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RESEARCH ARTICLE

Seroprevalence of *Dirofilaria immitis, Ehrlichia canis* and *Borrelia burgdorferi* in Dogs in Iğdır Province, Turkey [1]

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Summary

In this study, by using a Snap3dx test kit, 100 dogs sera were examined. *Dirofilaria immitis* infection was detected in 40 dogs (40%) and *Ehrlichia canis* antibodies were present in just 1 dog (1%), *Borrelia burgdorferi* antibodies were not detected in the test. Twenty-two of dogs (22%) are infested with ticks. A total of 42 ticks, 9 of female and 33 of male, were collected from dogs. Ticks collected from dogs were *R. sanguineus* 76.2% (32/42) and 23.8% of them (10/42) were *Rhipicephalus spp.* Considering the prevalence of potential vectors (mosquitoes and ticks), it is concluded that dirofilariosis and ehrlichiosis cases are often encountered in Iğdır province.

Keywords: *Dirofilaria immitis, Ehrlichia canis, Borrelia burgdorferi, Dog, Iğdır*

Iğdır Yöresinde Köpeklerde *Dirofilaria immitis, Ehrlichia canis* ve *Borrelia burgdorferi*'nin Seroprevalansının Araştırılması

Özet

Bu çalışma ile Iğdır yöresinde sahipli 100 köpekten elde edilen serumlarda Snap 3dx kiti kullanılarak *Dirofilaria immitis* antijenine %40, *Ehrlichia canis* antikoruna %1 oranında rastlanmış, *Borrelia burgdorferi* antikoru ise saptanamamıştır. Köpeklerin 22'si (22%) kenelerle enfeste bulunmuştur. Köpeklerden toplanan 42 adet kenenin 9'unun dişi, 33'ünün erkek olduğu görülmüştür. Kenelerin %76.2'sinin (32/42) *R. sanguineus* ve %23.8'inin (10/42) *Rhipicephalus spp.* türü olduğu belirlenmiştir. Iğdır yöresinde potansiyel vektörlerin (sivrisinek ve kene) yaygınlığı da göz önünde bulundurulduğunda, dirofilariosis ve ehrlichiosis vakalarıyla sıklıkla karşılaşılabileceği kanısına varılmıştır.

Anahtar sözcükler: *Dirofilaria immitis, Ehrlichia canis, Borrelia burgdorferi, Köpek, Iğdır*

INTRODUCTION

Dirofilariosis, ehrlichiosis and lyme borreliosis are arthropod-borne diseases that are seen in domestic dogs as in many species of animals. Dogs infected with these diseases; can be diagnosed as characteristic symptoms, are shown non-spesific clinical appearance or even asymptomatic. Therefore, factors are needed to seen directly as well as serological methods [1].

The adults of *Dirofilaria immitis* are known as the most pathogenic species in filarial nematodes. Parasite is commonly found in the pulmonary arteries, right ventricle, *V. cava cranialis, V. hepatica,* bronchioles, interdigital cyst and abscesses, brain arteries, spinal canal and eye of dogs, other canids and humans [2-6]. While in some dogs no symptom was observed clinically, in some, dyspnea, hoarseness, fatigue, rapid breathing, cough, collapse, asphyxia, anorexia, pathological sounds in heart and lungs, different types of dermatitis, cachexia, jaundice and hemoglobinuria are seen. The vectors of *D. immitis* are genus of female *Anopheles, Aedes, Culex, Myzorhynchus, Armigeres* and *Taeniorhychus* [2-6].

Dirofilaria immitis is described for the first time in the world in a dog from Alabama in 1856 by Joseph

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Leidy. It has been reported for the first time in 1951 in Turkey [2-7]. To diagnose *D. immitis* in dogs, Thick Drop, Modified Knott, Microhematocrit-Capillary Sedimentation, Saponin Concentration, Membrane Filtration-Aside Phosphates Histochemical Staining, radiology, angiography, ultrasonography, serological techniques (Indirect Flourescent Antibody Test, Counterimmunoelectrophoresis, Latex Agglutination, Hemagglutination), Polymerase Chain Reaction (PCR), and such as Dirochek, Petchek, Snap commercial ELISA test kits are used [2-6,8-10].

In studies, in different geographical regions of the world, using different diagnostic methods the prevalence of *D. immitis* in dogs were determined between 0-73.5% [1,11-20]. In Turkey, in studies based on microscopy, necropsy and serology the prevalence of *D. immitis* in dogs were determined between 0-46.2% [3,9,21-30].

Ehrlichiosis (tropical pancytopenia) is a rickettsial disease that dogs and human are infected with vector ticks and characterized by reduction of the blood-shaped elements. The name of the disease in dogs is canine monocytic ehrlichiosis and the factor is *Ehrlichia canis*. Cases of ehrlichiosis in dogs are found especially in tropical and subtropical regions [31-34]. The vector of disease is *Rhipicephalus sanguineus* ticks. The disease is transmitted to dogs by infected ticks or blood transmission from infected dogs and seen in acute, chronic and subclinical forms [35]. In acute form; weight loss, fever, dyspnea, lympadenopathy, edema in extremity and scrotum, epistaxis, anorexia, recession, eye-nasal discharge, irritability and neurological symptoms are seen. Generally no clinical signs are observed in subclinical form [34,36,37]. Peripheral blood examination, Western Blot and ELISA techniques can be performed to diagnose the disease. However for a definitive diagnose indirect flourescent antibody test IFAT is recommended to use [34,36,38]. In Turkey, in a study 67.8% with IFAT and 57.3% with dot-ELISA seropositivity were detected [39], and also case of ehrlichiosis in dogs was reported in another region of Turkey [40]. In Aegean region of Turkey the prevalence of ehrlichiosis was detected 41.5% by nested PCR [41]. Many studies have been done about the prevalence of *E. canis* infection in various countries [13,33,42-48].

Lyme disease, especially transmitted by Ixodes genus ticks, caused by *Borrelia* genus spirochetes is a zoonotic infection [49-51]. Young dogs are more susceptible to disease and the most obvious symptom is acute polyarthritis. In chronic cases lameness may be occured. In addition to that in dogs symptoms such as fever, lymphadenopathy, anorexia may be seen [52,53]. Disease can be identified by serological methods (IFAT, ELISA, Western Blot etc.) with the help of clinical findings [51,53,54].

It has been reported that Lyme disease is one of the most common disease transmitted by ticks in Europe (2.1-53.7%), Brazil (9.7%) and North America (2.3-76.3%) [13,33,47,48,54-56]. *Borrelia burgdorferi* is the factor of disease was also isolated from vector *Ixodes ricinus* species ticks [57-60]. While in a study [53] in a dog that 2 years old, male and race of Saint Bernard, Lyme disease was found, in another study [61] the infection rate was determined as 27.75% in Turkey.

Mosquito populations are common in Iğdır province [62]. This research was carried out to determine the seroprevalence of *D. immitis*, *E. canis*, and *B. burgdorferi* in Iğdır province where potential vectors are common.

MATERIALS and METHODS

A total of 100 owned and remain outside dogs, 16 of female and 84 of male, were randomly selected. Blood samples were drawn from the cephalic vein in four different focus of Iğdır province (Baharlı, Küllük, Pirli and Söğütlü). In relation to age, 66 of the dogs were 0.5-3 years old, 22 were 4-6 years old and 12 were 7 and older dogs. In addition to that all dogs were examined for ticks, and ticks were collected from dogs which are infested.

The prevalence of *D. immitis, E. canis* and *B. burgdorferi* were simultaneously determined by using a commercial *in-vitro* examination kit (Snap 3dx, Idexx Lab., USA) that detects *D. immitis* antigen, *E. canis* (P30 and P30-1outer membrane proteins), and *B. burgdorferi* (C_6 peptid) antibodies in dog sera.

C_6 ELISA test can be conducted in dog sera, plasma or whole blood. The C6 synthetic peptide was conjugated to bovine serum albumin (BSA) and to horseradish peroxidase (HRP) by using standard methods. The HRP-C6 peptide conjugate was contained in a conjugate diluent containing HRP-labeled antiheartworm antibody, HRP-labeled *E. canis* peptide conjugate, nonspecific proteins, and detergents. If *Borrelia burgdorferi* and/or *E. canis* antibody or *D. immitis* antigen present in the sample, bind to the synthetic peptide-HRP conjugate and to the synthetic peptide-BSA conjugate.

C_6 ELISA test construction is shown in the diagram below. Each kit contains 8 ml *D. immitis*/*E. canis*/*B. burgdorferi* Horseradish peroxidase conjugate, transfer pipette, sample tubes and Snap device. Each Snap device contains 0.4 ml washing and 0.6 ml substrate solution. First of all specimens and kit reagents are heated at room temperature (15-25°C). The latter stages are performed according to the kit procedure as *Fig. 1*.

Statistical analysis were conducted by using Chi-squared test [63].

RESULTS

In 40 of examined 100 dogs (40%) *D. immitis* antigens were detected (an example in *Fig. 2*). A 6 years old and

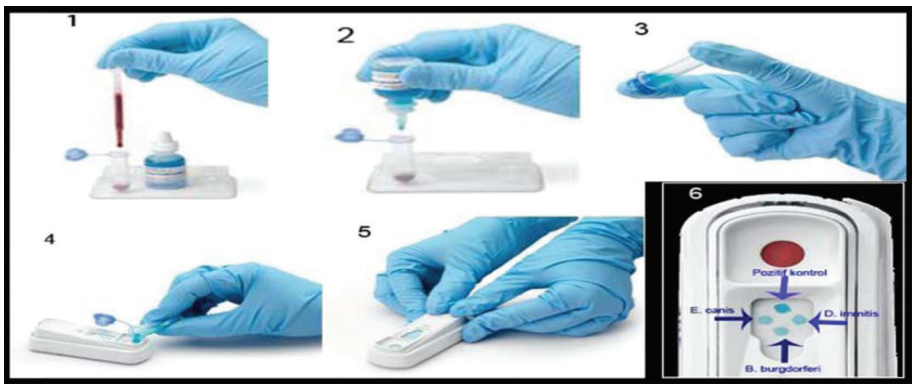

Fig 1. Procedure of Snap 3dx test

Şekil 1. Snap 3 dx testinin yapılışı

1) Three drops of suspicious sera are spotted to the sample tube with transfer pipette 2) Four drops of conjugate are spooted to the sample tube 3)The lid of sample tube is closed and mixed by inverting 3-5 times 4-) Snap device is placed horizontally on a flat surface, all of the content in sample tube is emptied to the sample well 5) Activator button is pressed when coloration in activation point starts 6) The results are evaluated in 8th min

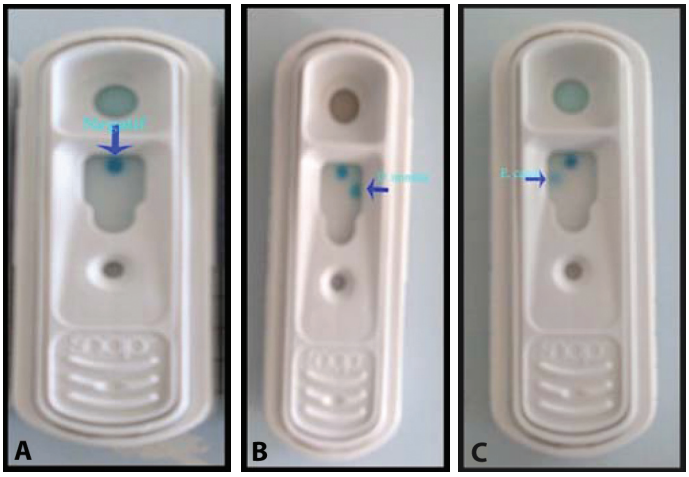

Fig 2. (ABC)- Appearance of positive and negative samples in Snap 3dx test kit

Şekil 2. (ABC)- Pozitif ve negatif örneklerin Snap 3dx test kitinde görünümü
(A: negative, B: *D. immitis* antigen, C: *E. canis* antibody)

Table 1. *The seroprevalence of D. immitis and E. canis correlated with sex and age in Iğdır province*

Tablo 1. *Iğdır yöresinde D. immitis ve E. canis seroprevalansı'nın yaş ve cinsiyet ile ilişkisi*

Dogs		*D. immitis* Antigen		*E. canis* Antibody	
		Infected/ Examined	(%)	Infected/ Examined	(%)
Sex	Female	9/16	(56.25)	0/16	(0.0)
	Male	31/84	(36.9)	1/84	(1.19)
Age	0.5-3	22/66	(33.3)	0/43	(0.0)
	4-6	13/22	(59.1)	1/45	(2.2)
	7 ≥	5/12	(41.7)	0/12	(0.0)
Total		40/100	(40.0)	1/100	(1.0)

Table 2. *Distribution of seroprevalence of D. immitis and E. canis correlated with foci in Iğdır province*

Tablo 2. *Iğdır yöresinde D. immitis ve E. canis seroprevalansı'nın odaklara göre dağılımı*

Province	*D. immitis* Antigen		*E. canis* Antibody	
	Infected/ Examined	(%)	Infected/ Examined	(%)
Küllük	14/25	(56.0)	0/25	(0.0)
Pirli	6/26	(23.1)	1/26	(3.8)
Baharlı	16/25	(64.0)	0/25	(0.0)
Söğütlü	4/24	(16.7)	0/24	(0.0)

male dog (1%) infested with *R. sanguineus* tick, and in which *E. canis* antibody was detected, while *B. burgdorferi* antibody was not determined. The seroprevalence of *D. immitis* and *E. canis* correlated with sex and age in Iğdır province were shown in *Table 1*. Foci and rates of infection in which they encountered were shown in *Table 2*.

Twenty-two of dogs (22%) are infested with ticks. A total of 42 ticks, 9 of female and 33 of male, were collected from dogs. Ticks collected from dogs were *R. sanguineus* 76.2% (32/42) and 23.8% of them (10/42) were *Rhipicephalus* spp.

DISCUSSION

In many countries of the world, many researches have been done to determine *D. immitis, E. canis* and *B. burgdorferi* in dogs by using different diagnostic techniques. Snap 3dx kit that can diagnose simultaneously these three diseases was used for this purpose. As a result of previous researches, the prevalence of *D. immitis* in dogs were determined between 0-46.2% [3,9,21-29] and *E. canis* were determined between 41.5-67.8% [39-41], while in a study [53] in a dog that 2 years old, male and race of Saint Bernard, *B. burgdorferi* was found, in another study [61] the infection rate was determined as 27.75% in Turkey. In this study, *D. immitis* infection was detected in 40 dogs (40%)

and *E. canis* antibodies were present in 1 dog (1%). But *B. burgdorferi* antibody was not determined.

In geographical regions where mosquito population is quite high and dogs are remained outside [62], the prevalences of *D. immitis* were reported in high percentages. Also, in this study the prevalence of *D. immitis* was determined highly (40%). In addition to that, it has been reported that the prevalence of *D. immitis* increased significantly together with age [11,13,23]. Also our findings seem to confirm this criterion. Because dogs between 0.5-3 age group has the infection rate as 33.3% (22/66), dogs between 4 years and older age group has the rate as 52.9% (18/34) (P=0.05).

No significant differences between the sexes were reported in some researches regarding *D. immitis* infections [12-14,28]. In this study, seropositivity was detected in 9 of 16 female (56.25%), and 31 of 84 male (36.9%) dogs. No significant differences between the sexes were observed (P>0.05).

In this study that carried out in Iğdır province, with Snap 3dx commercial ELISA kit, in only 1 of 100 dogs had *Ehrlichia canis* antibody in their sera. But *B. burgdorferi* antibody was not found in the sera. This situation can be explained by absence of *Ixodes ricinus* which is the vector of *B. burgdorferi* in dogs in Iğdır province.

In conclusion, because of Iğdır province has different geographical structure and season from region, also taking into account the population of potential vectors (mosquito and tick), arrived at an opinion that can be encountered with dirofilariosis and ehrlichiosis cases. Mosquitoes and ticks, that they are the vectors of many diseases, are common in this region. But there are not enough research about them. So, in order to determine the vector-disease relationships in all animals in this region, more detailed studies are needed.

REFERENCES

1. Solano-Gallego L, Llull J, Osso M, Hegarty B, Breitschwerdt E: A serological study of exposure to arthropod-borne pathogens in dogs from northeastern Spain. *Vet Res,* 37 (2): 231-244, 2006.

2. Güralp N: Helmintoloji, 2. Baskı, s.505-510, Ankara Üniv. Vet. Fak. Yayınları, Ankara Üniv. Basımevi, Ankara, 1981.

3. Yıldırım A: Ankara ve çevresindeki köpeklerde filarial etkenlerin prevalansı. *Doktora Tezi*, Ankara Üniv. Sağlık Bil. Enst., 2003.

4. Genchi C, Rinaldi L, Cringoli G: *Dirofilaria immitis* and *D. repens* in Dog and Cat and Human Infections, 1st ed., p.39-209, Litografia Vigilante srl, Rolando Editore Via Nuova Poggioreale, Naples, Italy, 2007.

5. Umur Ş, Hökelek M: Filariasis, dirofilariasis, gnathostomiasis, gongylonemiasis, lagochilascoriasis. **In,** Doğanay M, Altıntaş N (Eds): Zoonozlar, Hayvanlardan İnsanlara Bulaşan Enfeksiyonlar. s.1025-1031, Bilimsel Tıp Yayınevi, Ankara, 2009.

6. Johnstone C: Parasites and parasitic diseases of domestic animals: Heartworm. http://cal.nbc.upenn.edu/merial/hrtworm/hw_top.htm, *Accessed:* 22.01.2010.

7. Jackson RF: History of heartworm disease. *Calif Vet* (Special Edition), 6-7, 1989.

8. Peribanez MA, Lucientes J, Arce S, Morales M, Castillo JA, Garci MJ: Histochemical differantiation of *Dirofilaria immitis*, *Dirofilaria repens* and *Acanthocheilonema dracunculoides* microfilariae by staining with a commercial kit, Leucognost-SP®. *Vet Parasitol*, 102 (1-2): 173-175, 2001.

9. Taşçı GT: Kars yöresi köpeklerinde dirofilariosis'in yaygınlığı. *Yüksek Lisans Tezi*, Kafkas Üniv. Sağlık Bil. Enst., Kars, 2005.

10. Rishniw M, Barr SC, Simpson KW, Frongillo MF, Franz M, Alpizar JLD: Discrimination between six species of canine microfilariae by a single polimerase chain reaction. *Vet Parasitol*, 135 (3-4): 303-314, 2006.

11. Montoya JA, Morales M, Ferrer O, Molina JM, Corbera JA: The prevalence of *Dirofilaria immitis* in Gran Canaria, Canary Islands, Spain (1994-1996). *Vet Parasitol,* 75 (2-3): 221-226, 1998.

12. Rosa A, Ribicich M, Betti A, Kistermann JC, Cardillo N, Basso N, Hallu R: Prevalence of canine dirofilariosis in the city of Buenos Aires and its outskirts (Argentina). *Vet Parasitol*, 109 (3-4): 261-264, 2002.

13. Labarthe N, de Campos Pereira M, Barbarini O, Mckee W, Coimbra CA, Hoskins J: Serologic prevalence of *Dirofilaria immitis*, *Ehrlichia canis* and *Borrelia burgdorferi* infections in Brazil. *Vet Ther,* 4 (1): 67-75, 2003.

14. Song KH, Lee SE, Hayasaki M, Shiramizu K, Kim DH, Cho KW: Seroprevalence of canine dirofilariosis in South Korea. *Vet Parasitol*, 114 (3): 231-236, 2003.

15. Wu CC, Fan PC: Prevalence of canine dirofilariosis in Taiwan. *J Helminthol,* 77 (1): 83-88, 2003.

16. Garcez LM, de Souza NF, Mota EF, Dickson LAJ, Abreu WU, Cavalcanti VFN, Gomes PAF: Focus of canine heartworm disease in Marajo Island, North of Brazil: A risk factor for human health. *Rev Soc Bras Med Trop,* 39 (4): 333-336, 2006.

17. Carlos RS, Muniz Neta ES, Spagnol FH, Oliveira LL, de Brito RL, Albuguergue GR, Almosny NR: Frequency of antibodies anti-*Ehrlichia canis*, *Borrelia burgdorferi* and *Dirofilaria immitis* antigens in dogs from microrregion Ilhéus-Itabuna, State of Bahia, Brazil. *Rev Bras Parasitol Vet*, 16 (3): 117-120, 2007.

18. Davoust B, Normand T, Bourry O, Dang H, Leroy E, Bourdoiseau G: Epidemiological survey on gastro-intestinal and blood-borne helminths of dogs in north-east Gabon. *Onderstepoort J Vet Res,* 75 (4): 359-364, 2008.

19. Hoff B, Mcewen B, Peregrine AS: A survey for infection with *Dirofilaria immitis, Ehrlichia canis, Borrelia burgdorferi,* and *Babesia canis* in feral and client-owned dogs in the Turks and Caicos Islands, British West Indies. *Can Vet J,* 49 (6): 593-594, 2008.

20. Pantchev N, Norden N, Lorentzen L, Rossi M, Rossi U, Brand B, Dyachenko V: Current surveys on the prevalence and distribution of *Dirofilaria spp.* in dogs in Germany. *Parasitol Res,* 105 (1): 63-74, 2009.

21. Umur S, Arslan MO: Kars yöresi sokak köpeklerinde görülen helmint türlerinin yayılışı. *Türkiye Parazitol Derg*, 22 (2): 188-193, 1998.

22. Ağaoğlu Z, Akgül Y, Ceylan E, Akkan H: Van yöresi köpeklerinde *Dirofilaria immitis*'in yaygınlığı. *Yüzüncü Yıl Üniv Vet Fak Derg*, 11 (2): 41-43, 2000.

23. Balıkçı E, Sevgili M: Elazığ ve çevresindeki köpeklerde *Dirofilaria immitis*'in seroprevalansı. *Firat Üniv Sağ Bil Vet Derg*, 19 (2): 103-106, 2005.

24. Köse K: Erzincan yöresindeki köpeklerde *Dirofilaria immitis*'in prevalansı üzerine araştırmalar. *Yüksek Lisans Tezi*, Yüzüncü Yıl Üniv. Sağlık Bil. Enst., 2005.

25. Çakıroğlu D, Meral Y: Samsun bölgesinde köpeklerde *Dirofilaria immitis* enfestasyonu insidansı incelenmesi. *JIVS,* 2, 1-12, 2007.

26. Yalçın E, Şenlik B, Yılmaz Z, Alasonyalılar A, Akyol V: Bursa'daki köpeklerde *Dirofilaria immitis*'in prevalansı. *JTVS,* 13 (2): 23-27, 2007.

27. Yildirim A, Ica A, Atalay O, Duzlu O, Inci A: Prevalence and epidemiological aspects of *Dirofilaria immitis* in dogs from Kayseri province, Turkey. *Res Vet Sci,* 82 (3): 358-363, 2007.

28. Simsek S, Utuk AE, Koroglu E, Rishniw M: Serological and molecular studies on *Dirofilaria immitis* in dogs from Turkey. *J Helminthol,* 82 (2): 181-186, 2008.

29. Yıldız K, Yasa Duru S, Yağcı BB, Öcal N, Gazyağcı AN: The prevalence of *Dirofilaria immitis* in dogs in Kırıkkale. *Türkiye Parazitol Derg,* 32 (3): 225-228, 2008.

30. Taşçı GT, Kılıç Y: Kars ve Iğdır civarındaki köpeklerde *Dirofilaria immitis* (Leidy, 1856)'nin prevalansı ve potansiyel vektör sivrisinek türleri üzerine araştırmalar. *Kafkas Univ Vet Fak Derg*, 18 (Suppl-A): A29-A34, 2012.

31. Lee Pyle R: Canine Ehrlichiosis. *J Am Vet Med Assoc*, 177, 1197-1202, 1980.

32. Eng TR, Giles R: Ehrlichiosis. *J Am Vet Med Assoc*, 194, 497-500, 1989.

33. Beall MJ, Chandrashekar R, Eberts MD, Cyr KE, Diniz PP, Mainville C, Hegarty BC, Crawford JM, Breitschwerdt EB: Serological and molecular prevalence of *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, and *Ehrlichia* species in dogs from Minnesota. *Vector Borne Zoonotic Dis*, 8 (4): 455-464, 2008.

34. Harrus S, Waner T: Diagnosis of canine monocytotropic ehrlichiosis *(Ehrlichia canis)*: An overview. *Vet J,* 187 (3): 292-296, 2011.

35. Magnerelli LA, Anderson JF: Serologic evidence of canine and equine ehrlichiosis in Northeastern United States. *J Clin Microbiol*, 31 (11): 2857-2860, 1993.

36. Rikihisa Y, Ewing SA, Fox JC, Siregar Pasaribu FH, Malole BM: Analyses of *Ehrlichia canis* and canine granulotic ehrlichia infection. *J Clin Microbiol*, 30 (1): 143-148, 1992.

37. Harrus S, Waner T, Bark H, Jongejan F, Cornelissen AWCA: Minireview. Recent advances in determining the pathogenesis of canine monocytic ehrlichiosis. *J Clin Microbiol,* 37 (9): 2745-2749, 1999.

38. Warner T, Harrus S, Jongejan F, Bark H, Keysary A, Cornelissen AWCA: Significance of serological testing for ehrlichial diseases in dogs with special emphasis on the diagnosis of canine monocytic ehrlichiosis caused by *Ehrlichia canis*. *Vet Parasitol,* 95 (1): 1-15, 2001.

39. Erdeğer J, Sancak A, Ataseven L: Köpeklerde *Ehrlichia canis*'in Indirekt Fluoresan Antikor (IFA) Testi ve Dot-ELISA ile saptanması. *Turk J Vet Anim Sci,* 27, 767-773, 2003.

40. Dodurka HT, Bakırel U: Bir köpekte Ehrlichiosis olgusu. *İstanbul Üniv Vet Fak Derg*, 28, 11-16, 2002.

41. Karagenç T, Hoşgör M, Bilgiç HB, Paşa S, Kirli G, Eren H: Ege bölgesinde köpeklerde *E. canis, A. phagocytophila* ve *A. platys*'in prevalansının Nested PCR ile tespiti. *XIV. Ulusal Parazitoloji Kongresi, 18-25 Eylül, İzmir,* 2005.

42. O'Dwyer LH, Massard CL, Pereira De Souza JC: *Hepatozoon canis* infection associated with dog ticks of rural areas of Rio de Janeiro State, Brazil. *Vet Parasitol,* 94 (3): 143-150, 2001.

43. Dagnone AS, de Morais HS, Jojima FS, Vidotto O: Ehrlichiosis in anemic, thrombocytopenic, or tickinfested dogs from a hospital population in south Brazil. *Vet Parasitol,* 117 (4): 285-90, 2003.

44. Alexandre N, Santos AS, Nuncio MS, Sousa R, Boinas F, Bacellar F: Detection of *Ehrlichia canis* by polymerase chain reaction in dogs from Portugal. *Vet J,* 181 (3): 343-344, 2009.

45. Abd Rani PAM, Irwin PJ, Coleman GT, Gatne M, Traub RJ: A survey of canine tick-borne diseases in India. *Parasit Vectors*, 4 (141): 1-8, 2011.

46. Carlos RS, Carvalho FS, Wenceslau AA, Almosny NR, Albuquerque GR: Risk factors and clinical disorders of canine ehrlichiosis in the South of Bahia, Brazil. *Rev Bras Parasitol Vet*, 20 (3): 210-214, 2011.

47. Carrade D, Foley J, Sullivan M, Foley CW, Sykes JE: Spatial distribution of seroprevalence for *Anaplasma phagocytophilum, Borrelia burgdorferi, Ehrlichia canis,* and *Dirofilaria immitis* in dogs in Washington, Oregon, and California. *Vet Clin Pathol,* 40 (3): 293-302, 2011.

48. Villeneuve A, Goring J, Marcotte L, Overvelde S: Seroprevalence of *Borrelia burgdorferi, Anaplasma phagocytophilum, Ehrlichia canis,* and *Dirofilaria immitis* among dogs in Canada. *Can Vet J,* 52 (5): 527-530, 2011.

49. Keskin O, İzgür M: Lyme Hastalığı. *Etlik Vet Mikrobiyol Derg*, 10, 79-91, 1999.

50. Jaderlund KH, Egenvall A, Bergstrom K, Hedhammar A: Seroprevalence of *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* in dogs with neurological signs. *Vet Rec*, 160 (24): 825-831, 2007.

51. Kurt C: Lyme (Borreliosis); Kopek ve insanlarda kene kaynakli bakteriyel bir hastalik. http://www.tavsiyeediyorum.com/makale_666.htm. *Accessed:* 07.03.2008

52. Appel MJG: Lyme disease in dogs and cats. *Comp Cont Educ Prac Vet,* 12, 617-620, 1990.

53. Gülanber EG, Gülanber A, Albayrak R, Gülanber NG, Polat E: Lyme Disease (Borreliosis) in a Saint Bernard dog: First clinical case in Turkey. *Turk J Vet Anim Sci,* 31 (5): 367-369, 2007.

54. Bhide M, Travnicek M, Curlik J, Stefancikova A: The importance of Lyme borreliosis in eco-epidemiology of dogs: A review. *Vet Med,* 49 (4): 135-142, 2004.

55. Joppert AM, Hagiwara MK, Yoshinari NH: *Borrelia burgdorferi* antibodies in dogs from Cotia County, São Paulo State, Brazil. *Rev Inst Med Trop São Paulo,* 43 (5): 251-255, 2001.

56. Gary AT, Webb JA, Hegarty BC, Breitschwerdt EB: The low seroprevalence of tick-transmitted agents of disease in dogs from southern Ontario and Quebec. *Can Vet J,* 47 (12): 1194-1200, 2006.

57. Çalışır B, Polat E, Yücel A: Silivri ilçesinin bazı bölgelerindeki bir kısım evcil hayvanlardan toplanan kenelerin tür ayrımının yapılması ve *Ixodes ricinus*'larda *Borrelia burgdorferi*'nin araştırılması. *Türkiye Parazitol Derg*, 21 (4): 379-382, 1997.

58. Polat E, Çalışır B, Yücel A, Tüzer E: Türkiye'de *Ixodes ricinus*'lardan ilk defa ayrılan ve üretilen iki *Borrelia* kökeni. *Türkiye Parazitol Derg,* 22 (2): 167-173, 1998.

59. Baumgarten BU, Rollinghoff M, Bogdan C: Prevalence of *Borrelia burgdorferi* and granulocytic and monocytic ehrlichiae in *Ixodes ricinus* ticks from Southern Germany. *J Clin Microbiol,* 37 (11): 3448-3451, 1999.

60. Güneş T, Kaya S, Poyraz O, Engin A: The prevalence of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks in the Sinop region of Turkey. *Turk J Vet Anim Sci,* 31 (3): 153-158, 2007.

61. Bhide M, Yilmaz Z, Golcu E, Torun S, Mikula I: Seroprevalence of anti-*Borrelia burgdorferi* antibodies in dogs and horses in Turkey. *Ann Agric Environ Med,* 15 (1): 85-90, 2008.

62. Aldemir A, Demirci B, Kırpık MA, Alten B, Baysal A: Species composition and seasonal dynamics of mosquito larvae (Diptera: Culicidae) in Iğdır plain, Turkey. *Kafkas Univ Vet Fak Derg*, 15 (1): 103-110, 2009.

63. Özdamar K: SPSS ile Biyoistatistik. 4. Baskı, Kaan Kitabevi, Eskişehir, 2001.