moreover a recently introduced diagnostic test kit has used, thus, conclusion can not be made on the detection of CIV infection by using only antigen detection test in stray dogs in Bangladesh.

Heartworm disease

Heartworm disease (HWD) is also called dirofilariasis, caused by nematode parasite, *Dirofilaria immitis*, reported from many parts of the world[28-33] including Bangladesh.[19,34] However, the geographical distribution of HWD is associated with prevalent of intermediate host, mosquitoes. Adult parasites are found mainly in the right chamber of the heart and pulmonary artery. The female worms produce small, vermiform embryos called microfilaria. They can cross the capillary beds and so are found throughout the vascular circulation. Veterinarians rely on rapid in-clinic antigen tests to screen the heartworm infection in blood[35] A number of different types of commercial heartworm antigen test kits are widely used to detect heartworm infection in dogs elsewhere.[31,35,36-41] but it remains unpracticed in Bangladesh. The HW antigen test kit was used to detect HW infection in whole blood of 30 randomly selected stray dogs, both sexes (11 male and 19 female) and growing (n = 3) and adult (n = 27), according to the manufacturer's instructions.

Out of 30 dogs tested with heartworm antigen detecting test kit, 50.0% (n = 15/30) animals had *D. immitis* infection (Table 1). Significantly ($p < 0.05$) higher prevalence of *D. immitis* was recorded in male (72.73%) than female (36.84%), and in growing (0/3; 0.0%) than adult (55.56%) dogs (Table 1). Lower prevalence (9.09%) of heartworm infection in cats[34] but higher prevalence of 40% (2/5) in golden jackals based on necropsy examination has been reported from Bangladesh.[19] This study recorded high prevalence (50.0%) of heartworm infection in

stray dogs by using antigen detecting test kit and this result support the earlier report[35] who reported 50% prevalence of HWD in dogs in USA. However, lower prevalence of 13.4% from Taiwan,[31] 2.0% from Brazil,[36] 1.52% from Istanbul, Turkey,[38] 18.2% from Dominican Republic,[37] 26.0% from Hatay province, Turkey,[40] 12.8% from five Turkish province,[39] 30.4% from Spain,[42] 13.5% from Albania[41] have been reported in dogs. This high prevalence of *D. immitis* infection in stray dogs in Bangladesh is correlated with the widely prevalent mosquito vector in the environment. However, this high prevalence of HWD in stray dogs could be further evaluated by simultaneous detection of heartworm infection on necropsy examination and comparative efficacy of different commercial heartworm antigen test kits.[35,43]

This study recorded significantly ($p < 0.05$) higher prevalence of heartworm infection in adult (55.56%) than growing (0.0%), and male (72.73%) than female (36.84%) dogs (Table 1). This result on age-wise prevalence of heartworm infection support the earlier observations[29,31,39,40,44] who reported age as an important risk factor for this disease and accordingly, older dogs have a higher prevalence of heartworm infection than growing dogs. However, in some reports age did not appear to affect prevalence of heartworm infection.[38,41,45,46] The increase prevalence of heartworm infection in older dogs can be resulted in mainly due to inadequate age resistance in growing dogs and the older dogs has a longer exposure time to the mosquito bites.[38,47]

The higher prevalence of heartworm infection in male (72.72%) than female (36.84%) recorded in this study support the earlier finding of Selbey *et al*.[48] who reported that male dogs had the highest relative risk for heartworm infection than female dogs. However, some workers did not find any significant ($p > 0.05$) difference in seroprevalence between male and female dogs.[31,38-41]

The hot-humid weather of Bangladesh is highly suitable for the breeding of mosquitoes, and virtually inactive national mosquito control program causes re-emerged the prevalence of malaria, dengue fever and other mosquito borne diseases like D. immitis infection. As the dirofilariasis is a recognized mosquito borne zoonotic disease, there is a need to explore the disease prevalence simultaneously in small animals and humans.

In conclusion, the high prevalence of heartworm infection (50.0%) and canine influenza (13.33%) in a small population of stray dogs draws attention for further study in large population to prevent the threat to pet animals and public health.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES

01. Hossain M, Ahmed K, Marma ASP, Hossain S, Ali MA, Shamsuzzaman AKM and Nishizono A (2013). A survey of the dog population in rural Bangladesh. *Preventive Veterinary Medicine* 111: 134-138
02. Tenzin T, Ahmed R, Debnath NC, Ahmed G, and Yamage M (2015). Free-roaming dog population estimation and status of the dog population management and rabies control program in Dhaka city, Bangladesh. *PLoS Negl Trop Dis*. 9 (5): e0003784. [Doi:10.137/journal.pntd. 0003784]
03. Singh SK, Islam MR and Hasan MT (2011). The prevalence of clinical diseases in dogs of Sylhet Sadar, Bangladesh. *Journal of Pure and Applied Science & Technology* 5: 41-45
04. Das S, Alim MA, Sikder S, Gupta AD and Masuduzzaman M (2012). Prevalence and warm load of enteric helminthiasis in stray dogs in Chittagong Metropolitan, Bangladesh. *YYU Veteriner Fakultesi Dergisi* 23: 141-145
05. Talukder MH, Matsuu A, Iguchi A, Roy BC, Nishii N and Hikasa Y (2012). PCR-based survey of vector-borne pathogens in dogs in Dhaka, Bangladesh. *Journal of the Bangladesh Agricultural University* 10: 249-253
06. Hossain SSMR and Kayesh MEH (2014). Common diseases of pet animals in Dhaka city and their zoonotic importance. *International Journal of Natural and Social Science* 1: 81-84
07. Mahmud MAA, Belal SMSH and Uddin MJ (2014). Prevalence of protozoan diseases in pet dogs at district veterinary hospital, Sirajgonj, Bangladesh. *Bangladesh Journal of Veterinary Medicine* 12: 191-196
08. Parvez MA, Prodhan MAM, Das BC and Khatun R (2014). Prevalence of clinical conditions in dogs and cats at Teaching Veterinary Hospital (TV) in Chittagong Veterinary and Animal Sciences University, Bangladesh. *Research Journal of Veterinary Practice* 2: 99-104
09. Sarker MS, Ahaduzzaman M, Kabir MN, Rahman MK, Hossain F, Nath SK and Bupasha ZB (2015). Prevalence of clinical conditions in dogs and cats at Central Veterinary Hospital (CVH) in Dhaka, Bangladesh. *Van Veterinary Journal* 26 (2): 101-105
10. Habiba U, Islam R and Hossain M (2016). The emerging disease occurrence of pet animals in Bangladesh. *Journal of Advanced Veterinary and Animal Research* 3: 413-419
11. Talukder BC, Samad MA and Rahman AKMA (2011). Comparative evaluation of commercial serodiagnostic tests for the seroprevalence study of brucellosis in stray dogs in Bangladesh. *Bangladesh Journal of Veterinary Medicine* 9: 79-83
12. Sen S, Rahman MS, Nag M, Rahman MM, Sarker RR and Kabir SML (2016). Prevalence of canine parvovirus and canine influenza virus infection in dogs in Dhaka, Mymensingh, Feni and Chittagong districts of Bangladesh. *Asian Journal of Medicine and Biological Science* 2: 138-142
13. Samad, MA (2000). An overview of livestock research reports published during the twentieth century in Bangladesh. *Bangladesh Veterinary Journal* 34: 53-149
14. Tarafder, M and Samad, MA (2010). Prevalence of clinical diseases of pet dogs and risk perception of zoonotic infection by dog owners in Bangladesh. *Bangladesh Journal of Veterinary Medicine* 8: 163-174
15. Laurenson K, van Heerden J, Stander P and van Vuuren MJ (1997). Sero-epidemiological survey of sympatric domestic and wild dogs (*Lycaon pictus*) in Tsumkwe district, north-eastern Namibia. *Onderstepoort Journal of Veterinary Research* 64: 313-316
16. Levy JK, Crowford PC, Lappin MR, Dubovi EJ, Levy MG, Allenman R, Tucker SJ and Clifford EL (2008). Infectious diseases of dogs and cats on Isabella Island, Galapagos. *Journal of Veterinary Internal Medicine* 22: 60-65

17. Neumeister C, Nanan R, Cornu TI, Luder CGK, Meulen VT, Naim H and Niewiesk S (2001). Measles virus and target proteins into a TAP-independent MHC class 1-restricted antigen-processing pathway. *Journal of General Virology* 82: 441-444
18. Deem SL, Spelman LH, Yates RA and Montali RJ (2000). Canine distemper in terrestrial carnivores: A review. *Journal Zoology and Wildlife Medicine* 31: 441-451
19. Yousuf MA, Bashu J, Pervin M, Islam MT, Das PM and Khan MAHNA (2014). Identifying diseases of golden jackals of Bangladesh Agricultural University campus, Mymensingh, Bangladesh. *Bangladesh Journal of Veterinary Medicine* 12: 217-224
20. Avizeh R, Shapouri MRS and Akhlaghi N (2007). Antibody titers against Canine distemper virus in unvaccinated rural dogs from Ahvaz, Iran. *Pakistan Journal of Biological Sciences* 10: 3970-3972
21. Gencay A, Oncel T, Karaoglu T, Sancak AA, Demir AB and Ozkul A (2004). Antobody prevalence to canine distemper virus (CDV) in stray dogs in Turkey. *Revue Medicine Veterinary* 155: 432-434
22. Lai A, Chambers T, Holland RE, Morley PS, Haines DM, Townsend HG and Barrandequy M (2001). Diverged evolution of recent equine-2 influenza (H3N8) viruses in the Western Hemisphere. *Archieve Virology* 146: 1063-1074
23. Mumford E, Traub-Dargatz J, Carman J, Callan RJ, Collins JK, Goltz KL, Romn SR, Tarr SF and Salmon MD (2003). Occurrence of infectious upper respiratory tract disease and response to vaccination in horses on six sentinel premises in northern Colorado. *Equine Veterinary Journal* 35: 72-77
24. Crawford PC, Dubovi EJ, Castleman WL, Stepheson I, Gibbs EPJ, Chen L, Smith C, Hill RC, Ferro P, Pompey J, Bright RA, Medina MJ, Johnson CM, Olsen CW, Cox NJ, Klimov AL, Katz JM and Donis RO (2005). Transmission of equine influenza virus to dogs. *Science* 310: 482-485
25. Smith KC, Daly JM, Blunden AS and Laurence CJ (2005). Canine influenza virus (letter). *Veterinary Record* 157: 599
26. Newton R, Cooke A, Elton D, Bryant N, Rash A, Bowman S, Blunden T, Miller J, Hammond TA, Canon I and Day M (2007). Canine influenza virus: Cross-species transmission from horses (letter). *Veterinary Record* 161: 142-143
27. Kruth SA, Carman S and Weese JS (2008). Seroprevalence of antibodies to canine influenza virus in dogs in Ontario. *Canadian Veterinary Journal* 49: 800-802
28. Bidgood, A and Collins, GH (1996). The prevalence of *Dirofilaria immitis* in dogs in Sydney. *Australian Veterinary Journal* 73: 103-104
29. Montoya JA, Morales M, Ferrer O, Molina JM and Corbera JA (1998). The prevalence of *Dirofilaria immitis* in Gran Canaria, Canary Islands, Spain (1994-1996). *Veterinary Parasitology* 75: 221-226
30. Cringoli, G; Rinaldi, L; Veneziano, V and Capelli, G (2001). A prevalence survey and risk analysis of filariosis in dogs from the Mt. Vesuvius area of Southern Italy. *Veterinary Parasitology* 102: 243-252
31. Fan CK, Su KE, Lin YH, Liao CW, Du WY and Chiou HY (2001). Seroepidemiologic survey of *Dirofilaria immitis* infection among domestic dogs in Taipei City and mountain aboriginal districts in Taiwan (1998-1999). *Veterinary Parasitology* 102: 113-120
32. Araujo RT, Marcondes CB, Bastos LC and Sartor DC (2003). Canine dirofilariasis in the region of Conceicao Lagoon, Florianopolis and in the Military Police Kennel, Sao Jose, State of Santa Catarina, Brazil. *Veterinary Parasitology* 113: 239-242

33. Song KH, Lee SE, Hayasaki M, Shiramizu K, Kim DH and Cho KW (2003). Seroprevalence of canine dirofilariasis in South Korea. *Veterinary Parasitology* 114: 231-236
34. Samad MA and Rahman HR (1998). Investigation of endoparasites infection in stray cats in Bangladesh. *Bangladesh Veterinary Journal* 32: 142-151
35. Lee AC, Bowman DD, Lucio-Forster A, Beall MJ, Liotta JL and Dillon R (2011). Evaluation of a new in-clinic method for the detection of canine heartworm antigen. *Veterinary Parasitology* 177: 387-391
36. Labarthe N, de Campos PM, Barbarini O, McKee W, Coimbra CA and Hoskins J (2003). Serologic prevalence of *Dirofilaria immitis*, *Ehrlichia canis*, and *Borrelia burgdorferi* infections in Brazil. *Veterinary Therapeutics* 4: 67-75
37. Duran-Struuck R, Jost C and Hernandez AH (2005). Dirofilaria immitis prevalence in a canine population in the Samana Peninsula (Dominican Republic)- June 2001. *Veterinary Parasitology* 133: 323-327
38. Oncel T and Vural G (2005). Seroprevalence of *Dirofilaria immitis* in stray dogs in Istanbul and Izmir. *Turkish Journal of Veterinary and Animal Sciences* 29: 785-789
39. Simsek S, Utuk AE, Koroglu E and Rishniw M (2008). Serological and molecular studies on *Dirofilaria immitis* in dogs from Turkey. *Journal of Helminthology* 82: 181-186
40. Yaman M, Guzel M, Koltas IS, Demirkazik M and Aktas H (2009). Prevalence of *Dirofilaria immitis* in dogs from Hatay province, Turkey. *Journal of Helminthology* 83: 255-260
41. Rapti D and Rehbein S (2010). Seroprevalence of canine heartworm (*Dirofilaria immitis*) infection in Albania. *Parasitological Research* 107: 481-485
42. Montoya JA, Mellado Y, Cabrera-Pedrero ED, Morchon R and Simon F (2010). Canine dirofilariosis caused by *Dirofilaria immitis* is a risk factor for the human population on the island of Gran Canaria, Canary Islands, Spain. *Parasitological Research* 107: 1265-1269
43. Atkins CE (2003). Comparison of results of three commercial heartworm antigen test kits in dogs with low heartworm burdens. *Journal of the American Veterinary Medical Association*. 222: 1221-1223
44. Graham JM (1974). Canine filariasis in North Eastern Kansas. *Journal of Parasitology* 60: 322-326
45. Thrasher JP, Gould KG, Lynch MJ and Harris CC (1968). Filarial infections of dogs in Atlanta, Georgia. *Journal of the American Veterinary Medical Association* 153: 1059-1062
46. Martin TE and Collins GH (1985). Prevalence of *Dirofilaria immitis* and *Dipetalonema reconditum* in greyhounds. *Australian Veterinary Journal* 62: 159-163
47. Theis JH, Stvens F, Theodoropoulos G and Ziedins AC (1999). Studies on the prevalence and distribution of filariasis in dogs from Los Angeles County, California (1996-1998). *Canine Practice* 24: 8-16
48. Selbey LA, Corwin RM and Hayes HM (1980). Risk factors associated with canine heartworm infection. *Journal of the American Veterinary Medical Association* 176: 33-35