https://doi.org/10.52973/rcfcv-e34456



Revista Científica, FCV-LUZ / Vol. XXXIV, rcfcv-e34456

Molecular study of some vector-borne diseases in cattle raised in western Türkiye

Investigación molecular de algunas enfermedades transmitidas por vectores en ganado vacuno criado en el oeste de Turquía

Semiha Yalçın¹🗅, Neslihan Sürsal Şimşek²🕩, Seyda Cengiz¹*🕩

¹Mugla Sitki Kocman University, Milas Faculty of Veterinary Medicine, Department of Preclinical Sciences, Department of Microbiology. Mugla, Türkiye. ²Mugla Sitki Kocman University, Milas Faculty of Veterinary Medicine, Department of Preclinical Sciences, Department of Parasitology. Mugla, Türkiye. *Correspondence Author: <u>seydacengiz@mu.edu.tr</u>

ABSTRACT

Unfortunately, global warming, especially the global climate crisis, increases the rate of vector-borne infections. Among the causes of this infection are microorganisms in the Rickettsiales Order, which are Gram-negative and small coccobacillus microorganisms that can multiply within host cells and are dependent on their metabolism, in addition to bacterial infections, protozoa such as Babesia spp. and Theileria spp. are transmitted through vectors and cause serious diseases in animals. This study aimed to investigate the presence of some vector-borne bacterial and protozoan microorganisms in blood samples taken from cattle raised in Mugla province, located in the West of Türkiye, and to reveal relevant disease data for the region. In this study, blood samples taken from 100 cattle were examined using molecular methods. While Anaplasma phagocytophilum was detected in 15 blood samples (15%), Anaplasma ovis agent was detected in eight samples (8%). Anaplasma bovis agent (1%) was identified in only one blood sample. In the samples examined within the scope of the study, Ehrlichia and Rickettsia species from bacteria and Theileria spp. and Babesia spp. from parasitic agents could not be detected. Mugla province, located west of Türkiye, has a subtropical dry summer climate, so the probability of infections transmitted through arthropods is high. Since the agents are transmitted through ticks, conducting more studies on vector-borne diseases is essential. This includes mapping the region's vector ticks and determining and evaluating the tick carrier and disease maps in cattle. The data obtained is thought to help create regional and national vector-borne disease maps.

Key words: Anaplasma spp.; Babesia spp.; Ehrlichia spp.; Rickettsia spp.; Theileria spp.

RESUMEN

Desafortunadamente, el calentamiento global, especialmente la crisis climática global, aumenta la tasa de infecciones transmitidas por vectores. Entre las causas de esta infección se encuentran microorganismos del Orden Rickettsiales, que son microorganismos Gram negativos y cocobacilos pequeños que pueden multiplicarse dentro de las células huésped y son dependiente de su metabolismo las, además de infecciones bacterianas, protozoos como Babesia spp. y Theileria spp. se transmiten a través de vectores y causan enfermedades graves en los animales. Este estudio tuvo como objetivo investigar la presencia de algunos microorganismos bacterianos y protozoarios transmitidos por vectores en muestras de sangre tomadas de ganado criado en la provincia de Mugla, ubicada en el oeste de Turquía, y revelar datos relevantes sobre enfermedades para la región. En este estudio, se tomaron muestras de sangre de 100 bovinos y se examinaron mediante métodos moleculares. Mientras que Anaplasma phagocytophilum se detectó en 15 muestras de sangre (15%), el agente Anaplasma ovis se detectó en ocho muestras (8%). El agente Anaplasma bovis (1%) fue identificado en una sola muestra de sangre. En las muestras examinadas en el marco del estudio no se pudieron detectar especies de bacterias como Ehrlichia y Rickettsia y de parásitos como Theileria spp. y Babesia spp. La provincia de Mugla, situada al oeste de Türkiye, tiene un clima estival seco subtropical, por lo que la probabilidad de infecciones transmitidas a través de artrópodos es alta. Dado que los agentes se transmiten a través de las garrapatas, es esencial realizar más estudios sobre las enfermedades transmitidas por vectores. Esto incluye mapear las garrapatas vectoras de la región y determinar y evaluar los mapas de portadores de garrapatas y enfermedades en el ganado. Se cree que los datos obtenidos ayudarán a crear mapas regionales y nacionales de enfermedades transmitidas por vectores.

Palabras clave: Anaplasma spp.; Babesia spp.; Ehrlichia spp.; Rickettsia spp.; Theileria spp.



INTRODUCTION

Vector-borne diseases have been increasing in recent years, and global climate change and animal population movements are particularly effective in spreading such diseases. These infections occur especially in tropical and subtropical areas and are also seen in our country [1].

Microorganisms in the Order Rickettsigles, which cause infections transmitted through arthropods, are small coccobacilli that can multiply in host cells and show Gram-negative properties [2]. Rickettsia species belonging to the Rickettsiaceae family and Anaplasma and Ehrlichia species belonging to the Anaplasmataceae family, within the Order *Rickettsiales* [3], are important pathogens for farm animals. They are important for animal and public health because they contain some species that can cause human infection [4]. They are bacteria that settle in endothelial cells, immune system cells or erythrocytes, have obligate intracellular properties and are transmitted through blood [5]. Anaplasmosis is a bacterial infection that causes serious economic losses in animal husbandry and is also important for public health. Transmission is caused by the genera Ixodes, Dermacentor, Rhipicephalus and Amblyomma ticks. The infection is caused by Anaplasma spp. and infects the red blood cells of vertebrates [6]. The agent is transmitted biologically by ticks, mechanically by flies and contaminated materials.

The pathogenic species in cattle is A. marginale [7]. In addition to this species, A. centrale, A. bovis, A. ovis, A. phagocytophilum and A. platys cause infection defined as Anaplasmosis in cattle [8, 9, 10]. Ehrlichiosis is a disease caused by species of the Ehrlichia genus. Ehrlichia species settle intracytoplasmically in the leukocytes of their host [11]. Ehrlichiosis in cattle can be accompanied by fever, protruding tongue, floppy ears, turning around, excessive chewing, decreased feed consumption, conjunctival congestion and lymphadenitis symptoms [12, 13]. Bovine Ehrlichiosis is mainly caused by *E. ruminantium*. Transmission occurs from ticks of the genus Amblyomma, especially A. variegatum and A. habraeum [12]. Forms of the disease that progress with high mortality within a few hours in the peracute form and within 36–48 hours in the acute form have been reported [13].

Rickettsia genus bacteria has two main groups: the spotted fever group and the typhus group. In humans, infections from the spotted fever group can cause symptoms ranging from mild, like fever and rash, to life-threatening, depending on the specific agent. In ruminant animals, the infection tends to be self-limiting; therefore, *Rickettsia* infection has not received much attention in these animals [14, 15]. The primary vector of diseases in the spotted fever group is infected ticks. While various tick species of the *Dermacentor*, *Rhipicephalus*, and *Amblyomma* genera serve as vectors for *R. rickettsii* in America, *Rhipicephalus* sanguineus has been associated with *R. conorii* in Europe and the Mediterranean coasts, and *Amblyomma* ticks have been associated with *R. africae* in Africa. In Asia, *R. japonica* has been frequently isolated from various tick species belonging to the *Haemaphysalis*, *Ixodes* and *Dermacentor* genera [16].

The most common protozoan diseases transmitted by vectors in cattle are caused by *Babesia* and *Theileria* species [17, 18]. Babesiosis is an important parasitic disease for both animal and public health [19]. *Babesia* agents reproduce asexually within the erythrocytes of mammals, and the erythrocytic forms are called piroplasm. Sexual reproduction of the agent occurs in ticks in the *lxodidae* family [20]. Bovine babesiosis is also commonly called Texas Fever or Blood Urination Disease. *Babesia bovis*, *B. bigemina* and *B. divergens* species cause clinical babesiosis in cattle [21, 22]. *Babesia* infections are influenced by the host's age, immune system, co-infection status, and

genetic factors. Symptoms of acute infection include fever, anemia, hemoglobinuria, jaundice, weakness, lethargy, and anorexia, while chronic infection may be asymptomatic [20, 23].

Theileriosis is caused by Theileria agents, which are obligate intracellular protozoa mostly affecting ruminants and transmitted by ticks. The infection process involves entering the agents into the host's lymphocyte or macrophage cells, followed by asexual proliferation and development into piroplasmic forms found in erythrocytes at a later stage [24]. Theileria species are transmitted by ticks of the genus Hyalomma, Rhipicephalus, Dermacentor, Haemaphysalis, Amblyomma in the family Ixodidae, and ticks of the genus Ornithodorus in the family Argasidae. Especially T. parva (East Coast Fever) and T. annulata (Tropical theileriosis) are highly pathogenic species and can cause clinical disease in cattle. Symptoms may vary depending on factors such as infection with the pathogenic Theileria agent, tick infestation severity, other pathogen infections, host's immune system, age, race, and vaccination status [25]. The first symptom after a tick starts sucking blood is fever, followed by an enlargement of the nearest lymph node. Later symptoms include loss of appetite, increased heart rate, weakness, petechial bleeding, edema in the lymph and eyelids, decreased milk yield, and jaundice [26]. Although various studies have been conducted on the molecular epidemiology of diseases caused by vector-borne Rickettsial pathogens in cattle in Türkiye, there is still a lack of information regarding these factors [1]. In the Mugla region, which is included in the scope of the study, no studies on these diseases in cattle were found. This study aimed to investigate Anaplasma, Ehrlichia and Rickettsia, Babesia and Theileria species using molecular methods in blood samples taken from cattle raised in Mugla province, located in the west of Türkiye.

MATERIALS AND METHODS

Sampling

Blood samples were collected from apparently healthy dairy cattle between June and September 2023, when vector ticks were also active. A total of 100 cattle (*Bos taurus*) blood samples taken from 11 different farms were used as material. Blood samples were taken from the jugular veins of the animals into 10 mL tubes with di-sodium ethylenediamine tetra-acetate (EDTA) under aseptic conditions. Then, each blood sample collected was divided into sterile 1.5 mL eppendorf tubes and stored at -20°C (Grundig, GRNE 4302, Türkiye) until genomic DNA isolations were performed.

DNA extraction and molecular analysis

 $200 \,\mu$ L of blood was used to isolate genomic DNA(gDNA) from the blood sample taken from each cattle. At this stage, analyses were performed using a commercial kit (GeneJET Genomic DNA Purification Kit, Thermo Scientific, Waltham, MA, USA) in accordance with the manufacturer's instructions. The gDNAs obtained were stored at -20°C until PCR analysis.

Three different multiplex-PCR reactions (Rxn) were performed for bacteria A. centrale and A. marginale (Rxn1)[27], Ehrlichia spp. and Rickettsia spp. (Rxn2)[28], A. capra, A. bovis, A. ovis and A. phagocytophilum (Rxn3)(TABLE I)[29]. Ready-made PCR mix was used for multiplex PCR processes (DreamTaq Hot Start Green PCR Master Mix, Thermo Scientific, Waltham, MA, USA). Rxn1 was programmed as follows: 3 min at 95°C, 10 s at 98°C, 30 s at 55 °C, 30 s at 72°C (35 cycles) and a final extension at 72°C for 5 min. Rxn2 was programmed as follows: 95°C for 1 min, 95°C for 30 s, 56 °C for 30 s, 72°C for 30 s (40 cycles), and a final extension of 72°C for 7 min. Amplification

Agent	Oligonucleotide sequence	Amplicon size (bp)
Anaplasma centrale	F: CATGGGGCATGAATCTGTG R: AATTGGTTGCAGTGAGCGC	395
Anaplasma marginale	F: CATCTCCCATGAGTCACGAAGTGGC R: GCTGAACAGGAATCTTGCTCC	
	F: GCATTACAACGCAACGCTT R: ACCTTGGAGCGCATCTCTT	515-687
Ehrlichia spp.	F: CAATAGCAAGAGCCAATG R: TTAGAAGATGCTGTAGGATG	145
Rickettsia spp.	F: CAGACTTACCAAACTCAATC R: TACGCAAGAACCCTTGGA	437
Anaplasma capra	groE: TGAAGAGCATCAAACCCGAAG	874
Anaplasma bovis	groE: CTGCTCGTGATGCTATCGG groE: GTGGGATGTACTGCTGGACC	529
Anaplasma ovis	msp4: ATGGGGAGAGAGATATCCGCGA msp4: TGAAGGGAGCGGGGTCATGGG	347
Anaplasma phagocytophilum 16SrRNA:	GAGTAATTGCAGCCAGGCACTCT AGTGCTGAATGTGGGGGATAATTTATCTCCGTG CTAATCTCCATGTCAAGGAGTGGTAAGGTTT	172

conditions for Rxn3 were performed under the following 5 min at 94°C, 30 s at 94°C, 30 s at 63°C, 1 min at 72°C (35 cycles), and extension 1 min at 72°C conditions.

PCR was performed with specific primers Forward 3'-GACACAGGGAGGTAGTGACAAG-5' and Reverse 5'-CTAAGAATTTCACCTCTGACAGT-3'[30], which amplify approximately 403 bais pair (bp) of the 18S ribosomal rRNA (V4 hypervariable region) gene of *Babesia* spp. and *Theileria* spp. The reaction mixture should be $25 \,\mu$ L in accordance with the manufacturer's recommendation; 12.5 μ L of commercial master mix (DreamTaq Hot Start Green PCR Master Mix, Thermo Scientific, Waltham, MA, USA) was prepared by adding 0.5 μ M of each primer and 10-50 nanograms (ng) of genomic DNA (gDNA). The thermal profile is 2 min at 95°C; 35 cycles, denaturation: 30 s at 95°C, binding: 30 s at 57°C, extension: 1 min at 72°C, and final extension: 10 min at 72°C. The obtained PCR products were subjected to electrophoresis in 1.5% agarose gel and visualised in a UV transilluminator (Cleaver, Clear View, United Kingdom).

RESULTS AND DISCUSSION

Rickettsia spp. and *Ehrlichia* spp. could not be detected in all cattle blood samples collected from 11 different farms. Bacterial agents belonging to the *Anaplasma* genus were detected by molecular methods in 7 cattle farms. At least one *Anaplasma* spp. agent was detected in 18% of all cattle blood samples. While *A. phagocytophilum* was detected in fifteen blood samples (15%), the *A. ovis* agent was molecularly determined in eight samples (8%). Among these samples, *A. phagocytophilum* and *A. ovis* bacteria were molecularly detected as mixed infections in blood samples from six different cattle. *A. bovis* agent (1%) was identified in a blood sample from cattle (FIGS. 1 and 2). *Theileria* spp. and *Babesia* spp. could not be detected in the samples examined within the scope of the study. Information on bacterial agents detected molecularly in blood samples is given in TABLE II.

<i>TABLE II</i> The microorganisms detected by molecular method in blood samples			
Farm code	Sample no	Bacterial agent	
A	41	Anaplasma phagocytophilum	
	A1	Anaplasma ovis	
		Anaplasma phagocytophilum	
	A4	Anaplasma ovis	
	A5	Anaplasma phagocytophilum	
	B1	Anaplasma bovis	
В	B3	Anaplasma ovis	
	B4	Anaplasma phagocytophilum	
D	D4	Anaplasma ovis	
E		Anaplasma phagocytophilum	
	E1	Anaplasma ovis	
	E4	Anaplasma phagocytophilum	
		Anaplasma phagocytophilum	
	E5	Anaplasma ovis	
	E8	Anaplasma phagocytophilum	
	E9	Anaplasma phagocytophilum	
	E12	Anaplasma phagocytophilum	
	E15	Anaplasma phagocytophilum	
F	F2	Anaplasma phagocytophilum	
G		Anaplasma phagocytophilum	
	G1	Anaplasma ovis	
		Anaplasma phagocytophilum	
	G5	Anaplasma ovis	
М	M13	Anaplasma phagocytophilum	

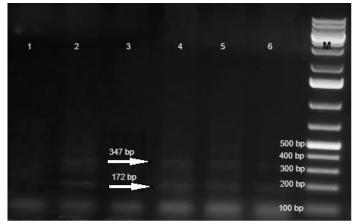


FIGURE 1. Gel electrophoresis image of *Anaplasma phagocytophilum* and *Anaplasma ovis*. 1: Negative control; 2, 4, 5, 6: Positive samples (*A. phagocytophilum* and *A. ovis*); 3: Negative sample; M: Marker DNA Ladder Plus

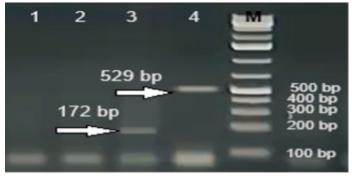


FIGURE 2. Gel electrophoresis image of *Anaplasma phagocytophilum* and *Anaplasma bovis*. 1: Negative Control; 3: *A. phagocytophilum*; 4: *A. bovis*; M: Marker DNA Ladder Plus

Due to climate change, global warming and increased humidity have enlarged the number of vectors, resulting in a rise in the incidence of vector-borne infections. These infections adversely affect cattle farming, causing economic losses by reducing productivity and even resulting in deaths worldwide [<u>31</u>]. Vector-borne infections can be detected through Giemsa-stained blood smear examinations and serological tests. However, microscopic examination can be misinterpreted, and serological tests can result in cross-reaction, leading to inaccurate diagnoses. As an alternative to traditional diagnosis, molecular techniques such as PCR are becoming more widespread due to their higher sensitivity and specificity, providing an accurate diagnosis [<u>31</u>, <u>32</u>].

Anaplasma agents, clinically most evident in cattle but can also infect other ruminant animals, are transmitted mechanically through fly bites, ticks, and surgical procedures such as dehorning and castration [33]. The main causative agent of bovine anaplasmosis is *A. marginale* [33, 34, 35]. Cattle that recover from infection remain permanently infected carriers and become a reservoir for other cattle [35]. Acute anaplasmosis is diagnosed by finding positive stained blood smears for infected erythrocytes. During this period, there is a significant decrease in hematocrit due to anemia in the first few days. In persistent infection, bacteria may not be detected [36]. The persistence of the infection subclinically in the herd may cause the infection to be overlooked, and therefore, anaplasmosis control programs cannot be designed [35]. *A. marginale* infections are endemic in Türkiye, and most animals are reservoirs for this infection [37]. *A. marginale* does not cause human disease [33].

Other Anaplasma species that can cause anaplasmosis in cattle are A. centrale, which causes a mild disease; A. bovis and A. phagocytophilum, also known as tick-borne fever. A. phagocytophilum is zoonotic. Congenital transmission of this agent to cattle has been reported. The severity of symptoms that occur after a latent period, such as fever, anemia, shortness of breath, loss of appetite, loss of productivity, abortion or stillbirth, are related to factors like the animal's immune status and co-infections [34]. In recent years, research on vector-borne diseases has been ongoing in various parts of the world. In a study conducted in Kyrgyzstan, the molecular prevalence of Anaplasma spp. was determined to be 1.7%, and the presence of A. centrale, A. phagocytophilum like-1 and human pathogenic new genotype A. capra agents was detected through sequence studies of the 16S rRNA gene in cattle in this region [38]. In another study conducted on cattle in Thailand, 20.8% of the blood samples were found positive for Anaplasma spp. by molecular

methods, and of these 20.8%, were determined to be A. marginale and 3.2% for A. platys. A. bovis agent was not detected [39]. In a study conducted in China, 3.2% of 493 blood samples taken from dairy cattle were found positive for Anaplasma spp. [40]. A study was conducted in the northern region of Türkiye's Black Sea to examine cattle blood samples with molecular methods for Anaplasma agents. The results showed that A. phagocytophilum was present in 30.8% of the samples, A. marginale in 18.8%, A. centrale in 18%, and A. bovis in only 0.7% of the samples [41]. In another, more comprehensive molecular-based study conducted with blood samples taken from cattle in the same region of Türkiye, the presence of A. marginale, A. centrale, and A. phagocytophilum agents was found at rates of 2.8%, 1.0%, and 1.0%, respectively [42]. In a recent study covering 16 provinces, mainly in the Central Anatolia and Southeastern Anatolia Regions of Türkiye, A. marginale was detected in 10.5% of the samples, A. phagocytophilum in 13.8%, A. bovis in 0.5%, and Anaplasma spp. in 2.9% [1]. In another study conducted in Malatya, eastern Türkiye, it was observed that the most common species in cattle was A. marginale (32.5%), followed by A. centrale (5.5%) and other Anaplasma agents [43]. Recent studies conducted in Türkiye show that the molecular prevalence of this pathogen in cattle is between 0% and 30.8% [1].

Consistent with these studies, in current study, the molecular prevalence of A. phagocytophilum agents in the Mugla region was determined to be 15%. Although there are studies on Anaplasma and other tick-borne diseases in small ruminants and pet animals in the Aegean Region provinces where this study was conducted [44, 45, <u>46, 47</u>] studies on cattle are limited in our region. No research on this subject has been found in Mugla. In a study conducted in Aydın region, a province close to our region, it was found that A. phagocytophilum species were found at a higher rate than A. margingle and A. Centrale; A. marginale infections peaked in March and September, and A. centrale infection started in March. It was determined that it continued to increase until September and then decreased. The A. phagocytophilum agent was detected regularly without fluctuation. Consistent with this study, the highest molecular prevalence in the Mugla region was seen in A. phagocytophilum (15%). A. marginale and A. centrale agents were not detected. It has been stated that this situation may be due to the presence of the agents in the blood of animals at varying levels depending on the months in the region or due to the low prevalence of these agents in the region [48]. Since current study was carried out on samples taken between June and September, it was thought that the absence of A. marginale and A. centrale agents when the previous study findings were examined may be due to the lower prevalence of the relevant agents in the summer months in the region. To have complete information about the prevalence of diseases, it is necessary to evaluate the results by sampling at regular intervals in the region over a broader period of time and to conduct further research on the subject.

In the literature, A. ovis is stated as the main agent responsible for the anaplasmosis of sheep and goats. Although the agent is not associated with cattle [36], in current study, it was found to be a mixed infection agent with A. phagocytophilum in 6 different samples and as a single agent in 2 different samples more, than one agent can commonly be detected in animals infested by more than one tick. This situation may cause the severity of the disease to increase [49]. Similar results in the world and our country confirm the existence of anaplasmosis due to A. ovis bacteria in cattle [40, 42]. In Türkiye, in the Black Sea region, Aktas et al. (2011), in the 16S rRNA sequence analysis, it was found that the sequence results of 3 samples from bovines were 100% similar to A. ovis, the causative agent of sheep anaplasmosis [42]. A. bovis, transmitted by Amblyomma and Rhipicephalus tick species, causes subclinical disease in cattle [10]. Recent studies conducted in Türkiye and around the world have revealed the presence of A. bovis in cattle [1, 40, 50], but this agent was not found in cattle in the study conducted in the Aydın region in our country. However, a low rate of A. bovis agent carriers was determined from ticks collected from animals [48]. A. bovis agent was detected at a low rate (1%) in the blood samples examined in current study, which suggests that it is parallel to this picture in the nearby region.

Babesia spp. and Theileria spp. were not detected in the blood samples collected within the scope of this study. In Türkiye, similar cases where the agents in question are negative have been reported in different studies [51, 52]. In addition, tick-borne anaplasmosis agents, unlike Babesia and Theileria agents, can be transmitted biologically by ticks as well as mechanically by some blood-sucking flies [53]. This may explain why blood samples are positive for anaplasmosis but negative for Babesia spp. and Theileria spp. Tick samples collected from cattle in Türkiye have been found to contain Ehrlichia spp. and Rickettsia spp. according to a study by Ji et al. (2022) [54]. There is insufficient research on Rickettsial disease in cattle in Turkey. In a recent study, Ceylan et al. (2024) reported E. minasensis for the first time with a prevalence rate of 0.5% (55). 51 tick species have been identified in Türkiye due to suitable climatic conditions and abundant wild and domestic animals [54]. Seasonal variation of ticks depends on the breed. Rhipicephalus and Hyalomma species are found in spring, summer and autumn, while Dermacentor, Hemaphysalis, Ixodes and Ornithodoros species are found in autumn, winter and spring. Dermacentor, Haemaphysalis, Hyalomma, Ixodes, Rhipicephalus (Boophilus) tick genera are commonly found throughout Türkiye [36, 54]. In a study conducted in the west of Türkiye and in the region where the samples were collected in current study, tick genera and species were defined according to months, and in the period between June and September, when the samples were collected in current study, it was determined that Hyalomma species, especially H. marginatum, were present at a higher rate, and Rhipicephalus genus ticks were found at a lower rate [56]. The occurrence and severity of these tick-borne diseases are associated with many factors, including seasonal or artificially induced fluctuations in the tick population and the resulting immune status of affected cattle [57]. In a study conducted in northern Türkiye [58], the region was grouped and examined according to climate characteristics and different carrier rates were determined in the same tick species. Different studies report tick-borne disease cases with different prevalences. This may be due to the change in vector population due to changing climate conditions or the agent carrier feature of tick species. Therefore, considering global climate change, it is important to prepare up-todate possible disease maps at the regional level by conducting more research on vector-borne diseases, vector diversity, seasonal vector distributions, and the agent carrier rates of these vectors.

CONCLUSION

These infections, both bacterial and protozoan, have been increasing in recent years, and global climate change and animal movements have a significant impact on the spread of such infections in farm animals. Studies have shown that these infections, which have been found to cause significant economic losses in cattle, are accompanied by fever, hemolytic anemia, abortion in pregnant animals and, in some cases, death. Mugla province, located in the west of Türkiye, has a subtropical dry summer climate; therefore, the probability of infections transmitted through arthropods is high. Since the agents are transmitted through ticks, it is important to conduct more studies on vector-borne diseases, create maps of vector ticks in the region, and determine and evaluate tick carriers and existing disease maps in cattle. The data obtained is thought to be useful in creating regional and national vector-borne disease maps.

Funding

This paper has been granted by Mugla Sıtkı Koçman University Research Support and Funding Office through Project Grant Number: (23/156/02/3/4).

Ethics approval

This research project was approved by the Animal Experiments Local Ethic Committee of Mugla Sitki Kocman University under number E-40051172-100-429028.

Conflict of interests

No conflicts of interest for all authors are declared.

BIBLIOGRAPHIC REFERENCES

- [1] Ceylan O, Ma Z, Ceylan C, Culha MH, Galon EM, Ji S, Li H, Zafar I, Mohanta UK, Xuan X, Sevinc F. Wide bovine tick-borne pathogen spectrum: Predominancy of *Theileria annulata* and the first molecular detection of *Ehrlichia minasensis* in Turkey. Vet. Res. Commun. [Internet]. 2024; 48(2):1037–1059. doi: <u>https://doi.org/g5rbvm</u>
- [2] Xu J, Gu XL, Jiang ZZ, Cao XQ, Wang R, Peng QM, Li ZM, Zhang L, Zhou CM, Qin XR, Yu XJ. Pathogenic Rickettsia, Anaplasma, and Ehrlichia in Rhipicephalus microplus ticks collected from cattle and laboratory hatched tick larvae. PLOS Negl. Trop. Dis. [Internet]. 2023; 17(8):e0011546. doi: https://doi.org/g5rbvr
- [3] Diop A, El Karkouri K, Raoult D, Fournier PE. Genome sequencebased criteria for demarcation and definition of species in the genus *Rickettsia*. Int. J. Syst. Evol. Microbiol. [Internet]. 2020; 70(3):1738–1750. doi: <u>https://doi.org/g5rbvv</u>
- [4] Lu M, Tian J, Wang W, Zhao H, Jiang H, Han J, Guo W, Li K. High diversity of *Rickettsia* spp., *Anaplasma* spp., and *Ehrlichia* spp. in ticks from Yunnan Province, Southwest China. Front. Microbiol. [Internet]. 2022; 13:1008110. doi: <u>https://doi.org/g5rbvw</u>
- [5] Osorio M, Miranda J, Gonzalez M, Mattar S. Anaplasma sp., Ehrlichia sp., and Rickettsia sp. in ticks: a high risk for public health in Ibagué, Colombia. Kafkas Univ. Vet. Fak. Derg. [Internet]. 2018; 24(4):557–562. doi: https://doi.org/g5rbvx
- [6] Soosaraei M, Haghi MM, Etemadifar F, Fakhar M, Teshnizi SH, Asfaram S, Esboei BR. Status of Anaplasma spp. infection in domestic ruminants from Iran: A systematic review with metaanalysis. Parasite Epidemiol. Cont. [Internet]. 2020; 11:e00173. doi: <u>https://doi.org/g5rbvz</u>
- [7] Vidotto MC, Kano SF, Gregori F, Headley SA, Vidotto O. Phylogenetic analysis of Anaplasma marginale strains from Paraná State, Brazil, using the msp1α and msp4 genes. J. Vet. Med. Ser. B. [Internet]. 2006; 53(9):404-411. doi: https://doi.org/bnfvnf

- [8] Dahmani M, Davoust B, Benterki MS, Fenollar F, Raoult D, Mediannikov O. Development of a new PCR-based assay to detect Anaplasmataceae and the first report of Anaplasma phagocytophilum and Anaplasma platys in cattle from Algeria. Comp. Immunol. Microbiol. Infect. Dis. [Internet]. 2015; 39:39-45. doi: https://doi.org/f68ja5
- [9] Park J, Han DG, Ryu JH, Chae JB, Chae JS, Yu DH, Park BK, Kim HC, Choi KS. Molecular detection of Anaplasma bovis in Holstein cattle in the Republic of Korea. Acta Vet. Scand. [Internet]. 2018; 60:1–5. doi: <u>https://doi.org/g5rbv4</u>
- [10] Peter SG, Aboge GO, Kariuki HW, Kanduma EG, Gakuya DW, Maingi N, Mulei CM, Mainga AO. Molecular prevalence of emerging Anaplasma and Ehrlichia pathogens in apparently healthy dairy cattle in peri-urban Nairobi, Kenya. BMC Vet. Res. [Internet]. 2020; 16:364. doi: <u>https://doi.org/g5rbv5</u>
- [11] Dumler JS. Anaplasma and Ehrlichia Infection. Ann. N.Y. Acad. Sci. [Internet]. 2005; 1063(1):361–373. doi: <u>https://doi.org/c6vrnc</u>
- [12] Basit MA, Ijaz M, Khan JA, Ashraf K, Abbas RZ. Molecular evidence and hematological profile of bovines naturally infected with ehrlichiosis in southern Punjab, Pakistan. Acta Parasit. [Internet]. 2022; 67(1):72–78. doi: https://doi.org/g5rbv9
- [13] Peter SG. Zoonotic Anaplasma and Ehrlichia infections and their potential reservoirs: A review. Int. J. Vet. Sci. [Internet]. 2020[cited 25 Apr. 2024]; 9(1):1–9. Available in: <u>https://goo.su/BcWQwM</u>
- [14] Adjadj NR, Cargnel M, Ribbens S, Quinet C, Malandrin L, Mignon B, Mori M. Prevalence of Anaplasma phagocytophilum, Borrelia burgdorferi sensu lato, Rickettsia spp. and Babesia spp. in cattle serum and questing ticks from Belgium. Ticks Tick-borne Dis. [Internet]. 2023; 14(4):102146. doi: https://doi.org/gr4nd6
- [15] Barradas PF, Mesquita JR, Ferreira P, Gärtner F, Carvalho M, Inácio E, Chivinda E, Katimba A, Amorim I. Molecular identification and characterization of *Rickettsia* spp. and other tick-borne pathogens in cattle and their ticks from Huambo, Angola. Ticks Tick-borne Dis. [Internet]. 2021; 12(1):101583. doi: https://doi.org/g5rbwb
- [16] Kim HK. Rickettsia-host-tick Interactions: Knowledge advances and gaps. Infect. Immun. [Internet]. 2022; 90:e00621-21. doi: https://doi.org/g5rbwc
- [17] Merino O, Almazan C, Canales M, Villar M, Moreno-Cid JA, Galindo RC, de la Fuente J. Targeting the tick protective antigen subolesin reduces vector infestations and pathogen infection by Anaplasma marginale and Babesia bigemina. Vaccine. [Internet]. 2011; 29(47):8575-8579. doi: https://doi.org/dvg4xq
- [18] Gosh S, Azhahianambi P, Yadav MP. Upcoming and future strategies of tick control: a review. J. Vector Borne Dis. [Internet] 2007[cited 24 Apr. 2024]; 44(2):79–89. PMID: 17722860. Available in: <u>https://goo.su/r0l52</u>
- [19] Homer MJ, Aguilar-Delfin I, Telford III SR, Krause PJ, Persing DH. Babesiosis. Clin. Microbiol. Rev. [Internet]. 2000; 13(3):451-469. doi: <u>https://doi.org/gqjng2</u>
- [20] Schnittger L, Rodriguez AE, Florin-Christensen M, Morrison DA. Babesia: a world emerging. Infect. Genet. Evol. [Internet]. 2012; 12(8):1788–1809. doi: <u>https://doi.org/f22qns</u>

- [21] Altay K, Aydin MF, Dumanli N, Aktas M. Molecular detection of *Theileria* and *Babesia* infections in cattle. Vet Parasit. [Internet]. 2008; 158(4):295–301. doi: <u>https://doi.org/bftwc8</u>
- [22] Inci A, Çakmak A, Karaer Z, Dinçer S, Sayin F, Iça A. Kayseri yöresinde sığırlarda babesiosis in seroprevalansı [Seroprevalence of bovine babesiosis around Kayseri]. Turk. J. Vet. Anim. Sci. [Internet]. 2002 [cited 29 Apr. 2024]; 26(6):1345–1350. Turkish. Available in: <u>https://goo.su/RW3H</u>
- [23] Kar S, Güven E, Karaer Z. Ankara'da Şubat ayında Babesiosis olgusu [A Babesiosis Case in February in Ankara]. Turkiye Parazitol. Derg. [Internet]. 2008 [cited 05 May 2024]; 32(4):379– 381. Turkish. Available in: <u>https://goo.su/6uORBzF</u>
- [24] Bishop R, Musoke A, Morzaria S, Gardner M, Nene V. Theileria: Intracellular protozoan parasites of wild and domestic ruminants transmitted by ixodid ticks. Parasitology. [Internet]. 2004; 129(1):271–283. doi: https://doi.org/ck8qks
- [25] Levine ND. Veterinary Protozoology. Ames (Iowa, USA): Iowa State University Press; 1985. Chapter 9, Apicomplexa: The Piroplasms. p. 291-328.
- [26] Taylor MA, Coop RL, Wall RL, editors. Veterinary Parasitology. 4th ed. Hoboken (New Jersey, USA): John Wiley & Sons, Inc; 2015. 1032 p.
- [27] Bell-Sakyi L, Palomar AM, Bradford EL, Shkap V. Propagation of the Israeli vaccine strain of Anaplasma centrale in tick cell lines. Vet. Microbiol. [Internet]. 2015; 179(3-4):270-276. doi: <u>https:// doi.org/f7p2vg</u>
- [28] Pérez Pérez JC, Montoya Ruiz C, Arroyave Sierra E, Paternina LE, Rodas JD. Design and analytical validation of a duplex PCR for *Ehrlichia* and *Rickettsia* detection in ticks. Rev. Colomb. Cienc. Pec. [Internet]. 2018; 31(4):285–294. doi: <u>https://doi.org/g5rbwj</u>
- [29] Peng Y, Zhao S, Wang K, Song J, Yan Y, Zhou Y, Shi K, Jian F, Wang R, Zhang L, Ning C. A multiplex PCR detection assay for the identification of clinically relevant *Anaplasma* species in field blood samples. Front Microbiol. [Internet]. 2020; 11:606. doi: <u>https://doi.org/g5rbwm</u>
- [30] Gubbels JM, De Vos AP, Van der Weide M, Viseras J, Schouls LM, De Vries E, Jongejan F. Simultaneous detection of bovine *Theileria* and *Babesia* species by reverse line blot hybridization. J. Clin. Microbiol. [Internet]. 1999; 37(6):1782–1789. doi: <u>https://doi.org/gg88dt</u>
- [31] Shrivastava P, Dehuri M, Mohanty B, Mishra C, Venkatesh KM, Biswal SS. Molecular characterization and prevalence of bovine hemoprotozoan and rickettsial organism from Bhubaneswar, Eastern India. Anim. Biotechnol. [Internet]. 2023; 34(7):2917– 2927. doi: <u>https://doi.org/g5rbwn</u>
- [32] Kaur R, Yadav A, Rafiqi SI, Godara R, Sudan V, Chakraborty D, Katoch R. Epidemiology, haematology and molecular characterization of haemoprotozoon and rickettsial organisms causing infections in cattle of Jammu region, North India. BMC Vet. Res. [Internet]. 2021; 17:219. doi: https://doi.org/g5rbwq
- [33] Aubry P, Geale DW. A review of bovine anaplasmosis. Transbound. Emerg. Dis. [Internet]. 2011; 58(1):1–30. doi: <u>https://doi.org/bpsfgv</u>
- [34] Parvizi O, El-Adawy H, Melzer F, Roesler U, Neubauer H, Mertens-Scholz K. Seroprevalence and molecular detection of bovine anaplasmosis in Egypt. Pathogens [Internet]. 2020; 9(1):64. doi: <u>https://doi.org/g5rbwr</u>

- [35] Spare MR, Hanzlicek GA, Wootten KL, Anderson GA, Thomson DU, Sanderson MW, Ganta RR, Reif KE, Raghavan RK. Bovine anaplasmosis herd prevalence and management practices as risk-factors associated with herd disease status. Vet. Parasitol. [Internet]. 2020; 277:100021. doi: https://doi.org/q5rbws
- [36] Ceylan O, Xuan X, Sevinc F. Primary tick-borne protozoan and Rickettsial infections of animals in Turkey. Pathogens [Internet]. 2021; 10(2):231. doi: <u>https://doi.org/g5rbwt</u>
- [37] Sevinç F, Xuan X. Major tick-borne parasitic diseases of animals: A frame of references in Turkey. Eurasian J. Vet. Sci. [Internet]. 2015; 31(3):132–142. doi: <u>https://doi.org/g5rbww</u>
- [38] Altay K, Erol U, Sahin OF, Aytmirzakizi A. First molecular detection of Anaplasma species in cattle from Kyrgyzstan; molecular identification of human pathogenic novel genotype Anaplasma capra and Anaplasma phagocytophilum related strain. Ticks Tickborne Dis. [Internet]. 2022; 13(1):101861. doi: https://doi.org/g5rbwx
- [39] Seerintra T, Saraphol B, Thanchomnang T, Piratae S. Molecular prevalence of Anaplasma spp. in cattle and assessment of associated risk factors in Northeast Thailand. Vet World. [Internet]. 2023; 16(8):1702–1707. doi: <u>https://doi.org/g5rbw2</u>
- [40] Yan Y, Jiang Y, Tao D, Zhao A, Qi M, Ning C. Molecular detection of Anaplasma spp. in dairy cattle in southern Xinjiang, China. Vet. Parasitol. Reg. Stud. Reports[Internet]. 2020; 20:100406. doi: <u>https://doi.org/g5rbw5</u>
- [41] Aktas M, Ozübek S. Bovine anaplasmosis in Turkey: First laboratory confirmed clinical cases caused by Anaplasma phagocytophilum. Vet. Microbiol. [Internet]. 2015; 178(3–4):246–251. doi: <u>https://doi.org/f7hcbg</u>
- [42] Aktas M, Altay K, Dumanli N. Molecular detection and identification of Anaplasma and Ehrlichia species in cattle from Turkey. Ticks Tick-borne Dis. [Internet]. 2011; 2(1):62-65. doi: https://doi.org/fkrb5g
- [43] Aktas M, Çolak S. Molecular detection and phylogeny of Anaplasma spp. in cattle reveals the presence of novel strains closely related to A. phagocytophilum in Turkey. Ticks Tick-borne Dis. [Internet]. 2021; 12(1):101604. doi: https://doi.org/g5rbw8
- [44] Bilgic HB, Bakırcı S, Kose O, Unlu AH, Hacılarlıoglu S, Eren H, Weir W, Karagenc, T. Prevalence of tick-borne haemoparasites in small ruminants in Turkey and diagnostic sensitivity of single-PCR and RLB. Parasit. Vectors. [Internet]. 2017; 10:211. doi: https://doi.org/g5rbw9
- [45] Ceylan O, Uslu A, Ozturk O, Sevinc F. Serological investigation of some vector-borne parasitic and rickettsial agents in dogs in the western part of Turkey. Pak. Vet. J. [Internet]. 2021[cited 25 Apr. 2024]; 41(3):386–392. Available in: <u>https://goo.su/prRgxq</u>
- [46] Haydardedeoğlu AE, Büyükleblebici O, Aksoy NH, Karaşahin T. Aksaray Malaklısı Çoban Köpeklerinde Ehrlichia Canis, Anaplasma Phagocytophilum, Borrelia Burgdorferi, Dirofilara immitis Enfeksiyonlarının Anlık Dağılımının Belirlenerek Hematolojik Bulguların Araştırılması [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 Üniv. Vet. Fak. Derg. [Internet]. 2019; 8(1):38–43. Turkish. doi: https://doi.org/g5rbxc

- [47] Ural K, Gultekin M, Atasoy A, Ulutas B. Spatial distribution of vector borne disease agents in dogs in Aegean region, Turkey. Revista MVZ Córdoba. [Internet]. 2014; 19(2):4086–4098. doi: <u>https://doi.org/g5rbxf</u>
- [48] Hoşgör M, Bilgiç HB, Bakırcı S, Ünlü AH, Karagenç T, Eren H. Detection of Anaplasma/Ehrlichia species of cattle and ticks in Aydın region. Turk. Parazitol. Derg. [Internet]. 2015; 39(4):291– 298. doi: <u>https://doi.org/g4ntd3</u>
- [49] Mohammadian B, Noaman V, Emami SJ. Molecular survey on prevalence and risk factors of *Anaplasma* spp. infection in cattle and sheep in West of Iran. Trop. Anim. Health Prod. [Internet]. 2021; 53(2):266. doi: <u>https://doi.org/g5rbxj</u>
- [50] Aktas M, Ozubek S. Molecular and parasitological survey of bovine piroplasms in the Black Sea region, including the first report of babesiosis associated with *Babesia divergens* in Turkey. J. Med. Entomol.[Internet]. 2015; 52(6):1344–1350. doi: <u>https://doi.org/f72c9m</u>
- [51] Altay K, Atas AD, Ograk YZ, Ozkan E. Survey of Theileria, Babesia and Anaplasma infections of cattle and ticks from Sivas region of Turkey. Erciyes Univ. Vet. Fak. Derg. [Internet]. 2020; 17(1):32–38. doi: <u>https://doi.org/g5rbxm</u>
- [52] Yavuz A, Inci A, Düzlü Ö, Bişkin Z, Yildirim A. Babesia bovis'in msa-2c Geninin Moleküler Karakterizasyonu [Molecular characterization of babesia bovis msa-2c gene]. Turkiye Parazitol. Derg. [Internet]. 2011; 35(3):140–144. Turkish. doi: <u>https://doi.org/bv5cjj</u>
- [53] Woldehiwet Z. The natural history of Anaplasma phagocytophilum. Vet. Parasit. [Internet]. 2010; 167(2–4):108–122. doi: <u>https://doi.org/fqcxwm</u>
- [54] Ji S, Ceylan O, Ma Z, Galon EM, Zafar I, Li H, Hasegawa Y, Sevinc M, Masatani T, Iguchi A, Kawase O, Umemiya-Shirafuji R, Asada M, Sevinc F, Xuan X. Protozoan and rickettsial pathogens in ticks collected from infested cattle from Turkey. Pathogens [Internet]. 2022; 11(5):500. doi: <u>https://doi.org/g5rbxq</u>
- [55] Ceylan O, Ma Z, Ceylan C, Culha MH, Galon EM, Ji S, Li H, Zafar I, Mohanta UK, Xuan X, Sevinc F. Wide bovine tick-borne pathogen spectrum: Predominancy of *Theileria annulata* and the first molecular detection of *Ehrlichia minasensis* in Turkey. Vet. Res. Commun. [Internet]. 2024; 48(2):1037–1059. doi: <u>https://doi.org/g5rbvm</u>
- [56] Bakirci S, Sarali H, Aydin L, Eren H, Karagenc T. Distribution and seasonal activity of tick species on cattle in the West Aegean region of Turkey. Exp. Appl. Acarol. [Internet]. 2012; 56(2):165– 178. doi: https://doi.org/crz99j
- [57] Henker LC, Lorenzett MP, Fagundes-Moreira R, Dalto AGC, Sonne L, Driemeier D, Soares JF, Pavarini SP. Bovine abortion, stillbirth and neonatal death associated with *Babesia bovis* and *Anaplasma* sp. infections in southern Brazil. Ticks Tick-borne Dis. [Internet]. 2020; 11(4):101443. doi: <u>https://doi.org/jzcz</u>
- [58] Aktas M, Altay K, Ozubek S, Dumanli N. A survey of ixodid ticks feeding on cattle and prevalence of tick-borne pathogens in the Black Sea region of Turkey. Vet. Parasitol. [Internet]. 2012; 187(3-4):567-571. doi: https://doi.org/f32t7p