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ORIGINAL RESEARCH ARTICLE

Molecular Detection of *Anaplasma ovis* and *Anaplasma capra* in Sheep Flocks from Gevaş District, Van Province, Türkiye

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Abstract. Tick-borne diseases, particularly anaplasmosis, have a significant impact on livestock production. This study aimed to investigate the incidence and prevalence of *Anaplasma ovis* and *Anaplasma capra* in sheep herds in the Gevaş district of Van Province, Türkiye. PCR analysis was performed on blood samples from 200 clinically healthy sheep using msp4 and gltA gene-targeted PCR primers. A total of 149 (74.5%) samples tested positive for *A. ovis*, whereas none were positive for *A. capra*. No significant associations were observed between *A. ovis* infection and host factors such as sex and age ($P > 0.05$). However, its prevalence was higher in the İnköy region ($P < 0.05$). Sanger sequencing confirmed partial msp4 gene sequences, which were submitted to GenBank (accession numbers PV231424-PV231428). The *A. ovis* isolates showed low genetic diversity compared to the strains from other countries. This study highlights the high prevalence of *A. ovis* in the region, in contrast to the absence of *A. capra*. Therefore, further research is needed to focus on the epidemiology and impact of *A. capra*.

Keywords: *Anaplasma ovis, Anaplasma capra, PCR, Sheep, Van*.

Detecţia moleculară a infecţiei cu *Anaplasma ovis* şi *Anaplasma capra* în turmele de oi din districtul Gevaş, provincia Van, Turcia

Rezumat. Bolile transmise de căpuşe, în special anaplasmoza, au un impact semnificativ asupra producţiei animaliere. Acest studiu şi-a propus să investigheze incidenţa şi prevalenţa infecţiei cu *Anaplasma ovis* şi *Anaplasma capra* în turmele de oi din districtul Gevaş din provincia Van, Turcia. Analiza PCR a fost efectuată pe probe de sânge recoltate de la 200 de oi clinic sănătoase, utilizând primeri PCR direcţionaţi către genele msp4 şi gltA. Un total de 149 (74,5%) probe au fost pozitive pentru *A. ovis*, în timp ce niciuna nu a fost pozitivă pentru *A. capra*. Nu s-au observat asocieri semnificative între infecţia cu *A. ovis* şi factorii gazdă, cum ar fi sexul şi vârsta ($P > 0,05$). Cu toate acestea, prevalenţa sa a fost mai mare în regiunea İnköy ($P < 0,05$). Secvenţierea Sanger a confirmat secvenţele parţiale ale genei msp4, care au fost trimise la GenBank (numerele de acces PV231424-PV231428). Izolatele de *A. ovis* au prezentat o diversitate genetică scăzută în comparaţie cu tulpinile din alte

ţări. Acest studiu evidenţiază prevalenţa ridicată a infecţiei cu *A. ovis* în regiune, spre deosebire de absenţa infecţiei cu *A. capra*. Prin urmare, sunt necesare cercetări suplimentare pentru studiul asupra epidemiologiei şi impactului *A. capra*.

Cuvinte cheie: *Anaplasma ovis, Anaplasma capra, PCR, Sheep, Van*.

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Introduction

Anaplasma is a group of bacteria that are obligate intracellular and gram-negative. These pathogens are transmitted to animals through certain ticks of the Ixodidae family. Accordingly, they represent a significant threat to veterinary and public health (Rymaszewska and Grenda, 2008; Silaghi et al., 2017; Ben Said et al., 2018; Rar et al., 2021). As of now, there are eight taxonomically accepted species included in this genus: *A. phagocytophilum*, *A. marginale*, *A. centrale*, *A. ovis*, *A. bovis*, *A. platys*, *A. odocoilei*, and *A. capra*. Most unclassified genetic variants with highly diverse genetic compositions have been reported (Ben Said et al., 2018; Rar et al., 2021). *Anaplasma ovis* is one of the best studied intraerythrocytic pathogens in sheep, goats and some wild ruminants. The primary vector of this pathogen is the tick species *Rhipicephalus* spp., however, its occurrence has also been documented in *Amblyomma* species, *Dermacentor*, and *Ixodes* (Chochlakis et al., 2010; Rymaszewska and Grenda, 2018). The impact of *A. ovis* is becoming increasingly recognized due to its correlation with production losses, the possibility of more severe clinical manifestations in certain cases, the risk of co-infections, and the increasing number of wild reservoir hosts that have been identified (Lu et al., 1997; Stoltsz, 2004; de la Fuente et al., 2007; Smith and Sherman, 2009; Yasini et al., 2012; Li et al., 2014). Moreover, recent studies have highlighted the impact of global climate change and interseasonal climatic fluctuations on the modification of *A. ovis* transmission dynamics in sheep herds (Lacasta et al., 2021; Ruiz et al., 2024). Changes in the environment are predicted to increase tick distribution and activity, contributing to the transmission of pathogens. Molecular epidemiological reports have also indicated the occurrence of high genetic diversity in *A. ovis* populations, particularly in investigations of msp4 and msp1a gene markers. These genes are

characterized by high conservation with region-specific polymorphisms and have potential applications in diagnostics and phylogenetic analysis. The observed variability among the isolates has a profound impact on understanding transmission patterns, improving diagnostic discrimination, and guiding control and management strategies (Belkahia et al., 2014; Aktaş and Özübek, 2018; Naeem et al., 2023; Stevanović et al., 2024). *Anaplasma capra* is a recently described tick-borne member of the *Anaplasma* genus with zoonotic potential. It has since been reported in a wide range of hosts, including domestic ruminants such as sheep, goats, and cattle, and wildlife, particularly deer species. This pathogen has been reported in several countries on different continents, including China, France, Japan, South Korea, Italy, and Türkiye, indicating its expanding geographical distribution (Altay et al., 2024). It is transmitted to humans primarily by two species of ticks: *Haemaphysalis longicornis* and *Rhipicephalus microplus*. However, more recent research has found that other ticks, including *Ixodes persulcatus*, *Haemaphysalis concinna*, and *Haemaphysalis qinghaiensis*, also carry this bacterium. It may also be wider than previously thought (Peng et al., 2018; Altay et al., 2022; Sahin et al., 2022, Oguz et al., 2024; Altay et al., 2024). Molecular and phylogenetic studies have established the occurrence of different genotypes and genetic variants of *A. capra* in small ruminant populations. A number of these genotypes have been demonstrated to have a strong genetic relationship with human isolates. This finding raises concerns about its potential public health implications (Yang et al., 2017; Peng et al., 2018; Shi et al., 2020; Lin et al., 2023). In addition, co-infection with other *Anaplasma* species in sheep is common, and some factors that affect infection patterns include the intensity of tick infestation, weather, and herd management (Yang et al., 2018; Razzaq et al., 2024). It remains unclear whether *A. capra* causes clinical disease in humans, and its pathogenic role in sheep has also not been fully elucidated. However, due to its relatively broad range of detection rates in some locations and the financial value of losses, this bacterium is believed to be of veterinary importance (Yang et al., 2018; Altay et al., 2022; Altay et al., 2024).

The need for continued monitoring and advanced research to improve the description of epidemiology in animal populations and guide effective control strategies is further highlighted by its increasing transmission and genetic diversity (Yang et al., 2017; Peng et al., 2018; Lin et al., 2023; Razzaq et al., 2024).

The present study aimed to investigate the prevalence and incidence of *Anaplasma ovis* and *Anaplasma capra* in sheep herds from the Gevas district of Van Province, Türkiye, using molecular diagnostic methods. This study also investigated the genetic diversity and phylogenetic analysis of the recovered *Anaplasma* isolates, thereby enhancing our understanding of their distribution and epidemiological relevance in this region.

Materials and methods

Animal Material

This study was conducted from October 2024 to April 2025 in the Gevaş district of Van Province in the Eastern Anatolia Region of Türkiye. It is the most populous province in the region and experiences a harsh continental climate, characterized by cold, snowy winters and hot, dry summers. The province is located at an average elevation of approximately 1,720 meters above sea level, while the Gevaş district lies on the south shore of Lake Van at around 1,650 meters (Kalelioğlu, 1991).

The study was conducted in ten villages of the Gevaş district (Van, Türkiye), where traditional small-ruminant husbandry is widely practiced. A total of 200 sheep were sampled from 10 independent flocks, with one flock selected from each village, and 20 randomly chosen animals per flock (Figure 1). All animals were registered as Akkaraman crossbreeds, a genotype well adapted to local environmental conditions. All flocks were managed under an extensive production system, in which animals grazed freely on natural pastures and were sheltered only at night or during adverse weather. Regarding parasite control, information on the antiparasitic treatments previously administered to the animals could not be reliably obtained from the farmers. During the study period, blood samples were taken from the jugular vein of each animal in 10 ml sterile vacuum tubes containing anticoagulant. The

samples were immediately labeled and transported on ice to the Molecular Parasitology Laboratory, Faculty of Veterinary Medicine, and Van Yuzuncu Yil University. Blood samples were stored at -20°C until DNA extraction. Ethics Committee approval for this research was obtained from the Local Ethics Committee for Animal Experiments of Van Yüzüncü Yıl University (2025/04-18).

Figure 1. Map of Gevaş showing 10 different sites.

Genomic DNA Extraction

Genomic DNA was extracted from 200 blood samples using the EcoPURE Genomic DNA Kit (EcoPURE, Türkiye) with a modified protocol. After erythrocyte lysis and protein digestion, the DNA was purified using column purification and stored at −20°C until PCR was performed.

PCR Amplification

To amplify *Anaplasma ovis*, primers for the locus of the msp4 gene were used to construct a product of 852 base pairs (bp) (De la Fuente et al., 2007). The nested PCR method with the gltA gene was applied to *Anaplasma capra* using outer primers, which yielded a product of 1031 bp. In addition, inner primers were utilised to produce a product of 594 bp (Li et al., 2015; Yang et al., 2016). All PCR reactions were prepared in a final reaction volume of 20 µL containing EcoTaq 2X Master Mix, forward and reverse primers (10 pmol/µL each), template DNA, and sterile distilled water. Amplification was performed using a thermal cycler under species-specific conditions. Previously extracted and sequenced DNA samples (accession number: PP625125 and OQ703060) from the Molecular Parasitology Laboratory were used as positive controls. For all PCR assays, a negative control was performed using double-distilled water. The PCR products were then subjected to electrophoresis through a 1.5% agarose gel, which had been stained with ethidium bromide. The gel was run in 1×TBE buffer at 80 V for 60 min, and then visualised under UV transillumination using a gel documentation system.

DNA Sequencing and Phylogenetic Analysis

Amplified successful PCR products for the msp4 gene were sent to Sentebiolab Biotechnology (Ankara, Türkiye) for sequence analysis. Edits were performed using BioEdit software (Hall, 1999) and compared with international isolates using the BLAST algorithm. Phylogenetic analysis was performed using MEGA version 7.0 software with the Maximum Likelihood method with 1,000 bootstrap iterations (Kumar et al., 2018). The sequences were deposited in GenBank with accession numbers PV231424–PV231428. *Anaplasma phagocytophilum* (GenBank accession no. KM205439) was used as an outgroup in the phylogenetic tree.

Statistical analysis

Statistical analyses were performed using IBM® SPSS® Statistics 25 software. The relationship between *A. ovis* molecular prevalence and age, gender, and regional groups in the sheep studied was tested

using Pearson's Chi Square test. $P < 0.05$ was accepted as the level of statistical significance.

Results

Molecular Findings and Prevalence

The present study screened 200 sheep blood samples taken from 10 villages in the Gevaş district of Van Province, Turkey, for the presence of *A. ovis* and *A. capra*. PCR amplification targeting the msp4 gene revealed *A. ovis* DNA in 149 of 200 samples (74.5%), whereas no amplification was observed for *A. capra* in any of the tested specimens (Figure 2).

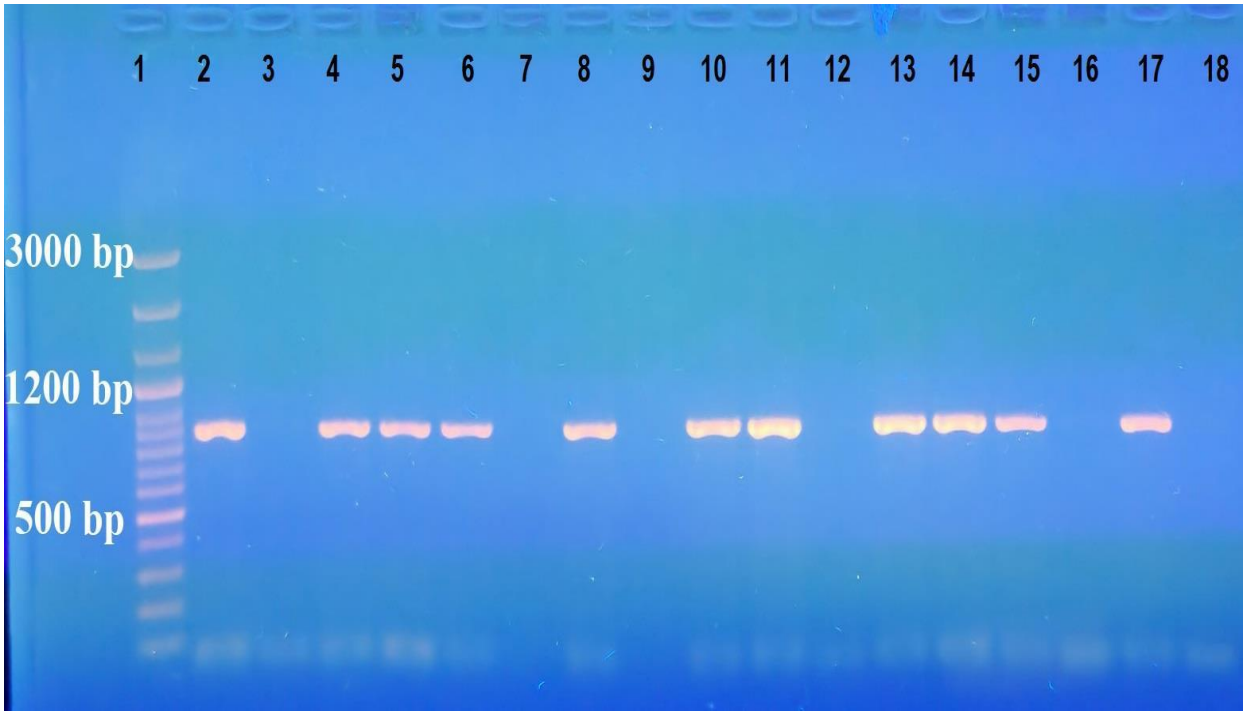

Figure 2. PCR amplification of *Anaplasma ovis* msp4 gene. Lanes: 1, 100 bp plus DNA marker; 2, Positive control; 3, Negative control; 4, 5, 6, 8, 10, 11, 13, 14, 15, 17. *A. ovis* positive sheep blood sample; 3, 7, 9, 12, 16, 18. *A. ovis* negative sheep blood sample.

Epidemiological Findings

The prevalence of *A. ovis* was higher in sheep aged ≤ 2 years than in those aged > 2 years; however, the difference was not significant ($P = 0.376$). Similarly, no significant difference in infection rates was observed between females and male animals ($P = 0.685$). The prevalence of *A. ovis* in the studied villages varied significantly. The highest positivity rates were in İnköy (95%), Yanıkçay (90%), and

Kurultu (90%), with the lowest in Değirmitaş (50%) and Kuşluk (55%). *A. ovis* prevalence among villages varied at a statistically significant level ($P < 0.05$) based on Pearson's chi-square test (Table 1).

Table 1. Prevalence of *Anaplasma ovis* in Sheep by Age, Sex, and Geographic Region

Variable	Category	n	Positive (n)	Prevalence (%)	P-value
Age	< 2 years	69	54	78.26%	0.376
	≥ 2 years	131	95	72.52	
Sex	Female	169	125	73.96%	0.685
	Male	31	24	77.42%	
Region	İnköy	20	19	95%	0.004
	Yanıkçay	20	18	90%	
	Kurultu	20	18	90%	
	Hasbey	20	17	85%	
	Dokuzağaç	20	16	80%	
	Göründü	20	14	70%	
	Abalı	20	14	70%	
	Gündoğan	20	12	60%	
	Kuşluk	20	11	55%	
	Değirmitaş	20	10	50%	
Total		**200**	**149**	**74.5**	

Sequencing and Phylogenetic Analysis

Five *A. ovis* PCR-positive samples were randomly selected for sequence analysis. The generated sequences (GenBank accession numbers: PV231424–PV231428) were 100% identical to those of previously reported isolates from China (OP503165), Mongolia (LC141078), and Türkiye (PP625125). Phylogenetic analysis of the msp4 gene indicated that these sequences were in the clade of *A. ovis* and well distinct from other *Anaplasma* species, including *A. capra*, *A. centrale*, *A. marginale*, and *A. phagocytophilum* (Figure 3).

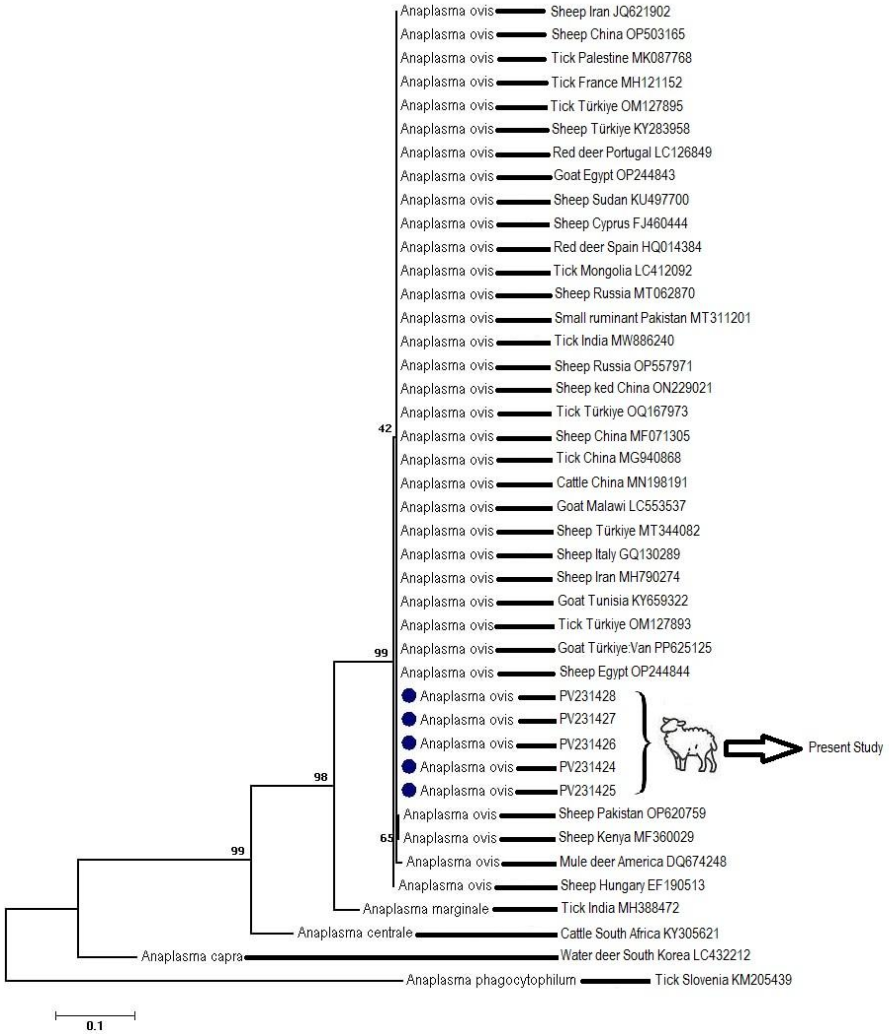

Figure 3. A phylogenetic tree of *A. ovis*, constructed using the maximum likelihood method and the Kimura 2-parameter model, is shown below. The tree was created using msp4 gene sequences and 1000 bootstrap replicates. The identification source (host) and country are given after the numbers.

Discussion

Ovine anaplasmosis is a tick-borne disease that has been spreading increasingly in recent decades, both in tropical and Mediterranean regions and in Europe. Climate change is a major contributing factor that promotes such spread by facilitating the survival, reproduction, and geographic expansion of vectors (Belkahia et al., 2017). PCR has become an invaluable tool for diagnosis due to its specificity and sensitivity. These are genetic markers of *A. ovis* and are capable of detecting it rapidly and accurately in samples with very low pathogen loads (Bilgiç et al., 2017). Previously, the epidemiological understanding of *A. ovis* in Türkiye was predominantly microscopic. Early investigations, such as the first reported case in Bursa (Lestoquard and Ekrem, 1931), observed low prevalence rates of 1.34% in symptomatic and 2.23% in asymptomatic animals in Central and Eastern Anatolia (Göksu, 1967), and 0.7% in clinically healthy sheep in Marmara (Güralp et al., 1975). In the following years, a microscopy-based prevalence rate of 0.2% was found in Southeastern Anatolia (Özer et al., 1993). However, significantly higher and more reliable prevalence rates have been identified since the introduction of molecular diagnostic techniques in 2013. For instance, Altay et al. (2014) reported an overall positivity rate of 67 % with a prevalence of 50.8% among sheep from Eastern Anatolia. Similarly, Öter et al. (2016) and Bilgiç et al. (2017) reported prevalence rates of 50.8% and 63.3%, respectively. Zhou et al. (2017) also reported 60 % prevalence in Central Anatolia. On the other hand, substantially reduced rates, such as 18% in southern Türkiye reported by Aktaş and Özübek (2018), may reflect the influence of ecological and environmental factors, that is, climatic variables, vector ecology, and herd management.

The present study found A. ovis DNA in 74.5% of the blood samples collected from sheep the Gevaş district of Van Province. This prevalence is comparable to previous reports in the region: 71.2% in Van (Oğuz and Değer, 2025), 67% in Eastern Anatolia (Altay et al., 2014), and 78.9% in Southeastern Anatolia (Ceylan and Ekici, 2023), confirming that *A. ovis* is predominantly and naturally endemic in the eastern regions of Türkiye.

Comparable prevalence rates have also been reported in other countries, such as Iraq (66.6%) (Renneker et al., 2013), Portugal (84.2%) (Renneker et al. 2013), Tunisia (70.1%) (Belkahia et al., 2014), Hungary (72.7%) (Hornok et al., 2007), and Italy (87%) (De la Fuente et al., 2005b). These findings suggest that the same ecological factors and vector dynamics may contribute to endemic *A. ovis* transmission in these regions. However, significantly lower prevalence rates have been reported in other regions, including Thrace, the Mediterranean, and certain southeastern provinces of Türkiye, with values as low as 15%. (Aktaş and Özübek, 2018). Similar low-level prevalence has been documented globally, such as in China (14.3–18.2%), Slovakia (22.6%), and Bangladesh (15.7%) (Derdáková et al., 2011; Han et al., 2017; Rahman et al., 2022). These geographic variations may be due to numerous ecological and host factors, such as the abundance of vector ticks, climate, host immunity, animal husbandry, and the existence of wildlife reservoirs for the bacteria, and therefore the pathogen (Rar and Golovljova, 2011; Torina et al., 2008; Liu et al., 2012). The findings of this study indicate the need for surveillance systems in each particular region, which are adapted to its epidemiological situation, and accompanying appropriate control measures.

Furthermore, a greater prevalence of infection was observed in sheep younger than two years, although this difference was not statistically significant. This trend is similar to previous reports from Iran (Yousefi et al., 2017), Egypt (Mahmoud et al., 2024), and Türkiye (Aktaş and Özübek, 2018), indicating the greater susceptibility of young animals based on immunological naivety. A higher prevalence is also commonly observed in older animals, where their condition may be influenced by herd management and environmental factors. No statistically significant difference was observed between the sexes regarding rates; males and females were both observed to have infection rates of 73.9% and 77.4%, respectively. These observations are consistent with the findings of Zhou et al. (2017). Nevertheless, some physiological stressors, such as inflammatory hormones associated with female reproduction, tend to change the probability of infection and require further research. Geographic

variations and host determinants were also apparent. The İnköy region had a significantly higher prevalence of *A. ovis* infection than the other sampling sites. Geographic variation is thought to be influenced by microclimatic and environmental determinants supporting greater tick vector populations and farm-level variation in livestock husbandry practices and biosecurity levels (Rar and Golovljova, 2011).

These findings highlight the importance of including host-associated and environmental factors in future epidemiological models and establishing geographically adapted control interventions. Despite statistically significant regional variation, *A. ovis* infection prevalence was not associated with the sex of the animals. The infection rates were 73.9% in females and 77.4% in males. This phenomenon was supported by Zhou et al. (2017), who reported higher prevalence rates in male sheep in Türkiye. Although the differences were not statistically significant in present study, susceptibility to *A. ovis* infection may still be regulated by hormones or physiology, for example through the reproductive cycles of females, which can influence immune responsiveness and infection dynamics. Further studies are needed to clarify and modify our understanding of this issue.

The molecular identification and evolutionary relationships of the local isolates were confirmed by partial sequence and phylogenetic analysis of msp4. The sequences were highly conserved, showing 99-100% similarity to *A. ovis* isolates from Pakistan, Egypt, Kenya, Turkiye, Hungary, and Tunisia, and comprised a phylogenetically distinct clade. In contrast, more distant phylogenetic relationships were established among isolates from Iran, China, and Palestine. These differences are likely reflective of geographical and ecological differentiation, but more notably for tick species variability and their transmission potential at local scale (Ben Said et al., 2018). These molecular results agree with the endemic status of *A. ovis* in the studied area and emphasize the necessity of continued molecular tracking to follow the diversity and evolution of the strain.

Given the growing interest in tick-borne disease agents worldwide, *A. capra* has been recognized as a zoonotic disease

agent. *Anaplasma capra* infection has been found in some ruminant hosts, such as sheep, cattle, and water buffalo, in some provinces of Türkiye, particularly in Sivas Province (Altay et al., 2022; Şahin et al., 2022). However, in Van Province, where the current study was performed, *A. capra* was present only in goats, and no evidence has yet been demonstrated to show that it is present in sheep. The two genotypes of *A. capra* (Genotypes I and II) have been identified, with all previously reported isolates in Turkey belonging to Genotype I (Altay et al., 2022). Further elucidation of the host spectrum, genetic variability, and zoonotic potential of *A. capra* in diverse ecological niches is warranted. No *A. capra* DNA was detected in any of the sheep samples analyzed in the present study. Although previous studies have determined the prevalence of *A. capra* among sheep in Türkiye as 3.22% (Altay et al., 2022), it has been reported higher prevalence rates have been documented worldwide, ranging from 7.8–56% in China to 30% in France. Our findings suggest that *A. capra* is either of very low prevalence or is entirely absent in sheep within Van Province. Similar to this study, a previous study in the same region found *A. capra* DNA in a single goat, corresponding to a prevalence of 0.5% (Oguz et al., 2023). These results suggest that *A. capra* transmission in Van Province is currently limited, although long-term monitoring is required.

Conclusions

This study confirmed that *A. ovis* infection is highly prevalent in sheep in Van Province and is a significant veterinary health problem. Molecular diagnostic methods, particularly those targeting msp4, are effective diagnostic tools against *A. ovis* and for the design of control measures for the disease. The absence of *A. capra* in sheep indicates the limited distribution of this pathogen in this region. Further comprehensive epidemiological and molecular studies are needed to identify the distribution, genetic heterogeneity, and zoonotic risks of *Anaplasma* spp. in Türkiye.

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Anaplasma capra türlerinin Moleküler Yöntemlerle Araştırılması" (2025).

Conflict of Interest

The authors declared that there is no conflict of interest

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