https://dergipark.org.tr/tr/pub/ataunivbd
2019; 14(2): 151-158

Research Article/Araştırma Makalesi
DOI: 10.17094/ataunivbd.580250

Atatürk Üniversitesi Veteriner Bilimleri Dergisi
Atatürk University Journal of Veterinary Sciences

A Serosurvey on Some Canine Vector-borne Zoonoses (*Anaplasma* spp., *Ehrlichia* spp.*, Borrelia burgdorferi*, *Dirofilaria immitis* and *Leishmania* spp.) in Osmaniye

Tülin GÜVEN GÖKMEN[1a]✉, Elçin GÜNAYDIN[2b], Nevin TURUT[3c], Bünyamin AKIN[4d], Özgür KOÇ[5e], Armağan Erdem ÜTÜK[6f]

1. University of Cukurova, Ceyhan Veterinary Faculty, Department of Microbiology, Adana, TURKEY.
2. University of Hitit, Alaca Avni Celik Vocational School, Corum, TURKEY.
3. Adana Veterinary Control Institute, Laboratory of Bacteriology, Adana, TURKEY.
4. Nature and Wildlife Conservation and Research Association, Osmaniye, TURKEY.
5. Municipality of Osmaniye, Directorate of Veterinary Affairs, Osmaniye, TURKEY.
6. University of Cukurova, Ceyhan Veterinary Faculty, Department of Parasitology, Adana, TURKEY.

ORCID: 0000-0001-9673-097X[a], 0000-0002-5247-7578[b], 0000-0003-2950-001X[c], 0000-0003-1074-652X[d], 0000-0002-6050-2286[e], 0000-0002-7986-3583[f]

Geliş Tarihi/Received	Kabul Tarihi/Accepted	Yayın Tarihi/Published
24.06.2019	29.08.2019	25.10.2019

Bu makaleye atıfta bulunmak için/To cite this article:
Güven Gökmen T, Günaydın E, Turut N, Akın B, Koç O, Ütük AE: A Serosurvey on Some Canine Vector-borne Zoonoses (*Anaplasma* spp., *Ehrlichia* spp.*, Borrelia burgdorferi*, *Dirofilaria immitis* and *Leishmania* spp.) in Osmaniye. Atatürk Üniversitesi Vet. Bil. Derg., 14(2): 151-158, 2019. DOI: *10.17094/ataunivbd.580250*

Abstract: Vector-borne diseases in dogs are of major global significance for their impact on animal and human health. Especially, it is necessary to determine the prevalence of agents found in reservoir animals by conventional, molecular and serological methods for the application of control programs for these diseases. Serosurvey studies are one of the reliable methods to know the presence and prevalence of these diseases in our country and region. In this study, it was aimed to detect the prevalence of *Ehrlichia canis/E.ewingii, Anaplasma platys/A.phagocytophilum, Borrelia burgdorferi, Dirofilaria immitis* and *Leishmania infantum* in dogs in Osmaniye. Five canine vector-borne diseases were investigated with a rapid in-clinic enzyme-linked immunosorbent assay in 100 clinically healthy dog serum samples in Osmaniye city center, Düziçi, Sumbas, Kadirli, Hasanbeyli, Bahçe and Toprakkale districts. Seroprevalence rate was detected as 3% for *E.canis/E.ewingii* and 1% for *D.immitis* by SNAP 4Dx PLUS. The prevalence of *A.platys/A.phagocytophilum*, *Borrelia burgdorferi* and *L.infantum* were determined as 0%. In conclusion, our study in which we determined the seroprevalence of dog vector-borne diseases in Osmaniye is the first study in which five agents are determined in one step and will contribute to the effective control programs prepared for animal and public health in our region.

Keywords: *Dirofilaria*, ELISA, *Ehrlichia,* PCR.

Osmaniye'de Bazı Köpek Vektör Kaynaklı Zoonozlar (*Anaplasma* spp., *Ehrlichia* spp. *Borrelia burgdorferi, Dirofilaria immitis* ve *Leishmania* spp.*)* Üzerine Serolojik Bir Araştırma

Öz: Köpeklerde bulunan vektör kaynaklı hastalıklar, hayvan ve insan sağlığı üzerindeki etkileri açısından büyük önem taşımaktadır. Özellikle belirli bölgelerde bu hastalıklara yönelik kontrol programlarının uygulanması için, öncelikle rezervuar hayvanlarda bulunan etkenlerin konvansiyonel, moleküler ve serolojik yöntemlerle prevalansının belirlenmesi gereklidir. Serolojik araştırma çalışmaları ülkemizde ve bölgemizde bu hastalıkların varlığını ve yaygınlığını bilmek için güvenilir yöntemlerden biridir. Bu çalışmada, Osmaniye ilinde köpeklerde *Ehrlichia canis / E.ewingii, Anaplasma platys / A.phagocytophilum, Borrelia burgdorferi, Dirofilaria immitis* ve *Leishmania infantum* prevalansının belirlenmesi amaçlanmıştır. Osmaniye merkez, Düziçi, Sumbas, Kadirli, Hasanbeyli, Bahçe ve Toprakkale ilçelerinde klinik olarak sağlıklı yüz köpekten alınan serum örneklerinde hızlı bir immünosorbent testi ile beş köpek vektör kaynaklı hastalık araştırılmıştır. SNAP 4Dx PLUS testi ile seroprevalans oranı *E.canis / E.ewingii* için %3, *D.immitis* için %1 olarak tespit edilmiştir. *A.platys / A.phagocytophilum, Borrelia burgdorferi* ve *L.infantum* prevalansı ise % 0 olarak belirlenmiştir. Sonuç olarak, Osmaniye'de köpek vektör kaynaklı hastalıkların seroprevalanslarını tespit ettiğimiz çalışmamız, bölgede beş etkenin tek aşamada belirlendiği ilk çalışmadır ve bölgemizde hayvan ve halk sağlığı için hazırlanan efektif kontrol programlarına katkı sağlayacaktır.

Anahtar Kelimeler: *Dirofilaria*, ELISA, *Ehrlichia,* PZR.

✉Tulin Gokmen Guven
University of Cukurova, Ceyhan Veterinary Faculty, Department of Microbiology, Adana, TURKEY.
e-mail: tulinguven01@hotmail.com

INTRODUCTION

Canine vector-borne diseases (CVBDs) are transmitted by arthropods, including phlebotomus, ticks, fleas and mosquitoes and have a worldwide distribution (1). The most well known CVBD agents are *Anaplasma* spp., *Ehrlichia* spp., *Borrelia burgdorferi* (*B.burgdorferi*), *Dirofilaria immitis* (*D.immitis*) and *Leishmania* spp. These agents are essential for public health due to their zoonotic potential and dogs, which in close contact with people in rural and urban areas serve as important reservoirs (2).

Anaplasma spp. are intracellular gram-negative bacteria. *Anaplasma phagocytophilum* (*A.phagocytophilum*) can infect many hosts such as ruminants, cats, horses, dogs and humans. While it causes symptoms such as thrombocytopenia, fever, anorexia, hypoalbuminemia, and anemia in animals, it also causes "Human Granulocytic Anaplasmosis" in humans. It infects granulocytes and is transmitted by *Dermacentor, Ixodes, Hyalomma* and *Rhipicephalus* tick species (3,4). *Anaplasma platys (A. platys)* cause "Canine cyclic thrombocytopenia" and infects canine platelets which *often causes* co-infections with other tick-borne agents, especially *Ehrlichia canis* (*E.canis*) transmitted by *Rhipicephalus sanguineus (R.sanguineus*) tick (5). *A. platys* is not confirmed as a disease agent in humans, but it was detected molecularly in some cases and this may indicate that it may have a zoonotic potential in the future (6).

E.canis is a gram-negative, intracellular bacterium that is transmitted by *R.sanguineus*. It causes to "Canine Monocytic Ehrlichiosis" characterized by hematological abnormalities and fever. In recent years, *E.canis* has been shown to cause "Human Monocytic Ehrlichiosis" in humans (7,8). *Ehrlichia ewingii* (*E. ewingii*) is transmitted by *Amblyomma* and *Dermacentor* tick species in the dogs, infects neutrophils and causes neutrophilic polyarthritis. It is also a human pathogen transmitted by *Amblyomma americanum* (*A.americanum*) at the same time. The bacteria cause "Human Monocytic Ehrlichiosis" which is an acute febrile disease with fever, headache, muscle pain and fatigue symptoms (9).

B.burgdorferi is transmitted by *Ixodes* species and is the causative agent of Lyme disease or borreliosis, characterized by anorexia, lethargy, lymphadenopathy, fever, arthritis, cardiac or neurological dysfunctions and glomerulonephritis in dogs, horses, cats and humans (10).

D.immitis is the causative agent of cardiopulmonary dirofilariosis or heartworm disease in dogs and transmitted by mosquitoes in the genus of *Culex, Aedes* and *Anopheles*. Pulmonary and ocular dirofilariasis are seen in humans (11).

Leishmaniosis is a zoonotic disease which is seen in vertebrate hosts and transmitted by sandflies. In Turkey, especially *Leishmania infantum* (*L.infantum*) and *Leishmania tropica* (*L.tropica*) agents are found. The disease is most common in humans and dogs among mammals. Dogs have critical importance in the spread of the disease because they both show the symptoms of the disease and act as reservoirs (12).

For the effective control of CVBDs, it is necessary to know the serological status of reservoir animals in a particular area. Therefore, in our study, we aimed to determine the seroprevalence of *E.canis/E.ewingii*, *A.platys/A.phagocytophilum*, *B.burgdorferi*, *D.immitis* and *L.infantum* in dogs in Osmaniye.

MATERIALS and METHODS

Sample Collection

This study was approved by the Unit Ethics Committee of the Ceyhan Veterinary Faculty of Çukurova University on 12.09.2017 with the 9-decision number. Blood samples of 100 farm dogs (30 female and 70 male) were collected from the countryside of seven districts, Osmaniye city center,

Düziçi, Sumbas, Kadirli, Hasanbeyli, Bahçe and Toprakkale at Osmaniye over 3 months from June to August 2017 (Figure 1). All dogs were clinically healthy. Dog owners filled out an information form, which includes information on the age, breed, sex, tick and flea infestation of each dog. The age of the dogs ranged from 2 months to 10 years. Three dogs had only tick infestation, 49 dogs had only flea infestation and 37 dogs had both tick and flea infestation. No infestation was observed in 11 dogs. The collected blood samples were centrifuged at 1200g for 10 minutes. The sera were separated and stored at -20°C until use.

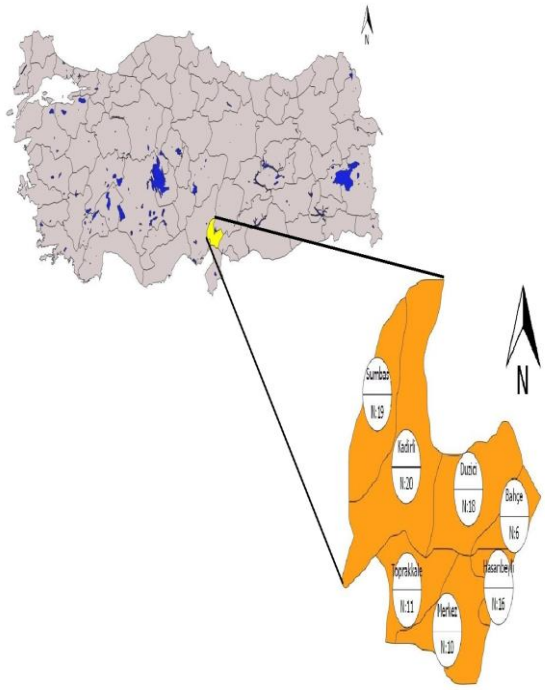

Figure 1. The distribution of samples taken in districts of Osmaniye (QGIS 3.6).
Şekil 1. Osmaniye ilçelerinden alınan örneklerin dağılımı (QGIS 3.6).

Sample Analysis

Serum samples from dogs were screened by a rapid in-clinic enzyme-linked immunosorbent assay (ELISA) kit (SNAP® 4Dx® Plus Test from IDEXX® Laboratories, Westbrook, Maine, USA) according to the instructions of the manufacturer. This test identifies the circulating carbohydrate of *D.immitis* and detects the antibodies against the immunodominant proteins of *A. phagocytophylum* (p44 and MSP2), *B. burgdorferi* (sensu lato) (C6) and *E. canis* (p30 and p30-1). Results were interpreted colorimetrically.

Furthermore, an ELISA kit (Leishmania 96, Biopronix, Agrolabo) was used for the detection of anti-*L.infantum* antibodies in dog sera. The test was done according to the instructions of the manufacturer. The mean optical density (OD) at 450 nm was determined with a microplate reader (EZ Read 400, Microplate Reader, Biochrom). Cut-off values were calculated (OD 450 nm positive control X 0.30 for negative cut-off and OD 450 nm positive control X 0.35 for positive cut-off) and samples with an OD lower than the negative cut-off value accepted as negative while samples with an OD higher than the positive cut-off value accepted as positive. Samples with an OD between both cut-off values were accepted as doubtful.

RESULTS

At the end of the study, the seroprevalence rate was detected as 3% for *E.canis/E.ewingii* and 1% for *D.immitis* by SNAP 4Dx PLUS. However, no antibodies were detected for *A.platys / A.phagocytophilum, B.burgdorferi* and *L.infantum. D.immitis* seropositive male dog in Düziçi was 2-year-old and Kangal breed. *Ehrlichia spp.* seropositivity was determined in Sumbas, Düziçi and Kadirli regions. In Sumbas and Kadirli, the Kangal breed female dogs were 18 months and 3 months old, respectively. Male Golden Retriever is 5-year-old in Düziçi (Table 1 and 2).

Table 1. Distribution of vector-borne agents according to Osmaniye districts.
Tablo 1. Vektör kaynaklı etkenlerin Osmaniye ilçelerine göre dağılımı.

	E.canis/ E.ewingii	*A.phagocytophilum/ A.platys*	*D.immitis*	*B.burgdorferi*	*Leishmania* spp.
Sumbas	1/19	0/19	0/19	0/19	0/19
Düziçi	1/18	0/18	1/18	0/18	0/18
Osmaniye city center	0/10	0/10	0/10	0/10	0/10
Toprakkale	0/11	0/11	0/11	0/11	0/11
Hasanbeyli	0/16	0/16	0/16	0/16	0/16
Kadirli	1/20	0/20	0/20	0/20	0/20
Bahçe	0/6	0/6	0/6	0/6	0/6
Total	3/100 (3%)	0/100 (0%)	1/100 (1%)	0/100 (0%)	0/100 (0%)

Table 2. Demographic information of dogs infected *Ehrlichia* spp. and *D.immitis.*
Tablo 2. *Ehrlichia* spp. *ve D. immitis* ile infekte köpeklerin demografik bilgileri.

E.canis/ E.ewingii positive dogs	Age	Breed	Sex	Region
Dog 1	18 month	Kangal	Female	Sumbas
Dog 2	5 age	Golden Retriever	Male	Düziçi
Dog 3	3 month	Kangal	Female	Kadirli
D.immitis positive dog	Age	Breed	Sex	Region
Dog 1	2 age	Kangal	Male	Düziçi

DISCUSSION and CONCLUSION

Different serological tests, ELISA, Dot-ELISA, indirect fluorescent antibody test (IFA), and micro-immunofluorescent assay, have been used for the detection of anti-*E.canis* antibodies. In Turkey, seroprevalences of *E.canis* were determined as 1-74 % (13-16). When we examine the neighboring countries, *E.canis* prevalences were determined as 37.5% (45/120) in Bulgaria (17) 16.6% (40/240) in Iran (18) and 10.3% (138/1335) in Southern Italy (19).

In this study, we detected anti-*E.canis/E.*ewingii antibodies in 3 out of 100 healthy dogs (3%) with ELISA (SNAP 4Dx Plus) test. Our result was similar to Iğdır 1% (1/100) and Diyarbakır 4.87% (4/82) (13,14). The common points of these three studies, including our work, sera samples were obtained from randomly selected dogs, same ELISA kits (SNAP 4DX plus, SNAP 3DX) and similar sample sizes were used and also climatic factors were similar. Although the same kit was used in the study in Italy, seroprevalence (10.3%) was higher than our study (3%). This higher seroprevalence may be associated with sampling. Hunting dogs were sampled in this study and these animals are more likely to come into contact with vectors, because they are constantly in the field.

In other studies, with high prevalences; there are marked differences from our study about the working parameters. For example, sample sizes are small in Balikesir (n: 38), İzmir (n: 32), Sanliurfa (n: 27), Adana (n: 26), Antalya (n: 18) and Ankara (n: 31) (15,16). Different serologic tests (IFA) and cut-off values were used (1/20, 1/40 and 1/100) in these studies (15,16,17). It has known that low cut-off values are associated with high prevalences. As a result, in these regions diseases may be endemic but at the same time, the high prevalence may be the result of the differences in the serological test, cut-off values, the number of samples, and the climatic factors.

We determined the prevalence of *D.immitis* as 1% (1/100) with an antigen-ELISA test. Prevalence values are quite different in different provinces of Turkey and determined as 0-30% (20-28). In studies conducted in neighboring countries, prevalences were determined as 4.1% (19/750) in Greece (29), 40.12% (69/172) in Iraq (30) and 5.4% (8/149) is Southern Iran (31) with the serologic examination and 33.3% in Bulgaria (32) with necropsy.

For the serologic examination antigen, ELISA tests were used in all examined studies. Except for İstanbul and İzmir province (PetCHEK HTWM PF, IDEXX) the same commercially ELISA kit was

(DiroCHEK, Synbiotics Corp. 96-0230 USA) preferred in Turkey (20). We choose SNAP 4DX plus ELISA kit for this study as in Greece (29), Iraq (30) and Southern Iran (31).

We think that differences in the seroprevalence rates of Dirofilariosis are a multifactorial concept. In most of the studies, the same serological method and also the same commercial kit were used in Turkey. It is said to be that, Dirofilariosis, in different parts of Turkey, is presented as in hypo and mesoendemic levels according to geographic, climatic factors and vector activity. In different times, researchers get different results from the same province with same ELISA test. For example, seroprevalences were 0% (0/19)-14.8% (4/27) in Ankara (23,26), 0% (0/29)-9.1% (11/120) in Elazığ (23,33), 3%(1/37)-10.5% (2/19) in Mersin (23,26), and 4.58% (11/240)- 10%(4/42) in Kars (25,26). So, sample size and sampling techniques also have an impact on the prevalence of the disease and may affect the results.

In our study, seroprevalences of *B.burgdorferi, Anaplasma* spp., *and Leishmania* spp. were 0%. In previous researches, the prevalence of *B.burgdorferi* in dogs were determined between 0-28.6% in Turkey (13,14,34-37). The prevalences in neighboring countries were as follows: 0% (0/200) in Greece (29), 2.4% (4/167) in Bulgaria (38) and 9.52% (16/168) in Iran (39).

In our country, some *Anaplasma* seroprevalence studies were found to be higher than ours. Seroprevalence of *A.phagocytophilum* was detected between 2.75-30% in Sinop and Thrace region (36, 40). Besides, seroprevalence of *A. platys* was determined as 4.75% in the Thrace region (40).

Although our region was an endemic region for *L.infantum*, it was interesting that the dogs were seronegative. According to the Ministry of Health in Turkey, according to the records years 1990-2010, 46 003 new human cases have been reported. 96% of these patients were reported from Sanliurfa, Adana, Osmaniye, Hatay, Diyarbakir, Icel and Kahramanmaras (41). Seroprevalence in dogs was determined as 7.2% in a study conducted in our country. However, similar to our results in the same study, the dogs were seronegative in Elazığ. There were also seronegative dogs in different studies in other provinces such as Ordu, Şanlıurfa, Diyarbakır, Erzurum, Edirne, Çanakkale, Burdur, Sinop, Amasya and Tokat (12). In a study conducted in Samsun, seroprevalence was 0.41%, close to our conclusion (42).

We thought that seronegativity might be due to various reasons for *B.burgdorferi, Anaplasma spp.,* and *Leishmania spp*. Firstly, low and higher seropositivities may be associated with the presence or absence of vectors and different endemic levels of the diseases in different provinces of Turkey and neighboring countries. According to Aydin and Bakirci (43), *Ixodes ricinus* (*I.ricinus*), the primary vector of *B.burgdorferi*, is seen in coastal areas and wetlands such as Marmara, Aegean, Mediterranean, Black Sea and East Anatolia regions of Turkey. In our region, the vector population may be small or, if present, may not carry *B.burgdorferi*. Another reason for seronegativity could be the decrease of specific antibody levels in the blood during sampling. When *Anaplasma* seronegativity was evaluated, it was known that antibody titers for *Anaplasma* reverted to undetectable levels by 7 to 8 months (44). Finally, another cause of seronegativity is attributed to the fact that serological tests in newly infected animals are not suitable for infection detection. In addition, immune sensitivity to a self-healing cellular immune reaction may be improved. Therefore, serological tests may lead to false-negative results (45). This is common in the serological detection of *Leishmania* species.

In conclusion, our study provided information about the serological status of Canine vector-borne diseases in Southern Turkey. In Osmaniye, there is not a study that examined together of five vector-borne disease agents and detected in a single step. Our results will contribute to the effective control program in our region. However, when we consider

the missing points of the study, some suggestions should be taken into consideration. Firstly, studies should be conducted in the region to examine the types, propagation of vectors and distributions according to environmental conditions. Furthermore, vector screening should be done according to the geographical location and climate of the regions. Vectors that select specific climatic and geographic regions should be examined. Finally, a standard procedure should be applied, especially by using tests with high sensitivity and specificity, and with a sufficient number of sampling.

Acknowledgments

In this study, we would like to thank Osmaniye Municipality for their contributions.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Angelou A., Gelasakis AI., Verde N., Pantchev N., Schaper R., Chandrashekar R., Papadopoulos E., 2019. Prevalence and risk factors for selected canine vector-borne diseases in Greece. Parasit Vectors, 12, 283.
2. Movilla R., Garcia C., Siebert S., Roura X., 2016. Countrywide serological evaluation of canine prevalence for Anaplasma spp., Borrelia burgdorferi (sensu lato), Dirofilaria immitis and Ehrlichia canis in Mexico. Parasit Vectors, 9, 421.
3. Carrade DD., Foley JE., Borjesson DL., Sykes JE., 2009. Canine Granulocytic Anaplasmosis: A review. J Vet Intern Med, 23,1129-1141.
4. Huber D., Reil I., Duvnjak S., Jurkovic D., Lukacevic D., Pilat M., Beck A., Mihaljevic Z., Vojta L., Polkinghorne L., Beck R., 2017. Molecular detection of Anaplasma platys, Anaplasma phagocytophilum and Wolbachia sp. but not Ehrlichia canis in Croatian dogs. Parasitol Res, 116, 3019-3026.
5. Low VL., Prakash BK., Lim YA., Tan TK., Vinnie Siow WY., Sofan Azirun M., AbuBakar S., 2018. Detection of Anaplasmataceae agents and co infection with other tick borne protozoa in dogs and Rhipicephalus sanguineus sensu lato ticks. Exp Appl Acarol, 75, 429-435.
6. Arraga-Alvarado CM., Qurollo BA., Parra OC., Berrueta MA., Hegarty BC., Breitschwerdt EB., 2014. Molecular evidence of Anaplasma platys infection in two women from Venezuela. Am J Trop Med Hyg, 91, 1161-1165.
7. Harrus S., Waner T., 2011. Diagnosis of canine monocytotropic ehrlichiosis (Ehrlichia canis): an overview. Vet J, 187, 292-296.
8. Perez M., Bodor M., Zhang C., Xiong Q., Rikihisa Y., 2006. Human infection with Ehrlichia canis accompanied by clinical signs in Venezuela. Ann NY Acad Sci, 1078, 110-117.
9. Harris RM., Couturier BA., Sample SC., Coulter KS., Casey KK., Schlaberg R., 2016. Expanded geographic distribution and clinical characteristics of Ehrlichia ewingii Infections, United States. Emerg Infect Dis, 22, 862-865.
10. Littman MP., Gerber B., Goldstein RE., Labato MA., Lappin MR., Moore GE., 2018. ACVIM consensus update on Lyme borreliosis in dogs and cats. J Vet Intern Med, 32, 887-903.
11. Simon F., Siles-Lucas M., Morchon R., Gonzalez-Miguel J., Mellado I., Carreton E., Montoya-Alonso JA., 2012. Human and animal dirofilariasis: the emergence of a zoonotic mosaic. Clin Microbiol Rev, 25, 507-544.
12. Utuk AE., Guven Gokmen T., Bolacali M., Balkaya I., Simsek A., 2018. Serologic survey on Canine Leishmaniasis in Kocaeli, Sakarya, Mersin and Elazığ Provinces of Turkey. Isr J Vet Med, 73, 3-7.
13. Sarı B., Taşçı G., Taşkın G., Kılıç Y., 2013. Seroprevalence of Dirofilaria immitis, Ehrlichia canis and Borrelia burgdorferi in dogs in Iğdır province, Turkey. Kafkas Univ Vet Fak Derg, 19, 735-739.

14. Icen H., Sekin S., Simsek A., Kochan A., Celik OY., Altas MG., 2011. Prevalence of Dirofilaria immitis, Ehrlichia canis, Borrelia burgdorferi infection in dogs from Diyarbakir in Turkey. Asian J Anim Vet Adv, 6,371-378.
15. Batmaz H., Nevo E., Waner T., Sentürk S., Yilmaz Z., Harrus S., 2001. Seroprevalence of Ehrlichia canis antibodies among dogs in Turkey. Vet Rec, 148, 665-666.
16. Erdeğer J., Sancak A., Ataseven L., 2003. Köpeklerde Ehrlichia canis'in indirekt fluoresan antikor (IFA) testi ve Dot-ELISA ile saptanması. Turk J Vet Anim Sci, 27, 767-773.
17. Tsachev I., Kontos V., Zarkov I., Krastev., 2006. Survey of antibodies reactive with Ehrlichia canis among dogs in South Bulgaria. Rev Med Vet, 157, 481-485.
18. Maazi N., Malmasi A., Shayan P., Nassiri SM., Salehi TZ., Fard MS., 2014. Molecular and serological detection of Ehrlichia canis in naturally exposed dogs in Iran: an analysis on associated risk factors. Rev Bras Parasitol, 23, 16-22.
19. Piantedosi D., Neola B., D'Alessio N., Di Prisco F., Santoro M., Pacifico L., Sgroi G., Auletta L., Buch J., Chandrashekar R., Breitschwerdt EB., Veneziano V., 2017. Seroprevalence and risk factors associated with Ehrlichia canis, Anaplasma spp., Borelia burgdorferi sensu lato, and D. immitis in hunting dogs from southern Italy. Parasitol Res, 116, 2651-2660.
20. Öncel T., Vural G., 2005. Seroprevalence of Dirofilaria immitis in stray dogs in İstanbul and İzmir. Turk J Vet Anim Sci, 29, 785-789.
21. Civelek T., Yıldırım A., Iça A., 2006. Bursa İli Gemlik yöresi köpeklerde kalp kurdu hastalığının prevalansı. Van Vet Bil Derg, 22, 65-68.
22. Yildirim A., Ica A., Atalay O., Duzlu O., Inci A. 2007. Prevalence and epidemiological aspects of Dirofilaria immitis in dogs from Kayseri province, Turkey. Res Vet Sci, 82, 358-363.
23. Simsek S., Utuk AE., Koroglu E., Rishniw M., 2008. Serological and molecular studies on Dirofilaria immitis in dogs from Turkey. J Helminthol, 82, 181-186.
24. Yaman M., Guzel M., Koltas IS., Demirkazik M., Aktas H., 2009. Prevalence of Dirofilaria immitis in dogs from Hatay province, Turkey. J Helminthol, 83, 255-260.
25. Taşçı TG., Kılıç Y., 2012. Kars ve Iğdır civarındaki köpeklerde Dirofilaria immitis'in prevalansı ve potansiyel vektör sivrisinek türleri üzerine araştırmalar. Kafkas Üniv Vet Fak Derg, 18, 29-34.
26. Köse M., Erdoğan M., 2012. Serological screening of canine heartworm (Dirofilaria immitis) infections in Turkey. Berl Munch Tierarztl Wochenschr, 125, 503-508.
27. Adanır R., Sezer K., Köse O., 2013. The prevalence of Dirofilaria immitis dogs with different breed, ages and sex. Ankara Üniv Vet Fak Derg, 60, 241-244.
28. Ataş AD., Altay K., Alim A., Özkan E., 2018. Survey of Dirofilaria immitis in dogs from Sivas province in the Central Anatolia Region of Turkey. Turk J Vet Anim Sci, 42, 130-134.
29. Diakou A., Kapantaidakis E., Tamvakis A., Giannakis V., Strus N., 2016. Dirofilaria infections in dogs in different areas of Greece. Parasit Vectors, 20, 508.
30. Al-Shabbani A., Al-Shabbani AHA., 2016. In Iraq, the first application of serological SNAP ELISA technique in detection of Canine Heartworms (Dirofilaria immitis) In Herder Dogs of Al-Qadisiyah and Dhi-Qar Provinces. Kufa J Vet Sci, 7,192-198.
31. Bamorovat M., Sharifi I., Harandi MF., Nasibi S., Sadeghi B., Khedri J., Mohammadi MA., 2017. Parasitological, serological and molecular study of Dirofilaria immitis in domestic dogs, Southeastern Iran. Iran J Parasitol, 12, 260-266.
32. Panayotova-Pencheva MS., Mirchev RL., Trifinova AP., 2016. Dirofilaria immitis infection in carnivores from Bulgaria: 2012-2013 update. Bulg J Vet Med, 19, 153-162.

33. Balıkçı E., Sevgili M., 2005. Elazığ ve çevresindeki köpeklerde Dirofilaria İmmitis'in seroprevalansı. FÜ Sağlık Bil Dergisi, 19, 103-106.
34. Küçüker S., Şahinduran Ş., 2018. Antalya İlinde bulunan köpeklerde Dirofilariazis, Borrelioziz, Ehrlichiazis ve Anaplazmozis'in hızlı test kitleri ile teşhisi ve insidansı üzerine araştırmalar. Atatürk Üniversitesi Vet Bil Derg, 13, 191-200.
35. Bhide M., Yilmaz Z., Golcu E., Torun S., Mikula I., 2008. Seroprevalence of anti-Borrelia burgdorferi antibodies in dogs and horses in Turkey. Ann Agric Environ Med, 15, 85-90.
36. Güneş T., Poyraz Ö., Babacan A., 2011. The seroprevalence of Borrelia burgdorferi sensu lato and Anaplasma phagocytophilum in clinically healthy dogs from Sinop region of Turkey. Cumhuriyet Tıp Derg, 33, 396-401.
37. Ural K., Gultekin M., Atasoy A., Ulutas B., 2014. Spatial distribution of vector borne disease agents in dogs in Aegean region, Turkey. Rev MVZ Cordoba, 19, 4086-4098.
38. Pantchev N., Schnyder M., Vrhovec MG., Schaper R., Tsachev I., 2015. Current surveys of the seroprevalence of Borrelia burgdorferi, Ehrlichia canis, Anaplasma phagocytophilum, Leishmania infantum, Babesia canis, Angiostrongylus vasorum and Dirofilaria immitis in dogs in Bulgaria. Parasitol Res, 114, 117-130.
39. Mosallanejad B., Avizeh R., Jalali MHR., Pourmahdi M., 2015. A serological survey on Borrelia burgdorferi infection among companion dogs in Ahvaz district, southwestern Iran. Comp Clin Pathol, 24, 1559-1563.
40. Çetinkaya H., Matur E., Akyazi I., Ekiz EE., Aydin L., Toparlak M., 2016. Serological and molecular investigation of Ehrlichia spp. and Anaplasma spp. in ticks and blood of dogs, in the Thrace Region of Turkey. Ticks Tick Borne Dis, 7, 706-714.
41. Gürel MS., Yeşilova Y., Ölgen MK., Özbel Y., 2012. Cutaneous Leishmaniasis in Turkey. Turkiye Parazitol Derg, 36, 121-129.
42. Bolukbas CS., Pekmezci GZ., Gurler AT., Pekmezci D., Guzel M., Hokele M., Acici M., Umur S., 2016. Evidence of Leishmania spp. antibodies and DNA in dogs in the Middle Black Sea Region of Turkey. Ankara Univ Vet Fak Derg, 63, 111-114.
43. Aydin L., Bakirci S., 2007. Geographical distribution of ticks in Turkey. Parasitol Res, 101, 163-166.
44. Egenvall AE., Hedhammar AA., Bjoersdorff AI., 1997. Clinical features and serology of 14 dogs affected by granulocytic ehrlichiosis in Sweden. Vet Rec, 140, 222-226.
45. Otranto D., Paradies P., Caprariis D., Stanneck D., Testini G., Grimm F., Deplazes P., Capelli G., 2009. Toward diagnosing leishmania infantum infection in asymptomatic dogs in an area where Leishmaniasis is endemic. Clin Vaccine Immunol, 16, 337-343.