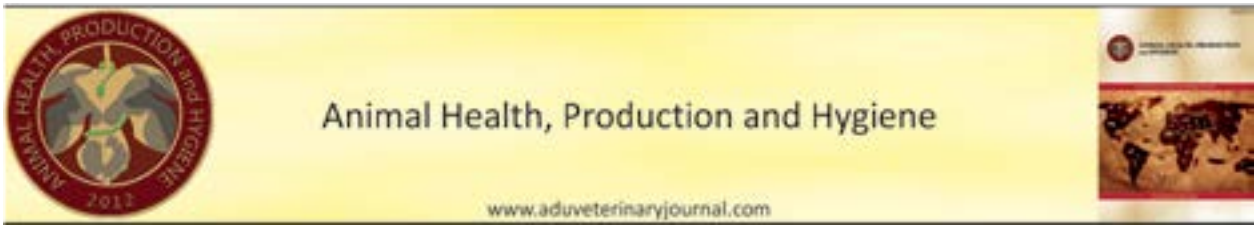
Animal Health Prod and Hyg (2020) 9(2) : 711 - 715



Research Article

Prevalence of *Dirofilaria immitis* infection in dogs from Aydin and Izmir Provinces, Turkey

Hakan Sarali[1], Huseyin Bilgin Bilgic[2], Serkan Bakirci[2], Tulin Karagenc[2*]

[1] *Gıda Tarım Ve Hayvancılık Bakanlığı Koçarlı İlçe Müdürlüğü, Orta Orta Mah., İncirliova Cd. No:13, 09970 Koçarlı/Aydın, Turkey,*
[2] *Department of Parasitology, Faculty of Veterinary Medicine, Aydın Adnan Menderes University, Batı Kampüsü, 09016 Isıklı/Aydın, Turkey*

A B S T R A C T

Mosquito-borne nematodes, *Dirofilaria immitis* and *D. repens* are among the most important filarial parasites of dogs. *D. immitis* is the causal agent of heartworm that primarily infects domestic and wild canids. Rarely, humans become accidental hosts to this parasite. *D. repens*, which is the main agent of human dirofilariosis, usually causes a non-pathogenic, subcutaneous infection in dogs. The aim of the present study was to investigate the prevalence of *Dirofilaria* spp. in the owned dogs in Aydin and Izmir regions using four different diagnostic techniques. To this end, blood samples were collected from 150 dogs in Aydin (n: 122) and Izmir (n: 28) provinces. The presence of the microfilariae of the parasite in the blood samples were examined by wet blood film method, modified Knott's test, membrane filtration-acid phosphatase staining techniques and polymerase chain reaction (PCR). A commercial antigen test kit (HESKA Solo Step CH Batch Test Strips) was also used to detect adult *D. immitis* antigens in serum samples. The overall prevalence of *D. immitis* was 10,0% as determined by both PCR and the antigen test kit. While the presence of the parasite determined by the modified Knott's and membrane filtration tests was 4,67%, only four animals were determined to be positive by direct microscopic examination (2,67%). All positive samples were detected in Aydin, majority in the Germencik district. The prevalence of *D. immitis* was higher in male dogs (11,93%) when compared to female dogs (4,87%). In conclusion, the present study demonstrates that canine dirofilariosis is prevalent in Aydın province (10,0%) of Turkey, even though the positivity is confined to a small area. Reliable epidemiological data is crucial to implement preventive control measures in order to decrease the incidence of canine dirofilariosis, which should be considered under the "One Health" concept in endemic areas.
Keywords: Dirofilaria immitis, prevalence, dog, diagnosis, epidemiology

Türkiye de Aydın ve izmir bölgelerindeki köpeklerde Dirofilaria immitis enfeksiyonunun prevalansı

ÖZET

Sivrisinek kaynaklı nematodlar, *Dirofilaria immitis* ve *D. repens* köpeklerin en önemli filarial parazitleri arasındadır. *D. immitis*, başlıca evcil ve yabani köpekleri enfekte eden kalp kurdunun nedensel ajanıdır. Nadiren, insanlar da bu parazite tesadüfi konaklık yapmaktadır. İnsan dirofilaryozunun asıl etkeni olan *D. repens*, köpeklerde genellikle patojenik olmayan, deri altı enfeksiyonunlara neden olur. Bu çalışmanın amacı, Aydın ve İzmir illerinde bulunan sahipli köpeklerde *Dirofilaria* spp.'nin prevalansını dört farklı tanı tekniği kullanarak araştırmaktır. Bu amaçla Aydın (n: 122) ve İzmir (n: 28) illerinde 150 köpekten kan örnekleri alınmıştır. Kan örneklerinde parazitin mikrofilaryalarının varlığı direkt kan muayene yöntemi, modifiye Knott testi, membran filtrasyon-asit fosfataz boyama tekniği ve polimeraz zincir reaksiyonu (PCR) ile incelenmiştir. Serum örneklerinde yetişkin D. immitis antijenlerini saptamak için ticari bir antijen test kiti (HESKA Solo Step CH Batch Test Strips) kullanılmıştır. Serum örneklerinde yetişkin D. immitis antijenlerini saptamak için ticari bir antijen test kiti (HESKA Solo Step CH Batch Test Strips) kullanılmıştır. Toplamda, *D. immitis'*in prevalansı hem PCR hem de antijen test kiti ile % 10,0 olarak belirlenmiştir. Modifiye Knott's ve membran filtrasyon testleri ile belirlenen parazit varlığı %4,67 iken, direkt mikroskobik inceleme ile sadece dört hayvan pozitif olduğu belirlenmiştir (% 2,67). Tüm pozitif örnekler Aydın'da, çoğunluğu Germencik ilçesinde tespit edilmiştir. Erkek köpeklerde (% 11,93) dişi köpeklere (% 4,87) göre *D. immitis* prevalansı daha yüksek olarak tespit edilmiştir. Sonuç olarak, bu çalışma, pozitifliğin küçük bir alanla sınırlı olmasına rağmen, Türkiye'nin Aydın ilinde (% 10,0) köpek dirofilaryozunun yaygın olduğunu göstermektedir. Bu tür çalışmalar, endemik bölgelerde "Tek Sağlık" kavramı altında ele alınması gereken köpek dirofilariozis insidansını azaltmak için önleyici kontrol tedbirlerinin uygulanmasında güvenilir epidemiyolojik verilerin ne kadar önemli olduğunu ortaya koymaktadır.
Anahtar Kelimeler: Dirofilaria immitis, prevalans, köpek, tanı, epidemiyoloji

Corresponding author: Tulin Karagenc Aydın Adnan Menderes University Faculty of Veterinary, Department of Parasitology, Isıklı, Aydin, Turkey. E-mail: tkaragenc@adu.edu.tr

Introduction

Dirofilaria immitis and *Dirofilaria repens* are among the most important filarial nematodes of dogs, transmitted by mosquito bites. *D. immitis* is the causative agent of the heartworm disease in dogs as well as in other carnivores. Adult *D. immitis* occupies mainly the right ventricle and pulmonary arteries of in carnivores, resulting in severe consequences like heart failure, pulmoner odeamo *etc*. In contrast, *D. repens* is usually non-pathogenic, and causes subcutaneous infection in dogs. Microfilariae of these parasites circulating in blood are ingested by several species of competent mosquito vectors during their blood-feeding (Otranto et al., 2015). While *D. immitis* rarely infects humans, *D. repens* is the main agent of dirofilariosis in humans, considered to be a public health concern (Pampiglione and Rivasi, 2000).

Dirofilaria immitis occurs mainly in tropical, sub-tropical as well as temperate zones (Martin and Collins, 1985; Cringoli et al., 2001). However, *D. repens* is present only in Old World (Pampiglione and Rivasi, 2000). Nevertheless, it was indicated that *D. repens* has been spreading to northern Europe from the endemic areas of southern Europe more rapidly than *D. immitis* probably due to the climate change (Capelli et al., 2018).

It is established that *D. immitis* and *D. repens* are present in different parts of Turkey, where *D. immitis* is more common than *D. repens* (Doğanay, 1983; Tasan, 1984; Ağaoglu et al., 2000; Oge et al., 2003; Yildirim et al., 2007). Early reports were based primarily on the detection of adult worm during postmortem examination (Doğanay, 1983; Tasan, 1984) or microfilariae detection by whole blood examination. There has been a steady increase in the number of epidemiological studies during the last two decades using various molecular and serological diagnostic tests, as well as conventional tests such as whole blood wet smear and modified Knott's tests (Yildirim et al., 2007; Yaman et al., 2009; Güven et al., 2017) . It should be pointed out that each diagnostic test has its own advantages and disadvantages. For example, the diagnostic test based on detection of microfilariae could give false negative results for amicrofilaremic dogs with occult infection of dirofilariosis.

We aimed in the present study to determine the prevalence of *Dirofilaria* spp. in dogs in Aydin and Izmir provinces located in the West Aegean Region of Turkey using conventional, immunological, and molecular diagnostic techniques at the same time. The diagnostic sensitivity of these tests was also compared.

Materials and Methods

Study areas and sample collection

The present study was conducted within provinces located in Aydin and Izmir, both located in the West Aegean region of Turkey. Randomly selected 150 dogs of different breeds, ages and sexes were examined in Aydin and Izmir provinces (Table 1). Whole blood samples (EDTA-treated) and serum samples were collected from owned and stray dogs, as well as from dogs admitted to the veterinary clinics in Aydin between August 2007-2009. The presence of *Dirofilaria immitis* was examined using various conventional, immunological, and molecular diagnostic techniques.

Parasitological investigation

Two conventional tests, namely wet blood film method and modified Knott's concentration technique, were used to detect the presence of microfilarial of the parasite as described by (Hendrix, 1998), subsequently followed by microfilaria identification by (Soulsby, 1982). The circulating microfilariae in the whole blood was also examined by the polycarbonate membrane filtration (Millipore, TMTP 02500) technique (Acevedo et al., 1981; Yildirim et al., 2007), combined with acid phosphates staining methods for the identification of microfilariae using a commercially available kit (Leucognost-SP, Merck, Germany) as described by the manufacturer.

In order to detect adult *D. immitis*, the presence of antigen in the serum samples were examined immunologically using an antigen kit (HESKA Solo Step CH Batch Test Strips, Colorado, USA) as described by the manufacturer. *D. immitis*-specific PCR was also used in the present study to determine the presence of the microfilariae in the blood samples as described below.

DNA extraction and PCR assays

DNA was isolated from EDTA-treated blood (300 ml) using the Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, USA) as described by the manufacturer. DNA samples were stored at −20°C until used in PCR.

A primer set targeting a 302 bp fragment of the ITS2 (internal transcribed spacer region 2) gene of the ribosomal DNA of *D. immitis*. Following primers described by (Rishniw et al., 2006) were used: D.imm-F1 (5'-CATCAGGTGATGATGTGATGAT-3') and D.imm-R1a (5'-TTGATTGGATTTTAACGTATCATTT-3').

For the detection of *Dirofilaria repens* by PCR, primers targeting a 348 bp fragment of the 5S SSU rRNA of D. repens (D.rep-F1 (5'-TGTTTCGGCCTAGTGTTTCGACCA-3') and D.rep-R1 (5'-ACGAGATGTCGTGCTTTCAACGTG-3') were used as described in (Favia et al., 2000) .

The PCR assays for *D. immitis* and *D. repens* were performed

Table 1. Overall heartworm prevalence in studied areas

Research area	Diagnostic techniques used						
	Sample No	Wet smear	Modified Knott's test	Membran Filtration	PCR	Serological Kit	Total
Aydın/Merkez	56	-	1	-	1	1	1
Aydın/Germencik	19	4	6	6	12[a]	12	12
Aydın/Karpuzlu	29	-	-	-	-	-	-
Aydın/ Clinics	18	-	-	-	2	2	2
Izmir	28	-	-	-	-	-	-
Total	150	4[b]	7[b]	6[b]	15[a]	15[a]	15
				χ^2	74,890		
				P	0,000		

χ^2Pearson's chi-square test.

[a,b] The different letters within the same column indicate significant difference among groups.

Table 2. The prevalence of *D. immitis* correlated with age and sex

	Examined dogs No	Infected dogs No	%	χ^2	*P*
Sex					
Female	41	2[a]	4,87	1,645	0,200
Male	109	13[a]	11,93		
Age (year)					
0,5-3	81	8[a]	9,88	0,043	0,979
4-6	47	5[a]	10,64		
≥ 7	22	2[a]	9,09		

χ^2Pearson's chi-square test.
[a,b] The different letters within the same column indicate significant difference among groups.

in a final volume of 25 µl consisting of 1× buffer (Promega, Madison, WI, USA), 2 mM $MgCl_2$ (Promega, Madison, WI, USA), 200 µm of each deoxynucleotide triphosphate (Promega, Madison, WI, USA), 25 pmol of each primer, 1.25 U of hot start polymerase (hot-start *Taq* polymerase (ThermoFisher Scientific, USA) and 2.0 µl template DNA. The reactions were performed in an automated DNA thermal cycler (Techne™ TC-512 Gradient Thermal Cycler, UK). Reactions consisted of an initial 5 min denaturation step at 95°C, followed by 32 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s. Final extension was performed at 72°C for 7 min, followed by a hold step at 4°C. Amplified DNA was subjected to electrophoresis in a 1.5% agarose gel, pre-stained with ethidium-bromide (10 µl/ml), in TAE buffer at 100 V and bands were visualized under UV light.

Statistical analysis

Pearson's chi-square (χ^2) test was used to compare the prevalence of *D. immitis* among district, sex, age. The sensitivity the diagnostic tests were also compared. Statistical analyses were performed using the statistical package SPSS (Version 21.0). A value of $p < 0.05$ was considered significantly different.

Ethical statement

This study was approved by the Animal Ethics Committee of Adnan Menderes University, Aydin, Turkey (Protocol number: B.30.2.ADÜ.0.06.00.00/124-HEK/2007/022).

Results

Several different diagnostic tests were used to determine the prevalence of the infected dogs in Aydin and İzmir regions (Table 1). As expected, different diagnostic techniques produced different results. The highest prevalence of the parasite was % 10,0 (15 /150 samples) as detected by both PCR and the antigen test kit (Table 1). The presence of the parasite determined by the modified Knott's test and membrane filtration test was 4,67% (7/150) and 4,0% (6/150), respectively. The lowest number of positive animals was detected by wet blood film method (2,67%; 4/150). The Figure 1 shows microfilariae detected either by wet blood, the filtration and/or the modified Knott's test and acid phosphatase staining. The staining of the microfilariae by acid phosphatase indicated that all the microfilariae were *D. immitis*, as evidenced by red staining of both excretory pore and anal pore. These results were confirmed by PCR. All samples, determined to be positive by wet blood film method and the modified Knott's test were also determined to be positive for *D. immitis* by PCR. None of the samples were positive for *D. repens.*

None of the samples obtained from İzmir were positive. All the positive dogs were detected in Aydın region. The number of positive dogs differed significantly among the districts in Aydın, the majority of positive samples (12/15 positive samples) originating from Germencik district (Table 1). There was also a marked difference in positivity between the genders of dogs, The prevalence of *D. immitis* was higher in males (11,93%) compared to females (4,87%) (Table 2). However, there was no differences among the age groups of animals.

Discussion

Vector-borne parasitic nematodes, *D. immitis* and *D. repens*, are prevalent worldwide especially in temperate, tropical, and subtropical climatic regions. The intensity and the distribution of dirofilariasis appear to expand to countries that were once considered to be free of the infection due mainly to climatic and ecological changes (Simon et al., 2012; Baneth et al., 2016). Therefore, these parasites pose a growing public health concern considering their effect on dogs and man.

The epidemiological studies conducted in different parts of Turkey have indicated a wide-range of prevalence rate, *viz.* 0-46,2% (Simsek et al., 2011; Tascı and Kilic, 2012; Guven et al., 2017). Several factors other than ecological and geographical differences of the study region on the base of vector population, such as the detection method used, survey periods, sample size, breed of dog population and the type of of infection (patent or occult) might have contributed to the wide-range discrepancies in prevalence rates. The overall prevalence of the parasite was 10% in the present study, which is similar to previous studies conducted in different parts of Turkey (Yildirim et al., 2007; Guven et al., 2017). However, it should be noted that positive samples were obtained only from Aydin, and mainly in the Germencik district, indicating the presence of factors at a local scale.

The prevalence of infection determined by PCR and antigen detection kit was markedly higher than that revealed by microscopic examination, modified Knott's technique as well as filtration methods. This finding is in accordance with previous observations demonstrating that the reliability and reproducibility of the PCR assay is higher than that of microscopic examination (Watts et al., 1999). It should also be noted that identification of microfilariae by microscopic examination is prone to error and could potentially lead to miss-diagnosis of three species of canine filarial parasites, namely *D. immitis, D. repens,* and *Acanthocheilonema reconditum* (Soulsby, 1982). Beside the relatively low sensitivity in comparison to PCR, the acid-phosphatase staining technique is an easy and reliable

method for the identification of microfilaria (Peribanez et al., 2001).

It is well known that PCR might give false negative results especially in cases of occult infection. Nevertheless, we did not come across any false negative results in the present study as all serologically positive samples were also PCR positive indicating the presence of microfilaria in the peripheral blood.

The results obtained in the present study indicated that the proportion of male dogs infected with *D. immitis* was higher than female dogs. However, the difference was not statistically significant. This finding is in agreement with several studies indicating that the prevalence of the parasites is not different between the males and females (Oge et al., 2003; Hou et al., 2011; Wang et al., 2016). Biological basis underlying the higher prevalence in males, as detected in the present study, is not known. However, it is suggested that male dogs encounter the intermediate host, mosquitoes, more often than females, since they are kept outdoors more often for defense (Song et al., 2003). According to Johnson and Harrel (1986), the outdoors provide more favorable environmental conditions for mosquitos. It was also postulated that preferential attraction to the mosquitoes might also play a role leading to higher infection rates in male dogs (Montoya et al., 1998).

There is evidence indicating that the chance of getting infected with *D. immitis* increases as the dog gets older (Montoya et al., 1998). This is most likely due the fact that the longer the life span of a dog higher the likelihood of exposure to mosquitoes infected with *D. immitis* (Rhee et al., 1998). Nevertheless, no significant differences were determined in the present study among the age groups. This finding is in agreement with the hypothesis that the infection risk remains the same throughout life span of the dog (Rhee et al., 1998).

Conclusion

In conclusion, the present study demonstrates that canine dirofilariosis is prevalent in Aydın province (10.0%) of Turkey, even though the positivity is confined to a small area. In the face of global climate change that leads to warmer temperatures supportive of extrinsic development of *D. immitis,* it is important to determine areas of risk and be aware of the spread of the vector into new environments. The availability of sensitive and specific diagnostic tests is the most important prerequisite for epidemiological studies. In this context, the present study highlights the diagnostic value of molecular and serological tests in determining the prevalence of *D. immitis*. Reliable epidemiological data is crucial to implement preventive control measures in order to minimize the incidence of canine dirofilariosis, which should be considered under the "One Health" concept in endemic areas.

Acknowledgements

This article is derived from a Master of Science thesis (VPR-YL-2009-0001) supported by Adnan Menderes University Research Foundation.

Conflict of interest

The authors declare that they have no competing interests.

References

Acevedo, R.A., Ciencias, L., Theis, J.H., Kraus, J.F., & Longhurst, W.M. (1981). Combination of filtration and histochemical stain for detection and differentiation of Dirofilaria immitis and Dipetalonema reconditum in the dog. American Journal of Veterinary Research, 42, 537–540. PMID: 7196718

Agaoglu, Z., Akgul, Y., Ceylan, E., & Akkan, H. (2000). The incidence of Dirofilaria immitis in dogs in Van province. Journal of the Faculty of Veterinary Medicine University of Yüzüncü Yıl 11, 41–43. DOI: 10.1501/Vetfak_0000002586

Baneth, G., Thamsborg, S.M., Otranto, D., Guillot, J., Blaga, R., Deplazes, P., & Solano-Gallego, L. (2016). Major parasitic zoonoses associated with ogds and cats in Europe. Journal of Comparative Pathology, 155(1 Suppl 1):S54-74. DOI: 10.1016/j.jcpa.2015.10.179.

Capelli, G., Genchi, C., Baneth, G., Bourdeau, P., Brianti, E., Cardoso, L., Danesi, P., Fuehrer, H.P., Giannelli, A., Ionică, A.M., Maia, C., Modrý, D., Montarsi, F., Krücken, J., Papadopoulos, E., Petrić, D., Pfeffer, M., Savić, S., Otranto, D., Poppert, S., & Silaghi, C. (2018). Recent advances on Dirofilaria repens in dogs and humans in Europe. Parasites Vectors 11, 663. DOI: 10.1186/s13071-018-3205-x

Cringoli, G., Rinaldi, L., Veneziano, V., Capelli, G., 2001. A prevalence survey and risk analysis of filariosis in dogs from the Mt. Vesivius area of southern Italy. Veterinary Parasitology 102, 243–252. DOI: 10.1016/s0304-4017(01)00529-5

Doganay, A. (1983). Ankara köpeklerinde görülen helmint türleri, bunlarin yayilisi ve halk sagligi yönündenönemi (Prevalence of Helminths in Ankara dogs and their potential public health significance). Ankara Üniversitesi Veteriner Fakültesi Dergisi, 30 (4), 550-561.

Favia, G., Bazzocchi, C., Cancrini, G., Genchi, C., Bandi, C. (2000). Unusual organization of the 5S ribosomal spacer in Dirofilaria repens: absence of a canonical spliced leader 1 sequence. Parasitology Research, 86, 497–499. DOI: 10.1007/s004360050700

Guven, E., Avcioglu, H., Cengiz, S., & Hayirli, A. (2017). Vector-borne pathogens in stray dogs in Northeastern Turkey. Vector-Borne and Zoonotic Diseases,17(8), 610-617. DOI: 10.1089/vbz.2017.2128

Hendrix, C. M. (1998). Diagnostic Veterinary Parasitology (2nd ed.). Mosby, St. Louis, Mo, USA,.

Hou, H., Shen, G., Wu, W., Gong, P., Liu, Q., You, J., Cai, Y., Li, J., & Zhang, X. (2011). Prevalence of Dirofilaria immitis infection in dogs from Dandong, China. Veterinary Parasitology, 29;183(1-2),189-93. DOI: 10.1016/j.vetpar.2011.06.016

Johnson, W.E., & Harrel, L. (1986). Further study on the potential vectors of Dirofilaria in Macon Country, Alabama. Journal of Parasitology, 72, 955–956. PMID: 3819971

Martin, T.E., & Collins, G.H. (1985). Prevalence of Dirofilaria immitis and Dipetalonema reconditum in greyhounds. Australian Veterinary Journal, 62, 159–163. DOI: 10.1111/j.1751-0813.1985.tb07278.x

Montoya, J.A., Morales, M., Ferrer, O., Molina, J.M. & Corbera, J.A. (1998). The prevalence of Dirofilaria immitis in Gran Canaria, Canary Islands, Spain (1994–1996). Veterinary Parasitology, 75, 221–226. DOI: 10.1016/s0304-4017(97)00175-1

Oge, H., Doganay, A., Oge, S., & Yildirim, A. (2003). Prevalence and distribution of Dirofilaria immitis in domestic dogs from Ankara and vicinity in Turkey. Deutsche Tierarztliche Wochenschrift, 110, 69–72. PMID: 12666502

Otranto, D., Cantacessi, C., Dantas-Torres, F., Brianti, E., Pfeffer, M., Genchi C, et al. (2015). The role of wild canids and felids in spreading parasites to dogs and cats in Europe. Part, II: Helminths and arthropods. Veterinary Parasitolology, 213, 24–37. DOI: 10.1016/j.vetpar.2015.04.020

Pampiglione, S., & Rivasi, F. (2000). Human dirofilariasis due to Dirofilaria (Nochtiella) repens: an update of world literature from 1995 to 2000. Parassitologi, 42:231–54. PMID: 8778658

Peribáñez, M.A., Lucientes, J., Arce, S., Morales, M., Castillo, J.A., & Gracia, M.J. (2001). Histochemical differentiation of Dirofilaria immitis, Dirofilaria repens and Acanthocheilonema dracunculoides microfilariae by staining with a commercial kit, Leucognost-SP. Veterinary Parasitology, 102(1-2)173-175. DOI: 10.1016/s0304-4017(01)00516-7

Rhee, J.K., Yang, S.S., & Kim, H.C. (1998). Periodicity exhibited by Dirofilaria immitis microfilariae identified in dogs of Korea. The Korean Journal of Parasitology, 36(4):235-239. doi: 10.3347/kjp.1998.36.4.235. DOI: 10.3347/kjp.1998.36.4.235

Rishniw, M., Barr, S.C., Simpson, K.W., Frongillo, M.F., Franz, M., & Alpizar, J.L.D. (2006). Discrimination between six species of canine microfilariae by a single polymerase chain reaction, Veterinary Parasitology, 135,303-314. DOI: 10.1016/j.vetpar.2005.10.013

Simón, F., Siles-Lucas, M., Morchón, R., González-Miguel, J., Mellado,

I., Carretón, E., et al. (2012). Human and animal dirofilariasis: the emergence of a zoonotic mosaic. Clinical Microbiology Reviews, 25, 507–544. DOI: 10.1128/CMR.00012-12

Simsek, S., Ozkanlar, Y., Balkayaö I., & Aktas, M.S. (2011). Microscopic, serologic and molecular surveys on Dirofilaria immitis in stray dogs, Turkey. Veterinary Parasitology, 183,109–113. DOI: 10.1016/j.vetpar.2011.06.012

Song, K.H., Lee, S.E., Hayasaki, M., Shiramizu, K., Kim, D.H. & Cho, K.W. (2003). Seroprevalence of canine dirofilariosis in South Korea. Veterinary Parasitology, 114, 231–236. DOI: 10.1016/s0304-4017(03)00137-7.

Soulsby, E.J.L. (1982). Helminths, Arthropods and Protozoa of Domesticated Animals. (7th ed) Baillere Tindall, London, 809 pp., ISBN 0-7020-0820-6.

Tasan, E. (1984). Elazig kirsal yöre köpeklerinde helmintlerin yayilisi ve insan sagligi yönünden önemi (The distribution and public health significance of dog helminths in rural districts of Elazig). Doga Bilim Dergisi, 8, 160-167.

Taşci GT, Kilic¸ Y. (2012). Kars ve Iğdır Civarındaki Köpeklerde Dirofilaria immitis (Leidy, 1856)'nin Prevalansı ve Potansiyel Vektör Sivrisinek Türleri Üzerine Arastırmalar. Kafkas Universitesi, Veteriner Fakültesi Dergisi, 18:29–34. DOI: 10.9775/kvfd.2011.5342

Yaman, M., Guzel, M., Koltas, I.S., Demirkazik, M., & Aktas, H. (2009). Prevalence of Dirofilaria immitis in dogs from Hatay province, Turkey. Journal of Helminthology, 83(3), 255-260. DOI: 10.9775/kvfd.2011.5342

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. DOI: 10.1016/j.rvsc.2006.08.006

Wang, S., Zhang, N., Zhang, Z., Wang, D., Yao, Z., Zhang, H., Ma, J., Zheng, B., Ren, H., & Liu S. (2016). Prevalence of Dirofilaria immitis infection in dogs in Henan province, central China. Parasite, 23, 43. DOI: 10.1051/parasite/2016054