Turkish Journal of Agriculture - Food Science and Technology, 10(10): 1832-1837, 2022
DOI: https://doi.org/10.24925/turjaf.v10i10.1832-1837.5338

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Molecular Investigation and Phylogenetic Analysis of *Ehrlichia canis* in Dogs in Siirt, Türkiye

Burçak Aslan Çelik[1,a,*], Özgür Yaşar Çelik[2,b], Ali Bilgin Yılmaz[3,c], Adnan Ayan[4,d], Özlem Orunç Kılınç[5,e], Ramazan Özdemir[6,f], Özge Oktay Ayan[7,g]

[1]*Department of Parasitology, Faculty of Veterinary Medicine, Siirt University, 56100 Siirt, Türkiye*
[2]*Department of Internal Medicine, Faculty of Veterinary Medicine, Siirt University, 56100 Siirt, Türkiye*
[3]*School of Health, Van Yuzuncu Yil University, 65090 Van, Türkiye*
[4]*Department of Genetics, Faculty of Veterinary Medicine, Van Yuzuncu Yil University, 65090 Van, Türkiye*
[5]*Özalp Vocational School, Van Yuzuncu Yil University, 65090 Van, Türkiye*
[6]*Adana Metropolitan Municipality, Adana, Türkiye*
[7]*Department of Medical Parasitology, Van Yuzuncu Yil University School of Medicine, 65090 Van, Türkiye*
[*]*Corresponding author*

ARTICLE INFO

Research Article

Received : 22/06/2022
Accepted : 29/08/2022

Keywords:
Ehrlichia canis
Phylogeny
PCR
Siirt
Türkiye

ABSTRACT

Ehrlichia canis is the primary etiologic agent of canine monocytic ehrlichiosis, a tick-transmitted disease of dogs. The aim of this study is to molecularly investigate the presence of *E. canis* and to reveal its prevalence in dogs in Siirt province. The animal material of the study is consisted of a total of 82 dogs. A region of the 16S ribosomal RNA gene of *E. canis* was targeted for PCR amplification. As a result of the conducted Nested-PCR, positivity was detected at the rate of 10.53% (4/38) in male dogs and 13.64% (6/44) in females, and *Ehrlichia canis* specific bands of size 389 bp were obtained in 10 (12.20%) dogs in total. The phylogenetic tree was constructed with the Maximum Likelihood (MCL) method, The nucleotide sequence was registered in the NCBI GenBank database with access numbers OK331365.1-OK331366. Early detection of the disease by means of hematological, serological, or molecular tests is very important in terms of prognosis. More studies should be performed to determine vector-disease relationships in this region about ticks that vector the disease.

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 10(10): 1832-1837, 2022

Siirt İli köpeklerinde *Ehrlichia canis*'in Moleküler Yöntemle Araştırılması ve Filogenetik Analizi

MAKALE BİLGİSİ

Araştırma Makalesi

Geliş : 22/06/2022
Kabul : 29/08/2022

Anahtar Kelimeler:
Ehrlichia canis
Filogeni
PZR
Siirt
Türkiye

ÖZ

Ehrlichia canis, köpeklerde kene aracılığıyla bulaşan bir hastalık olan köpek monositik ehrlichiosis'in birincil etiyolojik ajanıdır. Bu çalışmanın amacı, Siirt ilindeki köpeklerde E. canis varlığını moleküler olarak araştırmak ve yaygınlığını ortaya koymaktır. Araştırmanın hayvan materyalini toplam 82 köpek oluşturdu. *E. canis*'in 16S ribozomal RNA geninin bir bölgesi, PCR amplifikasyonu için hedeflendi. Yapılan Nested-PCR sonucunda erkek köpeklerde %10,53 (4/38), dişilerde %13,64 (6/44) oranında pozitiflik saptanmış ve toplamda 10 (%12,20) hayvanda 389 bp büyüklüğünde *Ehrlichia canis*'e özgü bantlar elde edilmiştir. Filogenetik ağaç, Maximum Likelihood (MCL) yöntemiyle oluşturulmuştur, Nükleotid dizisi, OK331365.1-OK331366 erişim numaralarıyla NCBI GenBank veritabanına kaydedilmiştir. Hastalığın hematolojik, serolojik veya moleküler testler ile erken teşhisi prognoz açısından oldukça önemlidir. Hastalığı vektörlüğünü yapan keneler hakkında bu bölgedeki vektör-hastalık ilişkilerini belirlemek için daha fazla çalışma yapılması gerektiği kanaatine varılmıştır.

[a] burcakaslan@siirt.edu.tr https://orcid.org/0000-0002-0130-970X
[b] oyc@siirt.edu.tr https://orcid.org/0000-0001-6365-2688
[c] alibilginyilmaz@yyu.edu.tr https://orcid.org/0000-0003-0749-2418
[d] adnanayan@yyu.edu.tr https://orcid.org/0000-0002-6564-3416
[e] ozlemkilinc@yyu.edu.tr https://orcid.org/0000-0001-6233-7109
[f] ozdemirramazan01@hotmail.com https://orcid.org/0000-0002-5896-2777
[g] ozgeokty09@gmail.com https://orcid.org/0000-0003-2577-3774

Introduction

Ehrlichia is a tick-transmitted, obligate intracellular, gram-negative bacteria associated with serious and sometimes fatal diseases in humans and some domestic and wild animals (Murphy et al., 1998; Ndip et al., 2005; Cihan et al., 2010; Ajaj et al., 2020). *E. canis* is the primary etiologic agent of canine monocytic ehrlichiosis (CME), a tick-transmitted disease of dogs (Rodriguez-Vivas et al., 2005; Sarı et al., 2009; Vargas-Hernández et al., 2012). CME is prevalent in tropical and subtropical regions around the world (Breitschwerdt et al., 1998; Sarı et al., 2009; İçen et al., 2011). The disease was first identified in Algeria and was reported later in some countries of Western Europe, America, Africa, and the Middle East (Brouqui et al., 1991; Waner et al., 1999; Erdeğer et al., 2003).

The disease is transmitted through infected ticks or by blood of infected dogs to other dogs (Sarı et al., 2009). The dominant type of tick *Rhipicephalus sanguineus* species in dogs in Türkiye (which is known as the brown dog tick), plays a role in the spread of the disease (Dodurka and Bakirel, 2002; Erdeğer et al., 2003; Ndip et al., 2005; Rodriguez-Vivas et al., 2005; Sarı et al., 2009; Aslantaş et al., 2020). All dog breeds can be infected with *E. canis*, however it is reported that German shepherd dogs are more susceptible to this disease than other breeds (Brouqui et al., 1991; Erdeğer et al., 2003).

CME progresses in acute, subclinical, and sometimes chronic forms after an incubation period of 8-20 days. Depression, lethargy, weight loss, anorexia, pyrexia, stagnation, nasal discharge, dyspnea, lymphadenopathy, edema of the extremities and scrotum, occasional epistaxis, hypersensitivity and the presence of tics are reported for the acute phase, as well as a series of central nervous system findings, including cranial nerve damage (Dodurka and Bakirel, 2002; Erdeğer et al., 2003; Sarı et al., 2009). Clinical signs are usually absent in the subclinical form (Sarı et al., 2009; Ajaj et al., 2020; Aslan Çelik et al., 2020). Thrombocytopenia, leukopenia and normocytic, normochromic anemia are the most important laboratory findings of the disease (Cihan et al., 2010).

It has been reported that the disease can become chronic and last for years if it cannot be diagnosed and treated in time, and this chronic phase may be asymptomatic in some dogs. More severe clinical findings may also occur in some dogs compared to the acute phase findings (Dodurka and Bakirel, 2002).

The diagnosis of ehrlichiosis is often difficult and laboratory examinations are needed. Blood smears, Immunofluorescence assay (IFA), Western Blot, Enzyme-Linked Immuno Sorbent Assay (ELISA) and Polymerase Chain Reaction (PCR) methods are used for this purpose (Dodurka and Bakirel, 2002; Erdeğer et al., 2003; Rodriguez-Vivas et al., 2005; Carvalho et al., 2008). PCR is used in the early diagnosis of ehrlichiosis (Carvalho et al., 2008; Düzlü et al., 2014) and it is a recommended method (Malik et al., 2018). It is reported that the most common gene region used in the differentiation of different genotypes of *E. canis* is the 16S rRNA region in this method (Carvalho et al., 2008; Düzlü et al., 2014).

It is very important to determine the presence and prevalence of tick-borne pathogens in dogs, and to reveal their molecular epizootiology, in order to take the necessary treatment, control and prevention measures (Düzlü et al., 2014). Studies on the investigation of E.canis in dogs by molecular methods are quite limited in Türkiye. The aim of this study is to molecularly investigate the presence of *E. canis* and to reveal its prevalence in dogs in Siirt province.

Materials and Methods

The Study Area

The Siirt province is located in the Southeastern Anatolia Region of Türkiye (37° 55' N, 41° 57' E). Siirt province is in a semi-arid climate region, where the average highest and lowest temperatures range between 36.9°C and 18.9°C in summer, and 8.7°C and -0.5°C in winter. Water shortages are frequent during the summer.

Animal Material and Sample Collection

The animal material of the study is consisted of a total of 82 dogs that appeared clinically healthy. The animals were examined to record their age, sex, and any presence of ticks. 2 mL blood samples were taken from the vena cephalica antebrachii of each of the dogs into EDTA tubes, which were then brought to the laboratory under cold chain conditions.

DNA extraction, PCR amplification and Sequence Analysis

DNA extraction from samples was performed with the PureLink™ Genomic DNA Mini Kit (USA, K182002). Nested PCR method was performed to amplify the 16S rRNA gene region of *Ehrlichia canis*. ECC (5′ AGAACGAACGCTGGCGGCAAGC-3′) and ECB (5′ CGTATTACCGCGGCTGCTGGCA-3′) primers were used in step 1 of Nested PCR, while ECAN5 (5-CAATTATTTATAGCCTCTGGCTATAGGA-3′) and HE3 (5′ TATAGGTACCGTCATTATCT) were used in step 2 (Murphy et al., 1998; Alves et al., 2014; Makino et al., 2015; Ayan et al., 2020). Protocol for both reactions was followed based on the suggestions of Ayan et al. (2020). Reaction Gradient PCR was performed on a SuperCycler (Kyratec, Australia) device. Subsequently, 1.5% agarose gel was prepared and stained with RedSafe™ Nucleic Acid Staining Solution. The PCR products were run on an agarose gel afterwards, and images were obtained on the gel imaging device (Syngene bio imaging system).

Sequence and Phylogenetic Analysis

Two positive PCR products were purified and sequenced for each sample. The sequences of each amplicon were manually aligned and edited. 16S rRNA gene sequences were subjected to GenBank's BLAST analysis. The evolutionary history was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the Maximum Composite Likelihood method. Evolutionary analyses were conducted in MEGA11. *Anaplasma platys* was used as outgroup.

Table 1. Molecular survey of *Ehrlichia canis* in dogs - analysis for sex, age and presence of ticks

Variable	Number of dogs (n)	Positive		P
		(n)	(%)	
Sex				
Female	44	6	13.64	NS
Male	38	4	10.53	
Age				
<1	27	3	11.11	
1-3	26	4	15.38	NS
>3	29	3	10.34	
Presence of ticks				
Yes	24	6	25.00	**
No	58	4	6.90	
Total	82	10	12.20	

NS: Non-significant, **: P<0.01

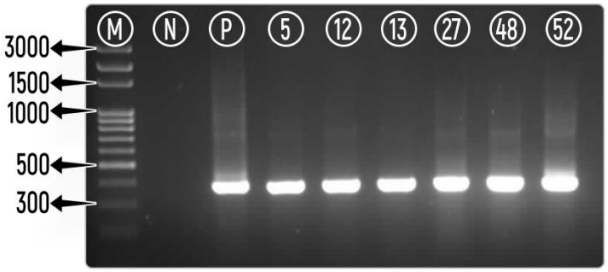

Figure 1. 16S rRNA amplification of *E. canis* using nested-PCR. Lanes M: Marker, N: Negative control, P: positive control, Lanes 5,12,13,27,48 and 52 represent *E. canis* positive samples (389 bp).

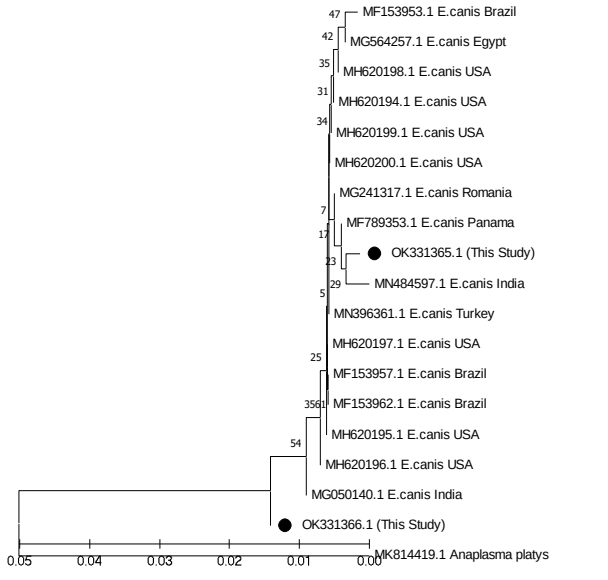

Figure 2. The evolutionary history was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method. Evolutionary analyses were conducted in MEGA11. Anaplasma platys was used as outgroup.

Statistical Analysis

The data obtained in the study were analyzed using the SPSS V16.0 program. The relationship between grouped variables was calculated using chi-square test.

Ethical Approval

Ethical approval for this study was obtained from the Siirt University Local Ethics Committee for Animal Experiments (Decision number 2021/01/11).

Results

Tick infestation was detected in 24 (29.27%) dogs during clinical examinations. The ticks were morphologically identified as *R. sanguineus*. As a result of the conducted Nested-PCR, positivity was detected at the rate of 10.53% (4/38) in male dogs and 13.64% (6/44) in females (Table 1), and Ehrlichia *canis* specific bands of size 389 bp were obtained in 10 (12.20%) of 82 dogs in total (Figure 1).

The phylogenetic tree was constructed with the Neighbor-Joining method, using the DNA sequences. Evolutionary analyses were conducted in MEGA11 (Figure 2). *E. canis* strains were found to be 100% similar with other (MN484597.1-MN396361.1-MH620200.1-MK507008.1) registered *E. canis* strains in GenBank, according to the BLAST analysis. The nucleotide sequence was registered in the NCBI GenBank database with access numbers OK331365.1-OK331366.

Discussion

In recent years, tick-borne diseases such as ehrlichiosis have become widespread worldwide, threatening the health of both humans and domestic and wild animals (Düzlü et al., 2014; Cetinkaya et al., 2016). The prevalence of *E. canis* depends on the distribution of the vector *R. sanguineus* which is largely seen in tropical and subtropical regions (Rodriguez-Vivas et al., 2005; Cihan et al., 2010; Kamani et al., 2013; Tanikawa et al., 2013; Iweriebor et al., 2017). Many studies have been conducted around the world to determine the epidemiology of CME in dogs, and most of them are based on serological methods (Ndip et al., 2005; Küçüker and Şahinduran, 2018). The results obtained from the studies differ based on to the specifics of the research and the tests implemented in the studies.

In studies carried out to determine the prevalence of *E.canis* worldwide, 3.1% prevalence was determined in Oklahoma (Murphy et al., 1998), while this number was 0.8% in North America (Beall et al., 2012), 69.4% by serological method and 3.7% by PCR method in Brazil (Tanikawa et al., 2013), and 82.4% by serological method (IFAT) and 40.6% by molecular method (PCR) in Colombia (Vargas-Hernández et al., 2012). Meanwhile, 44.1% (Rodriguez-Vivas et al., 2005) and 29.26% (Ojeda-Chi et al., 2019) *E. canis* prevalence was determined in Mexico in two different studies, and 6.7% prevalence was determined in Buenos Aires (Cicuttin et al., 2016). This rate was 32% in Cameroon (Ndip et al., 2005), 11% in Nigeria (Kamani et al., 2013), 6.36% in Algeria (Dahmani et al., 2015), 28% in Pakistan (Malik et al., 2018), 2% in Malaysia (Nazari et al., 2013), 30% in Israel (Baneth et al.,

1996), 10.2% in Iraq (Ajaj et al., 2020), 4.7% in Japan (Inokuma et al., 1999) and 16.18% in Italy (Ebani, 2019).

E. canis was first diagnosed in 1997 in Türkiye by Dodurka and Bakirel (2002)**,** and studies on the prevalence of *E. canis* were mainly conducted towards the detection of antibodies in dogs (Düzlü et al., 2014; Küçüker and Şahinduran, 2018; Haydardedeoğlu et al., 2019).

In studies conducted in Türkiye using rapid diagnostic test kits, 17.7% prevalence has been reported in Antalya (Küçüker and Şahinduran, 2018), while the ratio was 4.8% in Diyarbakır (İçen et al., 2011), 1% in Iğdır (Sarı et al., 2009), and 3% in Osmaniye (Gokmen et al., 2019).

In studies carried out using serological methods, on the other hand, a prevalence of 25.8% with the IFA method and 3.2% with the Dot-ELISA method was determined in and around Ankara by Erdeğer et al. (2003). The same researchers reported a prevalence of 74% with the IFA method and 65.4% with the Dot-ELISA method in Aydın and Muğla provinces. Cihan et al. (2010) reported a prevalence of 69.4% with the IFA method in Balıkesir and İzmir provinces. In Sinop, a prevalence of 18.28% was determined by ELISA method (Güneş et al., 2012). In studies carried out by Batmaz et al. (2001) in Bursa, Balıkesir, İzmir, Şanlıurfa, Adana and Antalya, seropositivity of 13.99%, 13.16%, 40.63%, 7.41%, 65.39% and 11.11% was reported, respectively. As a result of the serological study carried out by Haydardedeoğlu et al. (2019) on 40 Aksaray Malaklı shepherd dogs, all dogs were found to be negative for *E. canis*.

In studies carried out with PCR methods, 41.5% prevalence was determined in Aydın (Karagenç et al., 2005), while the ratio was 14.5% in Kayseri (Düzlü et al., 2014), 9.77% in Erzurum (Guven et al., 2017), and 30.56%, 8.89%, 14.29%, 0.00%, 0.00%, respectively in Mersin, Adana, Gaziantep, Hatay and Batman provinces (Aslantaş et al., 2020). Similarly, 0% seropositivity was reported in a study performed in Konya (Guo et al., 2017).

E. canis strain OK331366.1 was found to be 100% similar with MN484597.1 (India), MN396361.1 (Türkiye), MH620200.1 (USA) and MK507008.1 (Cuba) strains. Also OK331365.1 strain was found to be 99% similar with MH620200.1 (USA), MK507008.1 (Cuba) and MF789353.1 (Panama) strains registered in GenBank, according to the BLAST analysis.

Rhipicephalus sanguineus ticks are the vector of *E. canis*. These ticks are spread around worldwide, and are especially common in tropical and subtropical regions (Aguirre et al., 2004). Siirt province is under the influence of a typical Mediterranean climate with mild and rainy winters and hot and dry summers. This situation provides a suitable environment for vector ticks. In this study, as a result of morphological identification of ticks collected from 24 dogs, all ticks were determined to be the *R. sanguineus* type.

A prevalence of 12.20% was determined in this study using the PCR method. These results obtained are similar to some of studies by the following researchers: Batmaz et al. (2001), Güneş et al. (2012), Kamani et al. (2013), Düzlü et al. (2014), Küçüker and Şahinduran (2018), and Ajaj et al. (2020). Geographical conditions, climatic diversity, sample size, age range of the population, habitat of animals, presence of ticks and methods used can be counted among the reasons for the differences observed between other studies.

It is reported that no positivity was found in a study conducted with rapid diagnosis test kits in Siirt province (Aslan Çelik et al., 2020). In this study, a prevalence of 12.20% was determined by the nested-PCR method. The possible reasons for the difference between the two studies include the sampling period, sample size, and methods used. In addition, the use of PCR method is recommended for the accurate diagnosis of ehrlichiosis (Malik et al., 2018).

In terms of gender, the highest positivity rate was seen in females, but no statistically significant difference was detected between sexes in this study. These results support the works of researchers (Cihan et al., 2010; Guven et al., 2017; Malik et al., 2018).

Malik et al. (2018) showed that dogs younger than one year are more susceptible than those older than one year, while in another study carried out by Rodriguez-Vivas et al. (2005) it was reported that dogs aged 2-4 years were more susceptible. No statistically significant difference was found between the two age groups in any of these studies. In the present study, the highest positivity ratio was detected in the 1-3 age group (15.38%), and the lack of statistically significant difference between age groups supports the findings of previous research.

A positive correlation between infection and tick presence was reported in a study conducted by Aktas et al. (2013) while Malik et al. (2018) reported that there is no positive correlation between infection and the presence of ticks. The detection of a statistically significant difference between the presence of ticks and infection in this study supports the work carried out by Aktas et al. (2013).

Conclusion

The current prevalence of *E. canis* in Siirt province has been investigated by this study. CME should be considered in dogs presenting to the clinic with stated symptoms of the disease. Early detection of the disease by means of hematological, serological, or molecular tests is very important in terms of prognosis. More studies should be performed to determine vector-disease relationships in this region about ticks that vector the disease.

Acknowledgement

Conflicts of Interest

The authors declare that they have no competing interests

References

Aguirre E, Sainz A, Dunner S, Amusategui I, López L, Rodríguez-Franco F, Luaces I, Cortés O, Tesouro MA. 2004. First isolation and molecular characterization of Ehrlichia canis in Spain. Veterinary parasitology 125: 365-372.

Ajaj E, Mohammed H, and Al-Bayati L. 2020. Seroprevalence of ehrlichiosis in stray dogs in Nineveh Province. EurAsian Journal of BioSciences 14: 5883-5887.

Aktas M, Özübek S, Ipek DNS. 2013. Molecular investigations of Hepatozoon species in dogs and developmental stages of Rhipicephalus sanguineus. Parasitology research 112: 2381-2385.

Alves RN, Rieck SE, Ueira-Vieira C, Labruna MB, and Beletti ME. 2014. Isolation, in vitro propagation, genetic analysis, and immunogenic characterization of an Ehrlichia canis strain from southeastern Brazil. Journal of veterinary science 15: 241-248.

Aslan Çelik B, Çelik O, Şahin T, İrak K, Bolacalı M, Biçici Ö, Baldaz V. 2020. A Serologic Survey of Dirofilariasis, Leishmaniasis, Ehrlichiosis, and Anaplasmosis in Dogs in Siirt Province. Fırat Üniversitesi Sağlık Bilimleri Veteriner Dergisi 34: 103-108.

Aslantaş Ö, Çelebi B, Usluca S. 2020. Investigation of vector-borne diseases in dogs. Harran Üniv Vet Fak Derg 9: 154-160.

Ayan A, Kilinc O, Erdogan S, Akyildiz G, Bia M, Lee D. 2020. High prevalence of Ehrlichia canis in dogs in Van, Türkiye. Appl Ecol Environ Res 18: 1953-1960.

Baneth G, Waner T, Koplah A, Weinstein S, Keysary A. 1996. Survey of Ehrlichia canis antibodies among dogs in Israel. Veterinary Record 138: 257-259.

Batmaz H, Senturk S, Yilmaz Z, Harrus S, Nevo E, and Waner T. 2001. Seroprevalence of Ehrlichia canis antibodies among dogs in Türkiye. The Veterinary Record 148: 665.

Beall MJ, Alleman AR, Breitschwerdt EB, Cohn LA, Couto CG, Dryden MW, Guptill LC, Iazbik C, Kania SA, Lathan P. 2012. Seroprevalence of Ehrlichia canis, Ehrlichia chaffeensis and Ehrlichia ewingii in dogs in North America. Parasites and vectors 5: 1-11.

Breitschwerdt EB, Hegarty BC, Hancock SI. 1998. Sequential evaluation of dogs naturally infected with Ehrlichia canis, Ehrlichia chaffeensis, Ehrlichia equi, Ehrlichia ewingii, or Bartonella vinsonii. Journal of Clinical Microbiology 36: 2645-2651.

Brouqui P, Davoust B, Haddad S, Vidor E, and Raoult D. 1991. Serological evaluation of Ehrlichia canis infections in military dogs in Africa and Reunion Island. Veterinary microbiology 26: 103-105.

Carvalho F, Wenceslau A, Carlos R, and Albuquerque G. 2008. Epidemiological and molecular study of Ehrlichia canis in dogs in Bahia, Brazil. Genetics and Molecular Research 7: 657-662.

Cetinkaya 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 Türkiye. Ticks Tick Borne Dis 7: 706-714.

Cicuttin GL, De Salvo MN, Dohmen FEG. 2016. Molecular characterization of Ehrlichia canis infecting dogs, Buenos Aires. Ticks and tick-borne diseases 7: 954-957.

Cihan H, Temizel EM, Davoust B, Mariã L, Casali F, Parzy D, Aytuğ N. 2010. Silent Threat: Subclinical Canine Monocytic Ehrlichiosis in Stray Dogs in Türkiye Uludağ Üniversitesi Veteriner Fakültesi Dergisi 29: 15-19.

Dahmani M, Loudahi A, Mediannikov O, Fenollar F, Raoult D, Davoust B. 2015. Molecular detection of Anaplasma plays and Ehrlichia canis in dogs from Kabylie, Algeria. Ticks and tick-borne diseases 6: 198-203.

Dodurka HT, Bakirel U. 2002. A case of Ehrlichiosis in a dog. İÜ Vet. Fak Derg 28: 11-16.

Düzlü Ö, İnci A, Yıldırım A, Önder Z, Çiloğlu A. 2014. The investigation of some tick-borne protozoon and rickettsial infections in dogs by Real Time PCR and the molecular characterizations of the detected isolates. Ankara Üniv Vet Fak 61: 275-282.

Ebani VV. 2019. Serological survey of Ehrlichia canis and Anaplasma phagocytophilum in dogs from central Italy: An update (2013–2017). Pathogens 8: 3.

Erdeğer J, Sancak A, Ataseven L. 2003. Detection of Ehrlichia canis in Dogs by IFA Test and Dot-ELISA. Turkish Journal of Veterinary and Animal Sciences 27: 767-773.

Gokmen TG, Günaydin E, Turut N, Bünyamin A, Özgür K, Ütük AE. 2019. A serosurvey on some canine vector-borne zoonoses (Anaplasma spp., Ehrlichia spp., Borrelia burgdorferi, Dirofilaria immitis and Leishmania spp.) in Osmaniye. Atatürk Üniversitesi Veteriner Bilimleri Dergisi 14: 151-158.

Guo H, Sevinc F, Ceylan O, Sevinc M, Ince E, Gao Y, Moumouni PFA, Liu M, Efstratiou A, Wang G. 2017. A PCR survey of vector-borne pathogens in different dog populations from Türkiye. Acta parasitologica 62: 533-540.

Guven E, Avcioglu H, Cengiz S, Hayirli A. 2017. Vector-borne pathogens in stray dogs in Northeastern Türkiye. Vector-Borne and Zoonotic Diseases 17: 610-617.

Güneş T, Poyraz Ö, Babacan A. 2012. The seroepidemiologic survey of Ehrlichia canis and Rickettsia conorii in clinically healthy dogs from Sinop region. Cumhuriyet Med J 34: 17-22.

Haydardedeoğlu AE, Büyükleblebici O, Aksoy NH, Karaşahin T. 2019. Investigation of Hematological Findings by Determining the Spatial Distribution of Infections of Ehrlichia Canis, Anaplasma Phagocytophilum, Borrelia Burgdorferi, Dirofilaria immitis in Aksaray Malakli Dogs. Harran Üniversitesi Veteriner Fakültesi Dergisi 8: 38-43.

İçen H, Sekin S, Şimşek A, Koçhan A, Çelİk ÖY, Altaş Mg. 2011. Prevalence of Dirofilaria immitis, Ehrlichia canis, Borrielia burgdorferi infection in dogs from Diyarbakir in Türkiye. AJAVA 6: 371-378.

Inokuma H, Ohno K, Yamamoto S. 1999. Serosurvey of Ehrlichia canis and Hepatozoon canis infection in dogs in Yamaguchi Prefecture, Japan. Journal of Veterinary Medical Science 61: 1153-1155.

Iweriebor BC, Mmbaga EJ, Adegborioye A, Igwaran A, Obi LC, Okoh AI. 2017. Genetic profiling for Anaplasma and Ehrlichia species in ticks collected in the Eastern Cape Province of South Africa. BMC Microbiol 17: 45.

Kamani J, Lee CC, Haruna AM, Chung PJ, Weka PR, Chung YT. 2013. First detection and molecular characterization of Ehrlichia canis from dogs in Nigeria. Research in veterinary science 94: 27-32.

Karagenç T, Hoşgör M, Bilgiç H, Paşa S, Kırlı G, Eren H. 2005. *The determination of the prevalence of E. canis, A. phagocytophila, A. platys with nested PCR in dogs in the Aegean Region.* Paper presented at the Proceedings of the XIV National Parasitology Congress, İzmir.

Küçüker S, Şahinduran Ş. 2018. Reasearch in to the seroprevalance of Dirofilariasis, Borreliosis, Ehlichiosis and Anaplasmosis in Antalya. Atatürk Üniversitesi Vet. Bil. Derg 13: 191-200.

Makino H, Sousa VRF, Fujimori M, Rodrigues JY, Dias AFLR, Dutra V, Nakazato L, Almeida AdBPFd. 2015. Ehrlichia canis detection in dogs from Várzea Grande: a comparative analysis of blood and bone marrow samples. Ciência Rural 46: 310-314.

Malik MI, Qamar M, Ain Q, Hussain MF, Dahmani M, Ayaz M, Mahmood AK, Davoust B, Shaikh RS, Iqbal F. 2018. Molecular detection of Ehrlichia canis in dogs from three districts in Punjab (Pakistan). Veterinary medicine and science 4: 126-132.

Murphy GL, Ewing S, Whitworth LC, Fox JC, Kocan AA. 1998. A molecular and serologic survey of Ehrlichia canis, E. chaffeensis, and E. ewingii in dogs and ticks from Oklahoma. Veterinary parasitology 79: 325-339.

Nazari M, Lim SY, Watanabe M, Sharma RS, Cheng NA, Watanabe M. 2013. Molecular detection of Ehrlichia canis in dogs in Malaysia. PLoS neglected tropical diseases 7: e1982.

Ndip L, Ndip RN, Esemu S, Dickmu V, Fokam E, Walker D, McBride J. 2005. Ehrlichial infection in Cameroonian canines by Ehrlichia canis and Ehrlichia ewingii. Veterinary microbiology 111: 59-66.

Ojeda-Chi MM, Rodriguez-Vivas RI, Esteve-Gasent MD, de León AAP, Modarelli JJ, Villegas-Perez SL. 2019. Ehrlichia canis in dogs of Mexico: Prevalence, incidence, co–infection and factors associated. Comparative immunology, microbiology and infectious diseases 67: 101351.

Rodriguez-Vivas R, Albornoz R, Bolio G. 2005. Ehrlichia canis in dogs in Yucatan, Mexico: seroprevalence, prevalence of infection and associated factors. Veterinary Parasitology 127: 75-79.

Sarı B, Taşçı G, Kılıç Y. 2009. Seroprevalence of Dirofilaria immitis, Ehrlichia canis and Borrelia burgdorferi in Dogs in Iğdır Province, Türkiye. Kafkas Univ Vet Fak Derg 19: 735-739.

Tanikawa A, Labruna M, Costa A, Aguiar D, Justiniano S, Mendes R, Melo A, Alves C, Azevedo S. 2013. Ehrlichia canis in dogs in a semiarid region of Northeastern Brazil: Serology, molecular detection and associated factors. Research in veterinary science 94: 474-477.

Vargas-Hernández G, André M, Faria J, Munhoz T, Hernandez-Rodriguez M, Machado R, Tinucci-Costa M. 2012. Molecular and serological detection of Ehrlichia canis and Babesia vogeli in dogs in Colombia. Veterinary parasitology 186: 254-260.

Waner T, Keysary A, Bark H, Sharabani E, Harrus S. 1999. Canine monocytic ehrlichiosis-an overview. Israel Journal of Veterinary Medicine 54: 103-107.