**doi:** 10.30934/kusbed.1097664 **e-ISSN:** 2149-8571

**Kocaeli Üniversitesi Sağlık Bilimleri Dergisi**

**Özgün Araştırma / Original Article**

**http://dergipark.gov.tr/kusbed**

# INVESTIGATION OF *BABESIA* SPP. IN STRAY DOGS IN VAN PROVINCE BY POLYMERASE CHAIN REACTION

## *VAN İLİNDEKİ SOKAK KÖPEKLERİNDE BABESIA TÜRLERİNİN POLİMERAZ ZİNCİR REAKSİYONU YÖNTEMİ İLE ARAŞTIRILMASI*

Ömer Selçin[1], Bekir Oğuz[*2]

[1]Van Yüzüncü Yıl University, Institute of Health Sciences, Department of Veterinary Parasitology, Van, Turkey, [2]Van Yüzüncü Yıl University, Faculty of Veterinary Medicine, Department of Parasitology, Van, Turkey

**ORCID iD:** Ömer Selçin: 0000-0002-6462-9233; Bekir Oğuz: 0000-0001-8720-3940

***Sorumlu Yazar / Corresponding Author:** Bekir Oğuz **e-posta / e-mail:** bekiroguz@yyu.edu.tr

**Geliş Tarihi / Received:** 02.04.2022 **Kabul Tarihi / Accepted:** 26.05.2022 **Yayım Tarihi / Published:** 31.05.2022

## Abstract

**Objective:** Canine babesiosis is a tick-borne protozoal disease caused by *Babesia* spp., which can cause hemolytic anemia, splenomegaly, thrombocytopenia, and fever. As a result of molecular studies conducted in Turkey, the presence of *B. canis*, *B. gibsoni*, *B. vogeli*, *B. rossi*, and *B. vulpes* species has been revealed. To the best of our knowledge, there are no studies detecting *Babesia* species in dogs of Van province. This study was carried out to investigate the presence and prevalence of babesiosis in dogs of Van province using the Polymerase chain reaction (PCR) technique.
**Methods:** Blood samples were taken from vena cephalica antebrachii into EDTA tubes from a total of 100 randomly selected asymptomatic dogs. DNAs obtained from the samples were investigated by the PCR method in which 18S ribosomal RNA gene was amplified for the presence of Babesia spp.
**Results:** *Babesia* spp. DNA was not found in any of the 100 stray dogs examined according to PCR results.
**Conclusion:** In this study, *Babesia* spp. species were investigated molecularly for the first time in stray dogs of Van region.

**Keywords:** *Babesia, polymerase chain reaction, stray dogs, Van.*

## Öz

**Amaç:** Köpek babesiosisi *Babesia* spp.' nin neden olduğu hemolitik anemi, splenomegali, trombositopeni ve ateşe neden olabilen kene kaynaklı bir protozoal hastalıktır. Türkiye'de yapılan moleküler çalışmalar soncunda *B. canis*, *B. gibsoni*, *B. vogeli*, *B. rossi* ve *B. vulpes* türlerinin varlığı ortaya konulmuştur. Yapılan literatür taramalarına göre Van ilinde yaşayan köpeklerde *Babesia* türlerini ortaya koyan herhangi bir çalışma bulunmamaktadır. Bu çalışma, Van ilindeki köpeklerde babesiosisin varlığı ve yaygınlığının Polimeraz Zincir Reaksiyonu (PZR) tekniği ile araştırılması amacıyla yapılmıştır.
**Yöntem:** Rastgele seçilen toplam 100 asemptomatik köpeğin vena cephalica antebrachii'lerinden EDTA'lı tüplere kan örnekleri alınmıştır. Alınan örneklerden elde edilen DNA'lar, *Babesia* spp.' nin varlığı yönünden 18S ribozomal RNA geninin amplifiye edildiği PZR yöntemi ile araştırılmıştır.
**Bulgular:** Muayene edilen 100 köpeğin PZR sonuçlarına göre hiçbirinde *Babesia* spp. DNA'sına rastlanmamıştır.
**Sonuç:** Bu çalışma ile Van yöresi sokak köpeklerinde ilk kez *Babesia* spp. türleri moleküler olarak araştırılmıştır.

**Anahtar Kelimeler:** *Babesia, polimeraz zincir reaksiyonu, ,sokak köpeği, van.*

## Introduction

Canine babesiosis is a tick-borne, protozoal and hemoparasitic disease that may manifest with varying degrees of hemolytic anemia, splenomegaly, thrombocytopenia, and fever. *Babesia* species are frequently classified as large or small. *Babesia* spp. has been historically identified by morphological appearance in the erythrocytes of blood smears (intraerythrocytic merozoite stage) in dogs. While all large forms between 3 and 5 µm in length were originally classified as *B. canis*, all small forms between 1-3 µm were originally determined to be *B. gibsoni*, however, molecular methods and DNA sequence analyses revealed the presence of at least three small piroplasms in the infected dogs. These are *B. gibsoni*, *B. conradae* and recently reported "*Babesia vulpes*" species. Large *Babesia* species were classified as *B. canis*, *B. vogeli*, and *B. rossi*.[1,2]

The occurrence of canine babesiosis depends on the geographical distribution and habitat of the related vector tick species.[1] The disease causes varying degrees of hemolytic changes such as fever, numbness, loss of appetite, anemia, and hemoglobinuria and even it may jeopardize other organ systems, consequently, it may result in many various clinical findings. The course of canine babesiosis may range from mild anemia to death due to multiple organ failures depending on primarily *Babesia* species causing the infection, age of the animal, its immunity state, and the existence of secondary infections. The treated dogs carry disease factors lifelong even though the clinical symptoms disappear.[2-5] Species-based identification of *Babesia* species is important in the fight against the disease and treatment processes. The peripheral blood smear examination is the most commonly used technique for the diagnosis of canine babesiosis. Dogs can be diagnosed with too large or small *Babesia* species in the microscopic examination method.[6,7] It is not adequate for advanced diagnosis. The probability of overlooking is higher for particularly small *Babesia* species with the microscopic examination method. Both the specificity and sensitivity of this method are very low.[8] Therefore, it is considered that the use of molecular diagnostic methods would be more appropriate for the diagnosis of babesiosis.[1,2,7,9]

As a result of the molecular studies, the presence of *B. canis*, *B. gibsoni*, *B. vogeli*, *B. rossi*, and *B. vulpes* species has been identified in Turkey.[10-15] In light of the literature review, we have encountered no study that identified the *Babesia* species in the dogs living in Van Province. The present study aimed to investigate the species causing babesiosis in the stray dogs living in Van Province using molecular methods and to obtain comprehensive related evidence in this province for the first time.

## Methods

### Animal Material

The study was conducted on a total of 100 stray dogs between June and September 2020 in the Animal Shelter of Van Municipality (Figure 1). The age and sex of the stray dogs included in the sample were recorded (data not shown). Besides, the animals were examined for the presence of ticks before taking blood samples. The blood samples of the dogs included those taken for general examinations and during ovariohysterectomy and/or castration. In the study, the blood samples were taken to 5 ml sterile EDTA (disodium ethylenediamine tetraacetate) from a total of 100 stray dogs.

Blood samples were kept in a deep-freezer (-20°C) until analysis for DNA extraction. Ethics Committee approval for this research was obtained from Local Ethics Committee for Animal Experiments of Van Yüzüncü Yıl University (Approval number 2020/04-2021/12-09).

**Figure 1.** Map of Turkey; The sampling area is indicated by an arrow. (Map source: https://earth.google.com)


### DNA Extraction and Polymerase Chain Reaction

DNA extraction was performed according to the procedure of the Blood Genomic DNA Isolation Kit (MG-GDNA-01-250) belonging to Hibrigen Company. The genomic DNA extracts were subjected to PCR with BJ1 (5′-GTCTTGTAATTGGAATGATGG-3′) and BN2 (5′-TAGTTTATGGTTAGGACTACG-3') specific primers for *Babesia* spp. reproducing 486-520 bp gene region from 18S rRNA gene region.[16]

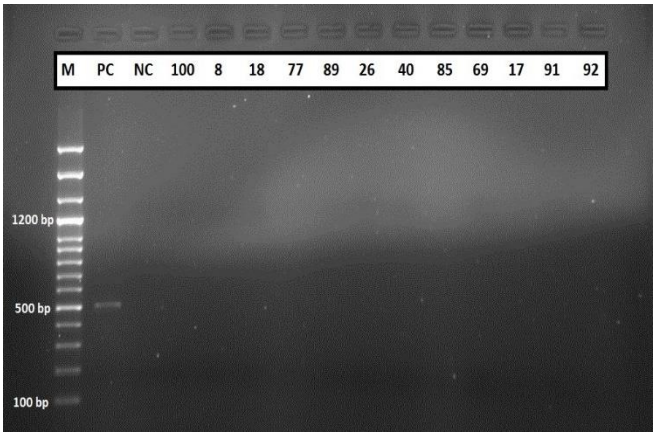
PCR mixture was prepared with a final volume of 25 µl containing 10 µl 5X MyTaq Reaction buffer, 1 µl 10 pmol for each primer, 5 µl template DNA, 0.5 µl Taq DNA polymerase, and 7.5 µl sterile distilled water. Then, the mixture was placed in the PCR device and the thermal profile was set as follows; initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 94°C for 60 sec, annealing at 55°C for 60 sec, extension at 72°C for 2 min and final extension at 72°C for 5 min.[16] A sample of 10 µl was taken from each PCR product, mixed with 1 µl 6X loading dye (Hibrigen, Turkey) to be loaded into the wells of the gel. The loaded samples were run for 50 minutes at a constant current of 100 V. At the end of time, the samples were viewed by a gel documentation system (Avegene, Taiwan).

DNA sample (Genbank Accession Number: OK035444) obtained from *Theileria* (*Babesia*) *equi* protozoa available in the Department of Parasitology of Van Yuzuncu Yıl University was used as the positive control to assess the validity of PCR test and to detect whether any contamination was present. Sterilized deionized water was used as the negative control.

## Results

DNA of *Babesia* spp. was identified in none of the collected blood samples according to the examination result of 100 blood samples taken from stray dogs living in Van Province (Figure 2). Totally two male adult ticks were picked from two different animals including one for each and these ticks were identified to be *Rhipicephalus turanicus* according to the taxonomic key of Walker *et al.*[17] and Estrada-Peña *et al.*[18]

**Figure 2**. Gel electrophoresis from PCR amplification of *Babesia* spp. M – molecular marker 100 bp DNA ladder, NC – negative control (no DNA), PC – PCR products from *Babesia* spp. (positive control), Negative samples – 100, 8, 18, 77, 89, 26, 40, 85, 69, 17, 91, 92.




## Discussion

Canine babesiosis caused by intraerythrocytic apicomplexan protozoans belonging to *Babesia* species as one of the various vector-borne diseases in dogs is a clinically important disease that can be observed very commonly worldwide including in Turkey. These blood protozoans are transmitted by ticks and therefore they may manifest a host variability extending from domestic and wild animals to humans. *Dermacentor reticulatus* species is the vector related to *B. canis*. *Babesia vogeli* is usually seen in the fields where *R. sanguineus* s.l. is the dominant tick species around Mediterranean Basin.[7,15] *B. gibsoni* is mainly transmitted by the ticks belonging to *Rhipicephalus sanguineus* s.l (Mediterranean Regions) and *Haemaphysalis longicornis* (Asia) species.[7,19-21] Although ticks belong to *Hae. leachi* species are the known vectors of *B. rossi*[22], DNA of *B. rossi* has been identified in the *Hae. parva* tick species in Turkey.[13] The potential vector of *B. microti*-like sp. is *Ixodes hexagonus* tick species.[1,23]

The examination of blood smears is a useful diagnostic tool for clinical canine babesiosis. Microscopic evaluation still remains as the easiest and most available diagnostic test for many veterinary physicians. However, the sensitivity of this method is less than molecular diagnosis and rather associated with the species infecting the dog. The two main forms of *Babesia* as large and small species can be differentiated using microscopic examination. However, it is not adequate for the diagnosis of large *Babesia* forms. Sequence analysis with other molecular biological methods is more convenient for these infections. Furthermore, observing small piroplasms with light microscope is very difficult and requires specialization.[6,7,24] In molecular biological techniques, phylogenetic analyses can be carried out by using 18S rRNA, mitochondrial cytochrome oxidase subunit 1 (Cox1), and internal transcribed spacer (ITS) gene regions as the target gene regions and sequencing the products isolated from 18S rRNA gene region more commonly. Numerous molecular prevalence and characterization studies have been conducted worldwide for identifying the spread of canine babesiosis and the species responsible for the disease.[25-33]

The studies carried out on canine babesiosis in Turkey are in the case reports between 2002 and 2013. It was first confirmed using molecular methods that a 2-year-old American Staffordshire Terrier dog without any disease symptoms were infected with *B. gibsoni* in Aydın.[11] Between 2002-2005, blood samples of three dogs were analyzed with RFLP-PCR method in Istanbul and two of those dogs were identified to be infected with *B. vogeli* according to the examination result.[10] In a study conducted in Kars Province, clinical, biochemical, and hematological findings were revealed in 3 dogs diagnosed with *B. canis* through PCR.[34] They also reported that the ticks picked from these three dogs were *D. reticulatus*. On the other hand, the number of epidemiological studies conducted with molecular techniques such as PCR and RLB has increased in recent years. *B. canis*, *B. gibsoni*, *B. vogeli*, and *B. rossi* were investigated by Real-Time PCR in 400 blood samples taken from the dogs in the region of Kayseri, and prevalence rates were found to be 12.9%, 9%, and 2.3%, respectively for *Babesia* species, whereas *B. rossi* was not encountered.[12] *Babesia* species were investigated in 757 dog blood samples collected from Sakarya, Kocaeli, Mersin, Giresun, Izmir, Elazığ, Diyarbakır, Erzurum, Ankara and Nevşehir Provinces using RLB method and *B. canis* was identified in only one dog (0.8%) in Erzurum Province.[14] It was reported in another study conducted in Erzurum that 7 (5.3%) of 133 asymptomatic dogs were infected with *B. canis* according to PCR and nested-PCR methods.[35] Canine piroplasm species were investigated by PCR-based RLB technique in 219 blood samples in Diyarbakır and *B. vogeli* (3/219; 1.4%) and *B. canis* (1/219; 0.4%) species were identified.[15] Tick-borne pathogens were investigated with PCR technique in 196 dogs in Konya and 4 (2.1%) of those were found to be infected with *B. vogeli* species.[36] In the most recent study on this subject, 186 blood samples collected from the dog shelters in Mersin, Adana, Hatay, Gaziantep, and Batman were investigated for vector-borne pathogens using molecular methods, and totally five (2.7%) dogs including four and one from Mersin and Hatay Provinces, respectively, were detected to be infected with *B. vogeli*.[37] The variation between prevalence rates depending on study sites is considered to be directly associated with the tick population in the sampling fields. It has been noticed that the prevalence rate of canine *Babesia* species is low throughout Turkey. It has been reported that the distribution and prevalence of vector-borne diseases are significantly affected by climate factors such as primarily extremely high and extremely low temperatures and rainfall pattern.[38] The ecology of *Babesia* was investigated in wild boars, wild rabbits, and foxes, and *B. vulpes* was first detected in the foxes in Turkey. In the same study, the presence of *B. rossi* was also identified in the ticks (*Hae. parva*) collected from wild boars and rabbits.[13] A tick should have ability to perform transstadial or transovarial transmission to be asserted as a vector for a pathogen.[39]

In the present study, the presence of canine babesiosis was investigated through PCR in Van Province where there is no previous study on the presence and prevalence of the disease. No positive result was detected in the tested dogs in this study that was first carried out using molecular methods in Van. As a matter of fact, the rates of canine *Babesia* species were found to be very low also in Erzurum and Kars which are the closest provinces to the region where this study was conducted. This partly supports the negative result obtained in the present study. It has been reported that the prevalence of *B. vogeli* across the different geographical regions in Turkey ranged between 1.4% and 8.7% [12,15,36,37,40]. The highest prevalence was determined in the Aegean Region.[40] *B. vogeli* could not be identified in Eastern Anatolian Region or in the region of Van where the present study was conducted. Upon the comparison of the habitats of the dogs, the distance between Van and Izmir is approximately 1,761 km. The geographical and climatic differences between the

regions, the current status of the vector tick species, and infection dynamics varying due to global warming may be responsible for the differences between prevalence rates of the regions. The *Babesia* species identified in Erzurum and Kars Provinces is *B. canis*. The infection rate was found to be 0.8% in Erzurum, whereas positivity was detected in only three dogs as case presentations in Kars Province. *Dermacentor reticulatus* is the known vector of *B. canis*. There is no adequate data on vector tick species considered to be responsible for disease and their prevalence in the study location. Upon the literature review, *D. reticulatus* was identified only in Ankara and Kars Provinces in Turkey.[34,41] Therefore, non-detection of *D. reticulatus* tick species in the region of Van in the previous studies supports the negative result of the present study.

Other species causing canine babesiosis are *Babesia vogeli* and *B. gibsoni*. These species are usually observed in the fields where *R. sanguineus* s.l. is the dominant taxon. The studies conducted in Eastern Anatolian Region have reported the presence of *R. sanguineus* s.l. in Van province.[42] The presence of vector tick species in the region of Van increases the probability of infection; however, no positive result related to this species was detected in the present study. Even though *Hae. leachi* tick species isthe known vector of *B. rossi*, DNA of *B. rossi* was identified in the tick species of *Hae. parva* in Turkey.[13,22] *Hae. leachi* observed in the African continent has not been seen in Turkey yet. Even though no DNA analysis result was detected about *B. rossi* in the dogs living in the region of Van, further comprehensive studies addressing vector-host interaction are needed.

*B. microti*-like piroplasm (Bml) which is usually accepted to be a synonym with Theileria annae is a small piroplasm that has been first identified in dogs in Spain. Some authors prefer to name this species as *B. vulpes* since it presents a high prevalence in red fox (*Vulpes vulpes*).[43] The potential vector of this species has been reported to be *I. hexoganus*.[23] It has been stated that this tick species become parasitic in wild life in Turkey.[44] The molecular characterization of *B. vulpes* was performed in three red foxes in Ankara and one of the foxes was determined to be infected with *Hae. parva* in the evaluation on tick. However, no DNA of *Babesia* spp. could be identified in the ticks.[15] In the present study, *B. vulpes* species could not be detected as a species in stray dogs. It is considered that particularly hunting dogs in close contact with foxes may contribute to the transmission of the related *Babesia* species to the local cycle. The eradication of ticks is not possible; however, periodical disinfestation is the best potential control strategy. The periodical disinfestation of the dogs using appropriate acaricides is another possible reasonable reason for the absence of *Babesia* positivity in the present study. Because it is known that stray animals are applied ectoparasiticides when they are brought to the Animal Shelter of Van Municipality from where our blood samples were supplied.

As a consequence, it was thought that the investigation of babesiosis in the dogs living in the region of Van made an epidemiological contribution to the data on the spread of the disease in Turkey. The present study has been the first molecular study to investigate canine *Babesia* species in Van Province. In addition to these data obtained from the dogs, advanced comprehensive studies that present long-term and systematic data collection on vector and pathogen distributions and investigate the natural cycles of infection, as well as the effect of climate variables on these cycles, are needed to demonstrate the status of the disease in the region in more details.

**Limitations**

The material of our study consisted only of blood samples taken from stray animals brought to the shelter and we couldn't get a positive result. Therefore, future studies performed by sampling the animals that are less frequently controlled by the veterinary physicians such as shepherd dogs, hunting dogs, and guard dogs fed for protection in the houses/gardens may contribute to the detection of the parasite.

**Acknowledgement**

This article was taken from the master thesis entitled “Van İlindeki Sokak Köpeklerinde *Babesia* Türlerinin Polimeraz Zincir Reaksiyonu Yöntemi ile Araştırılması” (2021).

**Conflict of Interest**

The author has no conflicts of interest.

**Compliance with Ethical Statement**

Ethics Committee approval for this research was obtained from the Local Ethics Committee for Animal Experiments of Van Yüzüncü Yıl University, (no. 2020/04-2021/12-09).

**Financial Support**

This study was sponsored by Van Yüzüncü Yıl University Scientific Research Project Fund and registered under Project No: TYL-2020-9169.

**Author Contributions**

ÖS, BO: Idea/Concept; ÖS, BO: Design; ÖS: Data Collection and/or Processing; ÖS, BO: Analysis and/or Interpretation; ÖS, BO: Literature Review; BO: Writing the Article; BO: Critical Review.

## References

1. Solano-Gallego L, Sainz A, Roura X, Estrada-Peña A, Miró G. A review of canine-babesiosis: the European perspective. *Parasit Vectors*. 2016;9:336. doi: 10.1186/s13071-016-1596-0.
2. Vishwakarma P, Nandini MK. Overview of canine babesiosis. In: Mani S, Bekoe SO, Adosraku RK, Ramkumar PK, editors. Veterinary Medicine and Pharmaceuticals. United Kingdom: IntechOpen; 2019. p.109-125. doi: 10.5772/intechopen.82243.
3. Birkenheuer AJ, Levy MG, Breitschwerdt EB. Efficacy of combined atovaquone and azithromycin for therapy of chronic *Babesia gibsoni* (*Asian genotype*) infections in dogs. *J Vet Intern Med.* 2004;18(4):494-498. doi: 10.1892/0891-6640(2004)18<494:eocaaa>2.0.co;2.
4. Irwin PJ. Babesiosis and Cytauxzoonosis. In: Shaw SE, Day MJ, Editors. Arthropod-borne infectious diseases of the dog and cat. London: Manson Publishing/The Veterinary Press; 2005. p.63-77.
5. Altay K, Aktaş M, Dumanlı N. Köpek ve Kedilerde Görülen Parazit Hastalıklar. İn: Özcel MA, İnci A, Köroğlu E, Karaer Z, Eren H, Yukarı BA, Dumanlı N, Aydın L, Yıldırım A (Editors). Veteriner Hekimliğinde Parazit Hastalıkları. Cilt 2. İzmir: Meta Basım Matbaacılık; 2016.
6. Solano-Gallego L, Trotta M, Carli E, Carcy B, Caldin M, Furlanello T. *Babesia canis canis* and *Babesia canis vogeli* clinicopathological findings and DNA detection by means of PCR-RFLP in blood from Italian dogs suspected of tick-borne disease. *Vet Parasitol.* 2008;157:211-221. doi: 10.1016/j.vetpar.2008.07.024.
7. Solano-Gallego L, Baneth G. Babesiosis in dogs and cats - expanding parasitological and clinical spectra. *Vet Parasitol.* 2011;181:48–60. doi.org/10.1016/j.vetpar.2011.04.023.
8. Miro G, Checa R, Paparini A, Ortega N, Gonzalez-Fraga JL. *Theileria annae* (syn. *Babesia microti*-like) infection in dogs in NW Spain detected using direct and indirect diagnostic

techniques: Clinical report of 75 cases. *Parasit Vectors*. 2015;8:217. doi.org/10.1186/s13071-015-0825-2.
9. Matjila PT, Leisewitz AL, Jongejan F, Penzhorn BL. Molecular detection of tick-borne protozoal and ehrlichial infections in domestic dogs in South Africa. *Vet Parasitol.* 2008;155:152-157. doi: 10.1016/j.vetpar.2008.04.012.
10. Gulanber A, Gorenflot A, Schetters TPM, Carcy B. First molecular diagnosis of *Babesia vogeli* in domestic dogs from Turkey. *Vet Parasitol*. 2006;139:224-230. doi.org/10.1016/j.vetpar.2006.02.035.
11. Aysul N, Ural K, Ulutas B, Eren H, Karagenc T. First detection and molecular identification of Babesia gibsoni in two dogs from the Aydin Province of Turkey. *Turk J Vet Anim Sci*. 2013;37:226-229. doi.org/10.3906/vet-1201-45.
12. Duzlu O, Inci A, Yildirim A, Onder Z, Ciloglu A. The investigation of some tick-borne protozoon and rickettsial infections in dogs by Real Time PCRand the molecular characterizations of the detected isolates. *Ankara Univ Vet Fak Derg.* 2014;61:275-282. doi.org/10.1501/Vetfak_0000002642.
13. Orkun Ö, Karaer Z. Molecular characterization of *Babesia* species in wild animals and their ticks in Turkey. *Infect Genet Evol.* 2017;55:8-13. doi: 10.1016/j.meegid.2017.08.026.
14. Aktas M, Özübek S, Altay K, İpek DNS, Balkaya İ, Ütük AE, et al. Molecular detection of tick-borne rickettsial and protozoan pathogens in domestic dogs from Turkey. *Parasit Vectors*. 2015;8:157. doi.org/10.1186/s13071-015-0763-z.
15. Aktas M, Ozubek S. A survey of canine haemoprotozoan parasites from Turkey, including molecular evidence of an unnamed *Babesia*. *Comp Immunol Microbiol Infect Dis*. 2017;52:36-42. doi:10.1016/j.cimid.2017.05.007.
16. Casati, SS, Sager, HH, Gern, LL, Piffaretti, JCJ-C. Presence of potentially pathogenic *Babesia* sp for human in Ixodes ricinus in Switzerland. *Ann Agric Environ Med*. 2006; 13(1):65.
17. Walker AR, Bouattour A, Camıcas JL, Estrada-Pena IG, Pegram RG, Preston PM. Ticks of domestic animals in Africa: a guide to identification of species. 1th ed. Edinburgh Scotland: U.K. The University of Edinburgh; 2003.
18. Estrada-Peña A, Bouattour A, Camicas JL, Walker AR. Ticks of veterinary and medical importance: The Mediterranean Basin. A guide of identification of species. Zaragoza: University of Zaragoza Press:2004.
19. Hatta T, Matsubayashi M, Miyoshi T, Islam K, Âlim MA, Anisuzzaman, et al. Quantitative PZR-based parasite burden estimation of Babesia gibsoni in the vector tick, *Haemaphysalis longicornis* (Acari: Ixodidae), fed on an experimentally infected dog. *J Vet Med Sci.* 2013;75:1-6. doi: 10.1292/jvms.12-0175.
20. Iwakami S, Ichikawa Y, Inokuma H. Molecular survey of *Babesia gibsoni* using *Haemaphysalis longicornis* collected from dogs and cats in Japan. *J Vet Med Sci.* 2014;76:1313-1316. doi: 10.1292/jvms.14-0210.
21. Jain J, Lakshmanan B, Nagaraj HV, Praveena JE, Syamala K, Aravindakshan T. Detection of *Babesia canis vogeli*, *Babesia gibsoni* and *Ehrlichia canis* by multiplex PCR in naturally infected dogs in South India. *Vet Arhiv*. 2018;88:215-224. doi: 10.24099/vet.arhiv.161229.
22. Apanaskevich DA, Horak IG, Camicas JL. Redescription of *Haemaphysalis* (Rhipistoma) *elliptica* (Koch, 1844), an old taxon of the *Haemaphysalis* (*Rhipistoma*) *leachi* group from East and southern Africa, and of *Haemaphysalis* (*Rhipistoma*) *leachi* (Audouin, 1826) (Ixodida, Ixodidae). *Onderstepoort J Vet Res*. 2007;74:181-208. doi: 10.4102/ojvr.v74i3.122.
23. Camacho AT, Pallas E, Gestal JJ, Guitian FJ, Olmeda AS, Telford SR, et al. Ixodes hexagonus is the main candidate as vector of *Theileria annae* in northwest Spain. *Vet Parasitol.* 2003;112:157-163. doi: 10.1016/s0304-4017(02)00417-x.
24. Bohm M, Leisewitz AL, Thompson PN, Schoeman JP. Capillary and venous *Babesia canis rossi* parasitaemias and their association with outcome of infection and circulatory compromise. *Vet Parasitol*. 2006;141:18-29. doi: 10.1016/j.vetpar.2006.05.002.
25. Jefferies R, Ryan UM, Muhlnickel CJ, Irwin PJ. Two Species of Canine *Babesia* in Australia: Detection and Characterization by PCR. *J Parasitol*. 2003;89(2):409-412. doi: 10.1645/0022-3395(2003)089[0409:TSOCBI]2.0.CO;2.
26. Sobczyk AS, Kotomski G, Górski P, Wędrychowicz H. Usefulness of touch-down PCR assay for the diagnosis of atypical cases of *Babesia canis canis* in dogs. Bull Vet Inst Pulawy. 2005; 49: 407-410.
27. Augustine S, Sabu L, Lakshmanan B. Molecular identification of *Babesia* spp. in naturally infected dogs of Kerala, South India. *J Parasit Dis.* 2017;41:459-462. doi: 10.1007/s12639-016-0827-9.
28. Ribeiro CM, Matos AC, Azzolini T, Bones ER, Wasnieski EA, Richini-Pereira VB, Lucheis SB, Vidotto O. Molecular epidemiology of *Anaplasma platys Ehrlichia canis* and *Babesia vogeli* in stray dogs in Paraná, Brazil. *Pesqui Vet Brasil*. 2017;37(2):129-136. doi.org/10.1590/S0100-736X2017000200006.
29. He L, Miao X, Hu J, Huang Y, He P, He J, et al. First molecular detection of *Babesia gibsoni* in dogs from Wuhan, China. *Front Microbiol*. 2017;8:1577. doi.org/10.3389/fmicb.2017.01577.
30. Barash NR, Thomas B, Birkenheuer AJ, Breitschwerdt EB, Lemler E, Qurollo BA. Prevalence of *Babesia* spp. and clinical characteristics of *Babesia vulpes* infections in North American dogs. *J Vet Intern Med*. 2019;33(5):2075-2081. doi: 10.1111/jvim.15560.
31. Checa R, Fidalgo LE, Montoya A, López AM, Barrera JP, Gálvez R, et al. The role of healthy dog carriers of *Babesia microti*-like piroplasms. *Parasit Vectors*. 2019;26: 12(1):127. doi.org/10.1186/s13071-019-3371-5.
32. Obeta SS, Ibrahim B, Lawal IA, Natala JA, Ogo NI, Balogun EO. Prevalence of canine babesiosis and their risk factors among asymptomatic dogs in the federal capital territory, Abuja, Nigeria. *Parasite Epidemiol Control*. 2020;11:e00186. doi.org/10.1016/j.parepi.2020.e00186.
33. Thomas RS, Santodomingo AM, Castro LR. Molecular detection of *Babesia canis vogeli* and *Hepatozoon canis* in dogs in the department of Magdalena, Colombia. *Rev Med Vet Zoot.* 2020;67(2): 7-122. doi.org/10.15446/rfmvz.v67n2.90701.
34. Gokce E, Kirmizigul AH, Taskin GT, Uzlu E, Gunduz N, Vatansever. Z: Clinical and Parasitological Detection of Babesia canis canis in Dogs: First Report from Turkey. *Kafkas Univ Vet Fak Derg.* 2013;19(4):717-720. doi:10.9775/kvfd.2013.7598.
35. Güven E, Avcıoğlu H, Cengiz S, Hayırlı A. Vector-Borne Pathogens in Stray Dogs in Northeastern Turkey. *Vector Borne Zoonotic Dis.* 2017;17(8):610-617. doi: 10.1089/vbz.2017.2128.
36. Guo H, Sevinç F, Ceylan O, Sevinç M, İnce E, Gao Y, et al. A PCR survey of vector-borne pathogens in different dog populations from Turkey. *Acta Parasitol.* 2017;62:533-540. doi: 10.1515/ap-2017-0064.
37. Aslantaş Ö, Çelebi B, Usluca S. Investigation of vector-borne diseases in dogs. *Harran Univ Vet Fak Derg.* 2020;9(2):154-160. doi:10.31196/huvfd.792147.
38. Gage KL, Burkot TR, Eisen RJ, Hayes EB. Climate and vectorborne diseases. *Am J Prev Med.* 2008;35:436-450. doi: 10.1016/j.amepre.2008.08.030.
39. Bakırcı S, Aktaş M, Vatansever Z, Aydın L. Keneler (Acarina: Ixodidae/Argasidae) Vektörlükleri ve Mücadelesi. Özbel Y editör. Vektör Artropodlar ve Mücadelesi. İzmir: Türkiye Parazitoloji Derneği, 2017. p.427-461.
40. Bilgic H, Pekel G, Hosgor M, Karagenc T. A Retrospective Epidemiological Study: The Prevalence of *Ehrlichia canis* and *Babesia vogeli* in Dogs in the Aegean Region of Turkey. *Acta Vet.* 2019;69(2):164-176. doi.org/10.2478/acve-2019-0013.
41. Orkun Ö. Molecular Characterization Based on 16S rDNA Phylogeny of Some Ixodid Ticks in Turkey. *Turkiye Parazitol Derg.* 2018;42:122-129. doi: 10.5152/tpd.2018.5882.
42. Deger MS, Bicek K, Oguz B. Infestation rate and distribution of hard ticks on cattle in the Eastern Anatolia Region of Turkey. Sci Parasitol. 2016;17:76-82.
43. Checa R, López-Beceiro AM, Montoya A, Barrera JP, Ortega N, Gálvez R, et al. *Babesia microti*-like piroplasm (syn. *Babesia vulpes*) infection in red foxes (*Vulpes vulpes*) in NW

Spain (Galicia) and its relationship with *Ixodes hexagonus*. *Vet Parasitol.* 2018;252:22-28. doi: 10.1016/j.vetpar.2018.01.011.
44. Aydın MF, Aktas M, Dumanlı N. Tick Infestations on Sheep and Goats in the Black Sea Region of Türkiye. *Kafkas Univ Vet Fak Derg.* 2012;18 (Suppl-A):A17-A22. doi: 10.9775/kvfd.2011.5209.