Assiut University web-site: www.aun.edu.eg

MOLECULAR INVESTIGATION OF SOME BACTERIA (*COXİELLA BURNETİİ, MYCOPLASMA HAEMOCANİS, CANDİDATUS* MYCOPLASMA HAEMATOPARVUM, WOLBACHİA) IN *RHIPICEPHALUS SANGUINEUS* TICKS IN SIIRT PROVINCE, TURKEY

BURÇAK ASLAN ÇELIK; ÖZGÜR YAŞAR ÇELIK; ADNAN AYAN; ALI BILGIN YILMAZ; ÖZLEM ORUNÇ KILINÇ; EZGI ŞABABOĞLU AND ÖZGE OKTAY AYAN

[1] Department of Parasitology, Faculty of Veterinary Medicine, Siirt University, Siirt, Turkey, +905428277616,
[2] Department of Internal Medicine, Faculty of Veterinary Medicine, Siirt University, Siirt, Turkey, +905373559889, oyc@siirt.edu.tr,
[3] Department of Genetics, Faculty of Veterinary Medicine, Van Yuzuncu Yil University, Van, Turkey, +905434399817, adnanayan@yyu.edu.tr,
[4] School of Health, Van Yuzuncu Yil University, Van, TURKEY, +905068860958, alibilginyilmaz@yyu.edu.tr,
[5] Özalp Vocational School, Van Yuzuncu Yil University, Van, Turkey, +905059380462, ozlemkilinc@yyu.edu.tr
[6] Department of Microbiology, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, Burdur, Turkey, +905063239825, ezgisababoglu@mehmetakif.edu.tr,
[7] Department of Medical Parasitology, Van Yuzuncu Yil University School of Medicine, Van, Turkey, +905535630865, ozgeokty09@gmail.com

Received: 24 July 2022; **Accepted:** 4 August 2022

ABSTRACT

Ticks harbor the largest diversity of microorganisms, ranging from viruses, prokaryotes, and eukaryotes. *Rhipicephalus sanguineus* ticks are the most common ticks worldwide. Although dogs are the main host of this tick species, it has been reported that it also infests humans in various parts of the world. This study aimed to examine some bacteria (*Coxiella burnetii*, *Mycoplasma haemocanis*, *Candidatus* Mycoplasma haematoparvum, Wolbachia) in *Rhipicephalus sanguineus* ticks sampled from dogs. In this study, 350 tick samples collected from 85 dogs in Siirt province were determined to be *Rhipicephalus sanguineus* ticks. *Coxiella* DNA was detected in 3 (0.85%) out of 350 ticks using Nested PCR (687 base pairs). None of the samples were found to contain *Mycoplasma haemocanis*, *Candidatus* Mycoplasma haematoparvum, and Wolbachia DNA. A partial sequence of the IS*1111* gene region was registered in GenBank with OM472143 accession numbers. Considering the zoonotic nature of the Q disease, it is very important for dog owners and related institutions to periodically spray animals against ticks, and to take any other necessary precautions. More samples are needed to determine the Mhc, CMhp, and Wolbachia prevalence.

Keywords: *C. burnetii, Mycoplasma haemocanis, Candidatus* Mycoplasma haematoparvum, *Rhipicephalus sanguineus, Siirt*

Corresponding author: Burçak Aslan Çelik
E-mail address: burcakaslan@siirt.edu.tr
Present address: Department of Parasitology, Faculty of Veterinary Medicine, Siirt University, Siirt, Turkey, +905428277616,

INTRODUCTION

Ticks, after mosquitoes, are the most prevalent arthropod vectors, capable of spreading the widest range of infections. For this reason, the detection of microorganisms carried by ticks is an important issue for human or animal health (Plantard *et al.*, 2012). Ticks are responsible for the transmission of a variety of infections that infect both people and animals, including bacteria, helminths, protozoa, and viruses (Dantas-Torres, 2010; Ayan *et al.*, 2019).

Q fever is an important highly infectious zoonotic disease caused by an obligate intracellular gram-negative bacterium called *Coxiella burnetii* (Leulmi *et al.*, 2016; Rezaei *et al.*, 2018; Ma *et al.*, 2020). *C. burnetii* can survive in outdoor environments for long periods since it is resistant to many physical and chemical factors (Maurin and Raoult, 1999; Kalender, 2001). A wide range of reservoirs exists for the disease that consists of domestic and wild mammals, birds, and arthropods (Rezaei *et al.*, 2018; Tukur *et al.*, 2019). Rodents, birds, and rabbits play an important role as reservoirs, but cattle, sheep, and goats are the primary reservoirs that are related to potential human infection (Webster *et al.*, 1995; Ma *et al.*, 2020). Animals acquire the infection by direct contact with diseased material, or through ticks (Kalender, 2001; Rezaei *et al.*, 2018). Infections caused by *C. burnetii* in animals are largely asymptomatic, but coxiellosis is known to cause decreased fertility, abortions, infertility, retained placenta, weak newborns, and perinatal deaths in ruminants (Woldehiwet, 2004; Cantas *et al.*, 2011; Ma *et al.*, 2020). People who come into contact with animals (such as veterinarians, and slaughterhouse workers) are at high risk (Kılıç, 2017). In humans, Q fever may occur subclinically, with no clinical signs of an acute or chronic disease that can cause life-threatening conditions or death (Cooper *et al.*, 2011). The most important clinical signs in humans are high fever and severe headaches (Kalender, 2001). Individuals who come into contact with infected asymptomatic animals, especially at the time of bearing, can become infected (Rezaei *et al.*, 2018). While Microagglutination (MA), Complement Fixation (CF), Indirect Fluorescent Antibody (IFA), and Enzyme-Linked Immunosorbent Assay (ELISA) tests are used for the serological diagnosis of the disease (Kalender, 2001), the PCR method is successfully used as a molecular method (Rezaei *et al.*, 2018).

Hemotropic Mycoplasmas (Haemoplasmas) are epierythrocytic parasites of mammals that are small, pleomorphic, cell wall-deficient, facultative intracellular bacteria in the group of non-cultured mycoplasma species (Sykes *et al.*, 2005; Barker *et al.*, 2010; Sababoglu *et al.*, 2021).
These bacteria cause asymptomatic intravascular infections in domestic and wild animals, however, they are not regarded particularly harmful (Maggi *et al.*, 2013).

The disease agent can be determined by Giemsa's staining of blood smears in the form of small coccoids, rings, or strings on the erythrocyte membrane, or they can be found free in the plasma (Lumb, 2001; Hosseini *et al.*, 2017). Although the pathogenic potential of hemotropic mycoplasmas as a cause of human disease is unknown, these zoonotic pathogens may constitute a greater public health threat than is currently recognized (Maggi *et al.*, 2013). Two types of hemotropic mycoplasma have been identified that infect dogs, which are *Mycoplasma haemocanis* (Mhc) and *Candidatus* Mycoplasma haematoparvum (CMhp) (Messick, 2004; Rosanna *et al.*, 2020; Sababoglu *et al.*, 2021). Mhc infection usually causes clinically significant anemia only in splenectomized or immunocompromised dogs, although latent infections can still cause subclinical anemia (Messick, 2004; Barker *et al.*, 2010). CMhp was first described in association with anemia in a splenectomized dog undergoing chemotherapy for leukemia (Barker *et al.*, 2010). The infection is characterized by

fatigue, depression, loss of appetite, weight loss, and anemia and it can cause death (Lumb, 2001; Hosseini *et al.*, 2017).

Wolbachia is classified within the Rickettsiale order and is obligate-intracellular bacteria transmitted by a wide range of arthropods (Chao *et al.*, 2021). Wolbachia was first detected in the ovaries and testicles of the mosquito *Culex pipiens*. Wolbachia is so frequent and omnipresent that some studies have estimated they have infected almost half of the earth-based arthropods, and more than half of the insects overall (Yildirim *et al.*, 2013; Chao *et al.*, 2021)**.** While their involvement with mosquitos has been well-established, their presence in ticks or tick-transmitted pathogens is not well understood (Chao *et al.*, 2021). Wolbachia have been detected in several studies in ticks (Hartelt *et al.*, 2004; Tijsse-Klasen *et al.*, 2011).

The objectives of this study were to examine some bacteria (*Coxiella burnetii*, *Mycoplasma haemocanis, Candidatus* Mycoplasma haematoparvum, Wolbachia) in *Rhipicephalus sanguineus* ticks sampled from dogs.

MATERIALS AND METHODS

Study area and Ticks Collection

Tick samples in this study were collected from 85 dogs in Siirt province. After the dogs were inspected, the ticks were collected into separately labeled 25 mL containers containing 70% alcohol and taken to the laboratory.

Tick Morphology and DNA separate

The detection of ticks was carried out by the method reported by Walker *et al.* (2000) and Estrada-Peña *et al.* (2004). Before DNA extraction, each sample was washed with 70% ethanol. Then, the ticks were taken into individual tubes and subjected to freezing and thawing processes. Ticks inside the tubes were crushed using a sterile glass rod. For DNA isolation, the Invitrogen PureLink™ Genomic DNA Mini Kit was used according to the manufacturer suggestion. The obtained DNA was stored at -20 °C until further analysis.

Detection of Coxiella burnetii

PCR was performed to amplify the IS1111 gene region of *Coxiella burnetii* of 687 bp. IS1111 is a multicopy transposon with a highly increased sensitivity for the detection of *C. burnetii*. The primers, Trans1 (5'TATGTATCCACCGTAGCCAGTC-3') forward and Trans2 (5'-CCCAACAACACCTCCTTATTC-3') reverse were used as previously described (Mares-Guia *et al.*, 2014). 200 µM dNTPs, 1.5 mM $MgCl_2$, 6 pmol forward and reverse primers, 0.1 U Taq Polymerase, and 10X PCR buffer (500 mM Tris-HCl, pH 8.8, 160 mM (NH4)SO4 and 0.1% Tween®20), Nuclease Free Water, and 4 µL DNA were used in a 25 µL master mix. The reaction was created by pre-denaturation for 15 min at 95°C, followed by 40 cycles of denaturation at 95°C for 30 s, bonding period at 60°C for 30 s, elongation period at 72°C for 1 min, and a final elongation period of 7 min at 72°C. The reaction was performed on a Kyratec Gradient Thermal cycler. The prepared 1.5% agarose gel was stained with RedSafe Nucleic Acid Staining Solution. The PCR products were then run on an agarose gel and the images were recorded with the gel imaging device (Syngene bioimaging system).

Detection of Mycoplasma haemocanis (Mhc)

The primers, 5′-GAAACTAAGGCCATAA ATGACGC-3′ forward and 5′-ACCTGTCA CCTCGATAACCTCTAC-3′ reverse were used to amplify the 309 bp 16S rRNA gene region of *Mycoplasma haemocanis* (Mhc). PCR reaction and Temperature cycling conditions were adapted according to Torkan *et al.* (2014).

Detection of Candidatus Mycoplasma haematoparvum (CMhp)

The primers, 5′-ACGAAAGTCTGATGGA GCAATAC-3′ forward and 5′- TATCTACG CATTCCACCGCTAC-3′ reverse were used to amplify the 328 bp 16S rRNA gene region

of *Candidatus* Mycoplasma haematoparvum (*CMhp*). PCR reaction and Temperature cycling conditions were adapted according to Torkan *et al.* (2014)

Detection of Wolbachia

The primers, 5'-TGGTCCAATAAGTGATG AAGAAACTAGCTA-3' forward and 5'-AAATTAAAGCTACTCCAGCTTCTGCA C-3' reverse were used to amplify the 590 bp to 632 bp wsp gene region of *Wolbachia* (Zhou *et al.*, 1998; Simsek and Ciftci, 2016). PCR reaction and Temperature cycling conditions were adapted according to (Zhou *et al.*, 1998). Primer pairs, target gene, and PCR product sizes are present in Table 1.

Sequence and Phylogenetic Analysis:

The QIAquick PCR Purification Kit (QIAGEN, Germany) was used to purify the PCR product according to the manufacturer's instructions. The same primers used in PCR amplification were utilized to sequence purified PCR products in both directions. Applied Biosystems' ABI 3100 Genetic Analyzer Automated Sequencer (Applied Biosystems, USA) was used to run the sequencing operations, which used ABI PRISM BigDyeTM terminator cycle sequencing kits (Applied Biosystems, Foster City, USA). The sequences were assembled and edited using Bioedit software (version 7.2). Molecular and evolution genetic analysis (MEGA X) software was used to accomplish multiple sequence alignment. Distances between sequences were calculated automatically using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993; Kumar *et al.*, 2018).

Ethical Approval

This study was approved by the Siirt University Animal Experiments Local Ethics Committee (Approval no: 2021.01.15).

RESULTS

An overall of 350 ticks were collected and identified down to the species level using morphological analysis.

All ticks were identified as *Rhipicephalus sanguineus* species. C*oxiella* DNA was detected in 3 (0.85%) out of 350 ticks using the Nested PCR method (687 base pairs) (Fig.1). *Mycoplasma haemocanis* (*Mhc*), *Candidatus* Mycoplasma haematoparvum (*CMhp*), and Wolbachia DNA were not detected in any of the samples. The phylogenetic tree was constructed with the Maximum Likelihood (MCL) method, using the DNA sequences (Fig.2). The statistics of the obtained phylogenetic tree were evaluated with 1000 repetitive bootstrap analyses. Partial sequences of the IS*1111* gene region were registered in GenBank with OM472143 accession numbers. *Legionella pneumophilla* (DQ897170.1) was selected as the out-group. The partial sequence of *C. burnetii* from this study was compared to the sequence available in the GenBank with BLAST search. The partial *C. burnetii* sequence obtained in this study showed 100% homology to *Coxiella burnetii* strain Coxi-IR-FM-112 insertion sequence IS1111A transposase gene, and *Coxiella burnetii* isolate goat_614 transposase gene, respectively. It was also found that the sequence obtained from this study had 99.84% similarity to *Coxiella burnetii* strain Coxi-IR-FM-101 insertion sequence IS1111A transposase gene, and *Coxiella burnetii* isolate Coxi-SM2/Iraq transposase gene, respectively.

Legends

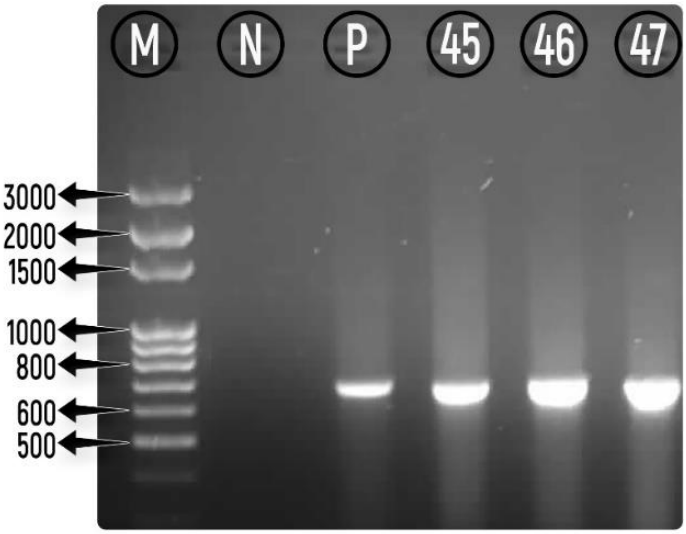
Table 1. Species-specific primers used in the study, and their genome sizes

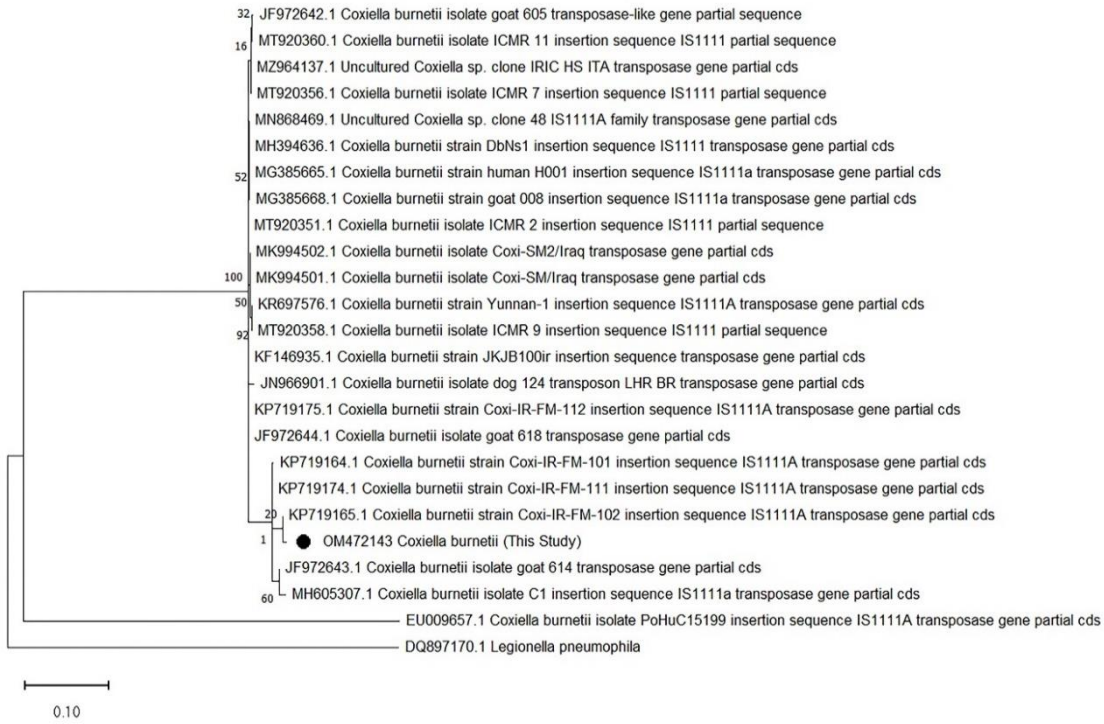
Figure 1. 16S rRNA amplification of *C. burnetii* in ticks using PCR. Lanes M: Marker, N: Negative control, P: positive control, Lanes 45,46,47 represent *C. burnetii* (687 bp).

Figure 2. Phylogenetic tree of *C. burnetii* with IS*1111* partial sequences using the Maximum Likelihood method and Tamura-Nei model. Evolutionary analyses were conducted in MEGAX. The nucleotide sequence determined in this study is indicated in the black dot. *Legionella pneumophila* was used as an out-group.

Table 1: Species-specific primers used in the study, and their genome sizes.

Target Gene	Specificity (%)	Primary	Sequence (5′-3′)	Product Length (bp)
IS*1111*	*C.burnetii*	Forward	5′-TATGTATCCACCGTAGCCAGTC-3′	687
		Reverse	5′-CCCAACAACACCTCCTTATTC-3′	
16S rRNA	*Mhc*	Forward	5′-GAAACTAAGGCCATAAATGACGC-3′	309
		Reverse	5′-ACCTGTCACCTCGATAACCTCTAC-3′	
16S rRNA	*CMhp*	Forward	5′-ACGAAAGTCTGATGGAGCAATAC-3′	328
		Reverse	5′-TATCTACGCATTCCACCGCTAC-3′	
wsp	*Wolbachia*	Forward	5'-TGGTCCAATAAGTGATGAAGAAACTAGCTA-3'	590-632
		Reverse	5'-AAAAATTAAACGCTACTCCAGCTTCTGCAC-3'	





DISCUSSION

One of the most prominent vectors along with mosquitos, especially considering zoonotic diseases, are the ticks. Due to their hematophagous nature, they are involved in transmitting numerous pathogens between animals and/or humans (Leulmi *et al.*, 2016). Ticks host the widest range of microorganisms, ranging from viruses, prokaryotes, and eukaryotes (Plantard *et al.*, 2012). *R. sanguineus* ticks are the most common type of ticks worldwide. Although dogs are the main host of this tick species, it has been reported that it also infests humans in various parts of the world (Chao *et al.*, 2021).

Q fever is a zoonotic infectious disease with a worldwide presence, caused by the obligate intracellular bacterium *Coxiella burnetii* (Andoh *et al.*, 2013; Oskam *et al.*, 2017). More than 40 types of ticks have been associated with *C. burnetii* and other Coxiella species to date (Oskam *et al.*, 2017; Khalili *et al.*, 2018).

Different prevalence has been reported by studies conducted in different parts of the world. Nine of 164 (5.5%) ticks in the Philippines (Ybañez, 2014), 5 of 209 (2.4%) tick pools in Italy (Satta *et al.*, 2011), 1 of 8 (12.5%) tick pools in Iran (Khalili *et al.*, 2018), 1 of 24 (4.17%) *R. sanguineus* ticks in Egypt (Loftis *et al.*, 2006), 26 of 44 (59%) *R. sanguineus* ticks in Malaysia (Watanabe *et al.*, 2015) and all of 199 *R. sanguineus* ticks (100%) in Australia (Oskam *et al.*, 2017) were determined to be positive. In a study conducted by Andoh *et al.* (2013) in Japan, all 261 ticks involved in the study were found to be negative concerning *C. burnetii.*

Even though there are seroprevalence studies in Turkey for the diagnosis of *Coxiella burnetii*, the number of studies performed on ticks is quite limited. It was reported that 46.15% and 1.89% positivity were detected in Denizli and Ankara provinces, respectively, in two studies (Capin *et al.*, 2013). *Coxiella burnetii* positivity was detected in 3 (0.85%) of 350 ticks examined in this study. The results of this study are similar to the results of the study conducted by Capin *et al.* (2013) and Satta *et al.* (2011). Differences between the findings may be due to geographical location, different climates, sample size, sampling period, tick species, number and stage of infected ticks, and availability of appropriate reservoirs and methods.

The PCR method has long been accepted as a highly sensitive and accurate determination process for *C. burnetii* in a wide range of sample types. The method offers some advantages compared to classical serological methods where the determinations can only be performed retrospectively and in a limited fashion (Capin *et al.*, 2013). In this study, the PCR method was used for the detection of agents in ticks. The partial *C. burnetii* sequence obtained in this study showed 100% similarity to *C. burnetii* strain registered to GenBank from Iran (KP719175.1, KP719174.1, KP719165.1), and Brazil (JF972643.1).

Hemoplasmosis is an infection caused by hemotropic mycoplasmas and *R.sanguineus* ticks are reported to be a possible vector for hemoplasmosis. Studies show that there is a significant relationship between the presence of *R. sanguineus* and hemoplasma infection (Willi *et al.*, 2007; Wengi *et al.*, 2008). *R. sanguineus* type ticks play an important role in the transmission of canine hemoplasmas and are reported to be found in arid regions of Turkey (Aydin *et al.*, 2015).

In a study conducted in Diyarbakır, it was reported that the Mhc rate was 26.2% and the CMhp rate was 6.7% (Aktas and Ozubek, 2017). In the study carried out by the same researchers in different provinces of Turkey the Mhc rate was 4.5% and the CMhp rate was 4.3% determined (Aktas and Ozubek, 2018). In a study conducted by Sababoglu *et al.* (2021) in Adana, it was reported that 8

(2.56%) of 312 ticks were positive for CMhp, while all samples were negative for Mhc.

Wolbachia bacteria can be found as endosymbionts in insects, arachnids, crustaceans, and filarial nematodes (Yetişmiş *et al.*, 2018). Some studies report as high as 65% infection rates for insects with *Wolbachia* (Yildirim *et al.*, 2013). In a study conducted in Taiwan, it was reported that the rate of the agent in *Rhipicephalus sanguineus* ticks was 46.1% (Chao *et al.*, 2021).
The examined ticks were found to be negative in terms of Mhc, CMhp, and *Wolbachia* as a result of this study. The reason for the negative result obtained from the study might depend on the tick species, the number of ticks involved with the study, and/or the sampling environment.

CONCLUSION

The data obtained from this study shows that dog-infesting ticks can be infected by C. burnetii. Considering the zoonotic nature of Q disease, it is very important for dog owners and related institutions to periodically drug animals against ticks and take any other necessary precautions. Efforts should be focused on understanding the role and epidemiological significance of dogs and infected ticks, especially for human Q fever, which can be a life-threatening disease. More tick samples are needed to determine the Mhc, CMhp, and Wolbachia status in Siirt province.

ACKNOWLEDGMENTS

The study has been presented as a poster presentation at the 22nd Parasitology Congress, 11-15 October 2021, Didim, Aydın, TURKEY.

COMPETING INTERESTS

Authors state no conflict of interest.

REFERENCES

Aktas, M.; and Ozubek, S. (2017): Molecular survey of haemoplasmas in shelter dogs and associations with Rhipicephalus sanguineus sensu lato. *Medical and veterinary entomology*, 31(4): 457-461. doi:https://doi.org/10.1111/mve.12244

Aktas, M.; and Ozubek, S. (2018): A molecular survey of hemoplasmas in domestic dogs from Turkey. *Veterinary microbiology*, 221: 94-97. doi:https://doi.org/10.1016/j.vetmic.2018.06.004

Andoh, M.; Andoh, R.; Teramoto, K.; Komiya, T.; Kaneshima, T.; Takano, A.; Hayashidani, H.; and Ando, S. (2013): Survey of Coxiella burnetii in ticks collected from dogs in Japan. *Journal of Veterinary Medical Science*, 75(8): 1115-1117. doi:https://doi.org/10.1292/jvms.12-0570

Ayan, A.; Kilinc, O.O.; Yilmaz, A.B.; and Babaoglu, A.R. (2019): Prevalence of ehrlichia spp in ticks collected from dogs in province of van in Turkey. *IJEES*, 9(3): 537-542. doi:https://doi.org/10.31407/ijees

Aydin, M.F.; Sevinc, F.; and Sevinc, M. (2015): Molecular detection and characterization of Hepatozoon spp. in dogs from the central part of Turkey. *Ticks and tick-borne diseases*, 6(3): 388-392. doi:https://doi.org/10.1016/j.ttbdis.2015.03.004

Barker, E.; Tasker, S.; Day, M.; Warman, S.; Woolley, K.; Birtles, R.; Georges, K.; Ezeokoli, C.; Newaj-Fyzul, A.; and Campbell, M. (2010): Development and use of real-time PCR to detect and quantify Mycoplasma haemocanis and "Candidatus Mycoplasma haematoparvum" in dogs. *Veterinary microbiology*, 140(1-2): 167-170. doi:https://doi.org/10.1016/j.vetmic.2009.07.006

Cantas, H.; Muwonge, A.; Sareyyupoglu, B.; Yardimci, H.; and Skjerve, E. (2011): Q fever abortions in ruminants and associated on-farm risk factors in northern Cyprus. *BMC veterinary research*, 7(1): 1-7. doi:https://doi.org/10.1186/1746-6148-7-13

Capin, G.A.; Emre, Z.; Canpolat, S.; Vatansever, Y.; and Duzgun, A. (2013): Detection of Coxiella burnetii from ticks by polymerase chain reaction and restriction fragment length polymorphism. *Ankara Üniv Vet Fak Derg*, 60: 263-268. doi:https://doi.org/10.1501/Vetfak_0000002590

Chao, L.-L.; Castillo, C.T.; and Shih, C.-M. (2021): Molecular detection and genetic identification of Wolbachia endosymbiont in Rhipicephalus sanguineus (Acari: Ixodidae) ticks of Taiwan. *Experimental and Applied Acarology*, 83(1): 115-130. doi:https://doi.org/10.1007/s10493-020-00574-3

Cooper, A.; Hedlefs, R.; Ketheesan, N.; and Govan, B. (2011): Serological evidence of Coxiella burnetii infection in dogs in a regional centre. *Australian Veterinary Journal*, 89(10): 385-387. doi:https://doi.org/10.1111/j.1751-0813.2011.00819.x

Dantas-Torres, F. (2010): Biology and ecology of the brown dog tick, Rhipicephalus sanguineus. *Parasites & vectors*, 3(1): 1-11.

Estrada-Peña, A.; Bouattour, A.; Camicas, J.; and Walker, A. *(2004): Ticks of Domestic Animals in the Mediterranean Region: A Guide to Identification of Species*. Spain: University of Zaragoza.

Hartelt, K.; Oehme, R.; Frank, H.; Brockmann, S.O.; Hassler, D.; and Kimmig, P. (2004): Pathogens and symbionts in ticks: prevalence of Anaplasma phagocytophilum (Ehrlichia sp.), Wolbachia sp., Rickettsia sp., and Babesia sp. in Southern Germany. *International Journal of Medical Microbiology Supplements*, 293: 86-92.

Hosseini, S.R.; Sekhavatmandi, A.; and Khamesipour, F. (2017): PCR based analysis of Haemobartonellosis (Candidatus mycoplasma haematoparvum and Mycoplacma haemocanis) and its prevalence in dogs in Isfahan, Iran. *Bioscience Biotechnology Research Communications*, 10(2): 187-191. doi:http://dx.doi.org/10.21786/bbrc/10.2/32

Kalender, H. (2001): Elazığ ve komşu illerdeki koyunlarda Coxiella burnetii enfeksiyonunuyaygınlığı. *Turk J Vet Anim Sci*, 25: 51-55.

Khalili, M.; Rezaei, M.; Akhtardanesh, B.; Abiri, Z.; and Shahheidaripour, S. (2018): Detection of Coxiella burnetii (Gammaproteobacteria: Coxiellaceae) in ticks collected from infested dogs in Kerman, Southeast of Iran. *Persian Journal of Acarology*, 7(1). doi:https://doi.org/10.22073/pja.v7i1.30699

Kılıç, A. (2017): Koyun ve Keçi Sütlerinde Coxiella burnetii Varlığının PCR ile Araştırılması. *Atatürk Üniversitesi Vet. Bil. Derg*, 12(2): 152-156. doi:https://doi.org/10.17094/ataunivbd.347968

Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; and Tamura, K. (2018): MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol Biol Evol*, 35(6): 1547-1549. doi:https://doi.org/10.1093/molbev/msy096

Leulmi, H.; Aouadi, A.; Bitam, I.; Bessas, A.; Benakhla, A.; Raoult, D.; and Parola, P. (2016): Detection of Bartonella tamiae, Coxiella burnetii and rickettsiae in arthropods and tissues from wild and domestic animals in northeastern Algeria. *Parasites &*

vectors, 9(1): 1-8. doi:https://doi.org/10.1186/s13071-016-1316-9

Loftis, A.D.; Reeves, W.K.; Szumlas, D.E.; Abbassy, M.M.; Helmy, I.M.; Moriarity, J.R.; and Dasch, G.A. (2006): Rickettsial agents in Egyptian ticks collected from domestic animals. *Experimental & applied acarology*, 40(1): 67-81. doi:https://doi.org/10.1007/s10493-006-9025-2

Lumb, W. (2001): More information on haemobartonellosis in dogs. *J Am Vet Med Assoc*, 219(6): 732-733. doi:https://doi.org/10.2460/javma.2001.219.732

Ma, G.C.; Norris, J.M.; Mathews, K.O.; Chandra, S.; Šlapeta, J.; Bosward, K.L.; and Ward, M.P. (2020): New insights on the epidemiology of Coxiella burnetii in pet dogs and cats from New South Wales, Australia. *Acta tropica*, 205: 105416. doi:https://doi.org/10.1016/j.actatropica.2020.105416

Maggi, R.G.; Compton, S.M.; Trull, C.L.; Mascarelli, P.E.; Mozayeni, B.R.; and Breitschwerdt, E.B. (2013): Infection with hemotropic Mycoplasma species in patients with or without extensive arthropod or animal contact. *Journal of Clinical Microbiology*, 51(10): 3237-3241.

Mares-Guia, M.A.M.d.M.; Rozental, T.; Guterres, A.; Gomes, R.; Almeida, D.N.d.; Moreira, N.S.; Barreira, J.D.; Favacho, A.R.; Santana, A.L.; and Lemos, E.R.S.d. (2014): Molecular identification of the agent of Q fever–Coxiella burnetii–in domestic animals in State of Rio de Janeiro, Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, 47(2): 231-234. doi:https://doi.org/10.1590/0037-8682-0076-2013

Maurin, M.; and Raoult, D.f. (1999): Q fever. *Clinical microbiology reviews*, 12(4): 518-553. doi:https://doi.org/10.1128/CMR.12.4.518

Messick, J.B. (2004): Hemotrophic mycoplasmas (hemoplasmas): a review and new insights into pathogenic potential. *Veterinary Clinical Pathology*, 33(1): 2-13. doi:https://doi.org/10.1111/j.1939-165x.2004.tb00342.x

Oskam, C.L.; Gofton, A.W.; Greay, T.L.; Yang, R.; Doggett, S.; Ryan, U.M.; and Irwin, P.J. (2017): Molecular investigation into the presence of a Coxiella sp. in Rhipicephalus sanguineus ticks in Australia. *Veterinary microbiology*, 201: 141-145. doi:https://doi.org/10.1016/j.vetmic.2017.01.021

Plantard, O.; Bouju-Albert, A.; Malard, M.-A.; Hermouet, A.; Capron, G.; and Verheyden, H. (2012): Detection of Wolbachia in the tick Ixodes ricinus is due to the presence of the hymenoptera endoparasitoid Ixodiphagus hookeri. *PLoS One*, 7(1): e30692.

Rezaei, M.; Khalili, M.; Shahrbabaki, F.B.; and Abiri, Z. (2018): Detection of C. burnetii in Uterine Samples Collected from Referred Dogs to the Veterinary Hospital of Shahid Bahonar University of Kerman by Nested Trans-PCR. *Acta Veterinaria Eurasia*, 44(1): 26-30.

Rosanna, Z.; Andrea, C.; Isabella, B.; Francesca, S.; Alberto, A.; Teresa, A.M.; and Luisa, P.P.M. (2020): Immune-Mediated Hemolytic Anemia Associated with Candidatus Mycoplasma Haematoparvum in a Splenectomized Dog in Italy. *Acta Veterinaria*, 70(2): 277-284. doi:https://doi.org/10.2478/acve-2020-0020

Sababoglu, E.; Ayan, A.; Orunc Kilinc, O.; Yilmaz, A.B.; Tekindal, M.A.; Akkaya, H.; and Aslan Celik, B. (2021): Molecular Detection of Mycoplasma Haemocanis and Candidatus Mycoplasma Haematoparvum in Rhipicephalus Sanguineus Tick

Species Collected From Dogs in Adana Turkey. *Fresenius Environ. Bull*, 30(06A): 6485-6489.

Satta, G.; Chisu, V.; Cabras, P.; Fois, F.; and Masala, G. (2011): Pathogens and symbionts in ticks: a survey on tick species distribution and presence of tick-transmitted micro-organisms in Sardinia, Italy. *Journal of medical microbiology*, 60(1): 63-68. doi:https://doi.org/10.1099/jmm.0.021543-0

Simsek, S.; and Ciftci, A.T. (2016): Serological and molecular detection of Dirofilaria species in stray dogs and investigation of Wolbachia DNA by PCR in Turkey. *Journal of arthropod-borne diseases*, 10(4): 445.

Sykes, J.E.; Ball, L.M.; Bailiff, N.L.; and Fry, M.M. (2005): 'Candidatus Mycoplasma haematoparvum', a novel small haemotropic mycoplasma from a dog. *International journal of systematic and evolutionary microbiology*, 55(1): 27-30. doi:https://doi.org/10.1099/ijs.0.02989-0

Tamura, K.; and Nei, M. (1993): Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular biology and evolution*, 10(3): 512-526. doi:https://doi.org/10.1093/oxfordjournals.molbev.a040023

Tijsse-Klasen, E.; Braks, M.; Scholte, E.-J.; and Sprong, H. (2011): Parasites of vectors-Ixodiphagus hookeri and its Wolbachia symbionts in ticks in the Netherlands. *Parasites & Vectors*, 4(1): 1-7.

Torkan, S.; Aldavood, S.J.; Sekhavatmandi, A.; and Moshkelani, S. (2014): Detection of haemotropic Mycoplasma (Haemobartonella) using multiplex PCR and its relationship with epidemiological factors in dogs. *Comparative Clinical Pathology*, 23(3): 669-672. doi:http://dx.doi.org/10.1007%2Fs00580-012-1668-2

Tukur, S.M.; Mohammed, K.; Watanabe, M.; Rani, P.A.M.A.; and Watanabe, M. (2019): Coxiella burnetii detection in stray dogs in Klang Valley, Malaysia. *Journal of Advances in Microbiology*, 14(2): 1-7. doi:https://doi.org/10.9734/JAMB/2019/46311

Walker, J.B.; Keirans, J.E.; and Horak, I.G. (2000): The genus Rhipicephalus (Acari, Ixodidae): a guide to the brown ticks of the world: Cambridge University Press.

Watanabe, M.; Nakao, R.; Amin-Babjee, S.; Maizatul, A.; Youn, J.; Qiu, Y.; Sugimoto, C.; and Watanabe, M. (2015): Molecular screening for Rickettsia, Anaplasmataceae and Coxiella burnetii in Rhipicephalus sanguineus ticks from Malaysia. *Trop Biomed*, 32: 390-398.

Webster, J.; Lloyd, G.; and Macdonald, D. (1995): Q fever (Coxiella burnetii) reservoir in wild brown rat (Rattus norvegicus) populations in the UK. *Parasitology*, 110(1): 31-35. doi:https://doi.org/10.1017/s0031182000081014

Wengi, N.; Willi, B.; Boretti, F.S.; Cattori, V.; Riond, B.; Meli, M.L.; Reusch, C.E.; Lutz, H.; and Hofmann-Lehmann, R. (2008): Real-time PCR-based prevalence study, infection follow-up and molecular characterization of canine hemotropic mycoplasmas. *Veterinary microbiology*, 126(1-3): 132-141. doi:https://doi.org/10.1016/j.vetmic.2007.06.018

Willi, B.; Boretti, F.S.; Tasker, S.; Meli, M.L.; Wengi, N.; Reusch, C.E.; Lutz, H.; and Hofmann-Lehmann, R. (2007): From Haemobartonella to hemoplasma: molecular methods provide new insights. *Veterinary microbiology*, 125(3-4): 197-209. doi:https://doi.org/10.1016/j.vetmic.2007.06.027

Woldehiwet, Z. (2004): Q fever (coxiellosis): epidemiology and pathogenesis. *Research in veterinary science*, 77(2): 93-100. doi:https://doi.org/10.1016/j.rvsc.2003.09.001

Ybañez, A.P. (2014): First molecular evidence of Coxiella spp. from Rhipicephalus sanguineus ticks in Cebu, Philippines. *Eurasian Journal of Veterinary Sciences*, 30(1): 35-38.

Yetişmiş, G.; Düzlü, Ö.; Yıldırım, A.; Çiloğlu, A.; Önder, Z.; and Abdullah, İ. (2018): Sultan Sazlığı yöresinde sivrisinek türlerinde Wolbachia endobakterisinin moleküler yöntemlerle araştırılması ve genotiplendirilmesi. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 65(3): 229-237. doi:https://doi.org/10.1501/Vetfak_0000002851

Yildirim, A.; Abdullah, I.; Duzlu, O.; Onder, Z.; and Ciloglu, A. (2013): Detection and molecular characterization of the Wolbachia endobacteria in the Culex pipiens (Diptera: Culicidae) specimens collected from Kayseri province of Turkey. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 60(3): 189-194.

Zhou, W.; Rousset, F.; and O'Neill, S. (1998): Phylogeny and PCR–based classification of Wolbachia strains using wsp gene sequences. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265(1395): 509-515. doi:https://dx.doi.org/10.1098%2Frspb.1998.0324