Iranian Journal of Microbiology
Volume 18 Number 3 (June 2026) 393-402
DOI: http://doi.org/10.18502/ijm.v18i3.21666

ORIGINAL ARTICLE

Molecular detection of *Anaplasma* spp. in blood and ticks collected from sheep and goat in West Azerbaijan province, Iran

Ahmad Enferadi[1], Amir Tukmechi[1*], Katayoon Nofouzi[2], Zahraalsadat Tavakolian[1]

[1]Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
[2]Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

Received: May 2025**, Accepted:** May 2026

ABSTRACT

Background and Objectives: Anaplasmosis, an ailment affecting both captive and free-ranging small ruminants, is instigated by *Anaplasma* spp., a tick-vectored, obligate intracellular rickettsial bacterium. Iran's ovine and caprine populations, numbering roughly 71 million, are vital to its financial structure.
Materials and Methods: This study investigated the prevalence of *Anaplasma* species in sheep and goats within West Azerbaijan province. Additionally, nucleic acid specimens were isolated from gathered ticks and examined for *Anaplasma* spp. Through polymerase chain reaction (PCR) utilizing the major surface protein gene (*groEL*).
Results: *Anaplasma* was detected in 161 (69.0%) of 919 ovine and 82 (71.3%) of 243 caprine blood DNA extracts. Subsequently, genetic material from 426 ticks comprised of *Rhipicephalus sanguineus* (n=146), *Rhipicephalus turanicus* (n=63), *Hyalomma asiaticum* (n=56), *Hyalomma anatolicum* (n=74), and *Hyalomma egebtiom* (n=87) was screened for *A. ovis* utilizing the same methodology.
Conclusion: This research not only confirmed the presence of *A. ovis* within Iranian sheep and goats but also implicated ticks as a possible vector for its transmission. The findings emphasize the importance of monitoring the health status of Iran's small ruminants to detect clinical manifestations of anaplasmosis and of implementing effective tick control strategies worldwide.

Keywords: *Anaplasma*; Ticks; Sheep; Goats; Blood; Iran

INTRODUCTION

The health of both humans and animals may be negatively impacted by ticks and tick-borne diseases (TBDs), an increasing worldwide concern (1, 2). Ticks rank as the second most prevalent vectors globally, making the monitoring of tick-borne diseases (TBDs) like piroplasmosis (which includes babesiosis and theileriosis), anaplasmosis, and rickettsiosis vital for safeguarding both public and animal health (3). Ticks and TBDs have a detrimental impact on animal production, leading to pruritus, loss of weight, impaired immune response, pyrexia, anemia, and potentially fatal outcomes (4).

Tick-borne infections have spread in recent years in Asian countries, including India, Pakistan, China, Tajikistan, and Mongolia (5). Anaplasmosis, a pathology stemming from bacteria within the *Anaplasma* genus (Rickettsiales: Anaplasmaceae), presents a significant obstacle to animal husbandry due to its financial burden and possible zoonotic potential (6). Despite the fact that these six *Anaplasma* species are

*Corresponding author: Amir Tukmechi, DVSc., Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. Tel: +98-9141473201 Fax: +98-4432752741 Email: tukmachi@urmia.ac.ir

obligate intracellular Gram-negative organisms parasitizing mammalian blood cells, each demonstrates disparate host predilections and cellular tropism (7). *Anaplasma ovis* replicates within the erythrocytes of small ruminants like sheep and goats. *Anaplasma ovis* (*A. ovis*) induces bovine anaplasmosis by targeting monocytes, whereas *Anaplasma marginale* and *Anaplasma centrale* reside within the red blood cells of cattle (8). Furthermore, *Anaplasma phagocytophilum* represents a novel human pathogen, invading neutrophils in both humans and other mammals, thus engendering disease across diverse species (8).

Various tick genera, specifically *Ixodes*, *Dermacentor*, *Rhipicephalus*, and *Amblyomma* species, have been characterized as vectors transmitting *Anaplasma marginale* (9). The clinical manifestations of anaplasmosis in small ruminants may encompass a gradual onset of anemia, pyrexia, icterus, lethargy, anorexia, diminished lactogenesis, reproductive losses through abortion, and mortality in critical instances (10). Anaplasmosis, a tick-vectored illness (TBD) impacting both human and animal populations, is of considerable concern. Specifically, in small ruminants, the Gram-negative rickettsial bacterium *Anaplasma ovis* is propagated through the bite of *Rhipicephalus bursa*, alongside other tick species within the Old World, whereas transmission in the New World is chiefly facilitated by *Dermacentor andersoni* (11). *A. ovis* was initially described by Bevan in 1912 and has subsequently been observed across numerous geographical regions (12). Despite the apparent ubiquity of this disease, limited data exist on the scope of infection and its subsequent effects on livestock productivity. A possible contributing factor to this knowledge gap is the historical neglect of *A. ovis* infections, often attributed to their purported subclinical presentation, leading to an underestimation of their economic relevance (13). Nevertheless, severe pathologies have been documented in bighorn sheep and goats, with acute disease episodes frequently correlating with concurrent stressors including co-infections, elevated ambient temperatures, immunization procedures, anthelmintic treatments, substantial tick burdens, extended transportation durations, and animal relocations (14). Moreover, a frequently overlooked element in this and numerous other tick-borne pathogens is the potential for concurrent infections (15).

Ticks and tick-borne diseases, such as anaplasmosis, are a growing global health concern affecting both humans and animals. Anaplasmosis, caused by various *Anaplasma* species, affects different animal species with varying cellular tropism. Clinical signs in small ruminants include anemia, fever, and reproductive issues, resulting in economic losses. Despite its widespread presence, *Anaplasma ovis* is understudied due to its perceived subclinical presentation, which often masks its true impact.

MATERIALS AND METHODS

Study areas. The province of West Azerbaijan, in the northwest of Iran, features a diverse Geographical distribution and spatial spread of landscapes, encompassing mountainous areas, fairly level ground, and the shores of Urmia Lake. The weather patterns associated with the Atlantic and Mediterranean have a major impact on the province's climate (Fig. 1) (16).

Sample collection. A total of 429 ticks were collected from goats and sheep in West Azerbaijan province, situated in the northwest of Iran. In several provinces of West Azerbaijan, the sampling procedure was carried out, comprising three distinct geographic regions (north, center, and south). Tick samples were collected by physically restraining sheep and goats with the assistance of animal handlers, followed by careful removal of ticks from the body surface using sterile tweezers. Throughout the spring and summer of 2022, Arthropod ectoparasites were procured from select anatomical regions of ovine and caprine hosts within the West Azerbaijan province. Specimens were individually harvested into aseptic borosilicate containers pre-filled with a 95% ethanol solution for subsequent taxonomic classification. The collected samples were subsequently transported to the Parasitology Laboratory, Faculty of Veterinary Medicine, Urmia University, under appropriate conditions for further analysis. Upon arrival at the laboratory, morphological examination utilizing a magnifying loupe and established taxonomic keys was performed to ascertain tick species identity (18).

After ticks were collected, 973 blood samples were obtained via jugular venipuncture in the same animal hosts. These were subsequently dispensed into a tube containing ethylenediaminetetraacetic acid (EDTA). The samples collected were brought to the laboratory using controlled hypothermic conditions and afterward stored at -20°C awaiting molecular assays (17).

Fig. 1. Geographic map of the study locations in West Azerbaijan, Iran (17).

DNA extraction: blood DNA extraction process. Blood specimens were procured from the jugular veins of each ovine and caprine subject and dispensed into aseptic tubes pre-loaded with the anticoagulant ethylenediaminetetraacetic acid (EDTA). DNA was subsequently isolated from the resuspended cellular pellet using the Blood Genomic DNA Extraction Mini Kit (Favorgen, Taiwan) according to the manufacturer's protocol. A NanoDrop 2000c spectrophotometer (Thermo Scientific, USA) was employed to quantify and evaluate the purity of the recovered DNA. The extracted DNA samples were stored at -20°C until polymerase chain reaction (PCR) analysis. In the DNA extraction procedure, the elution buffer from the extraction kit was incorporated as a negative control.

Ticks DNA extraction process. Prior to cryofixation in liquid nitrogen, the arthropod ectoparasites were subjected to two washes with phosphate-buffered saline (PBS) to eliminate residual ethanol. Subsequently, the specimens were comminuted in an aseptic environment using a scalpel blade and transferred to microtubes for nucleic acid extraction. After removal from 70% ethanol, ticks were air-dried on sterile filter paper under a laminar flow hood and subsequently subjected to DNA extraction. Genomic DNA was extracted using a commercially available DNA Extraction Kit (MBST, Iran), according to the manufacturer's instructions. The concentration and purity of the extracted DNA were evaluated using a NanoDrop 2000c spectrophotometer (Thermo Scientific, USA). The extracted DNA samples were stored at -20°C until further use in PCR assays. As a negative control, the elution buffer provided in the extraction kit was included in the nucleic acid extraction procedure.

Molecular detection of *Anaplasma* spp. Using standard nested PCR, the DNA was examined to detect the specific bacteria. Targeted genes were the *groEL* along with the intergenic gene for *Anaplasma* spp., Amplifx software (Version 1.7) was used to design primer sets, as shown in Table 1. *Anaplasma* gene sequences were retrieved from the NCBI website to design primers.

PCR was performed in a final volume of 25 µL, using PCR-grade water (Merck) as the source for 4 µL of template DNA (50-100 ng/µL), 1 µL of each primer (0.1 nmol/µL), and 12.5 µL of premixed master mix. The rest of the volume was filled with sterile distilled water. To reduce nonspecific product amplification, the reaction was performed in a thermal cycler (Quanta Biotech, England). The touchdown PCR protocol started with a higher annealing temperature, which was lowered during subsequent cycles. This increase is further accompanied by a decrease in nonspecific amplification from this assay. For the PCR assay, 2.5 µL of the DNA product was used as the template in the amplification. Following amplification, the PCR products were resolved by electrophoresis on a 1% agarose gel containing a DNA-safe intercalating dye (Labnet, ENDURO, USA). The separated bands were subsequently visualized and documented using the Genius Gel Documentation System (Syngene Bio-Imaging, UK) (Figs. 2 and 3).

Nucleotide diversity and phylogenetic tree construction. Following the identification of some positive specimens, those samples were forwarded to Pishgam Biotechnology Company (Tehran, Iran) for Sanger sequencing. The resulting DNA sequences were then submitted to the National Center for Biotechnology Information (NCBI) database for analysis and Basic Local Alignment Search Tool (BLAST) comparisons. These sequences were queried against NCBI to identify similar reference sequences, and the geographic origins, defined by Country-of-Origin Information (COI), were determined using NCBI's

Table 1. Primer for identification of *Anaplasma* spp. in blood and ticks of sheep and goats

Protocol	Primer Name	Sequence 5'----3'	PCR product size (bp)	PCR condition (cycle)
Normal-PCR	*groEL* -F	AGGACTGACGGTATGCAGTT	866	95c for 5m, 95c for 90s, anling 58c for 90s, extention 72c for 90s for 72c for 7m. (38 cycle)
	groEL -R	TCGTTCTGCTTCAGCAAGTG		

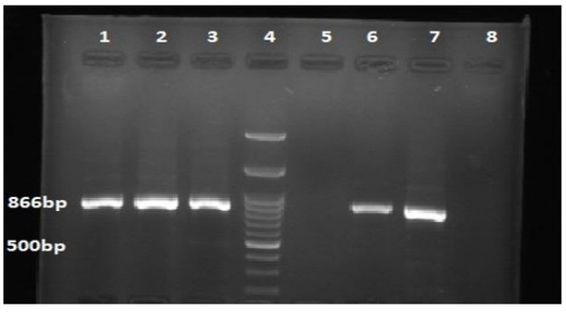

Fig. 2. Visualization of amplified *Anaplasma* spp. *groEL* gene (866 bp) via agarose gel electrophoresis of PCR products from sheep and goat blood and ticks. Lane M: 100 bp DNA marker (Smobio Technology Inc, Taiwan), Lanes 1,2,3,6 and 7 positive and 5 negative samples, 8 Lane of negative control).

BLAST algorithm. For phylogenetic analysis, all accessible *Anaplasma* spp. cytochrome oxidase subunit I (COI) sequences from GenBank were retrieved. Multiple sequence alignment was performed with Clustal W, with manual edits to correct any discrepancies, and then exported as MEGA and FASTA-formatted files (19). All obtained nucleotide sequences were deposited in GenBank and assigned accession numbers. Evolutionary relationships were inferred by constructing a phylogenetic tree using Molecular Evolutionary Genetics Analysis (MEGA) software, version 10, based on the maximum likelihood method. The robustness of the inferred tree topology was evaluated using 1,000 bootstrap replicates. In addition, DNA sequence polymorphism and nucleotide diversity were analyzed using MEGA software, version 11 (20).

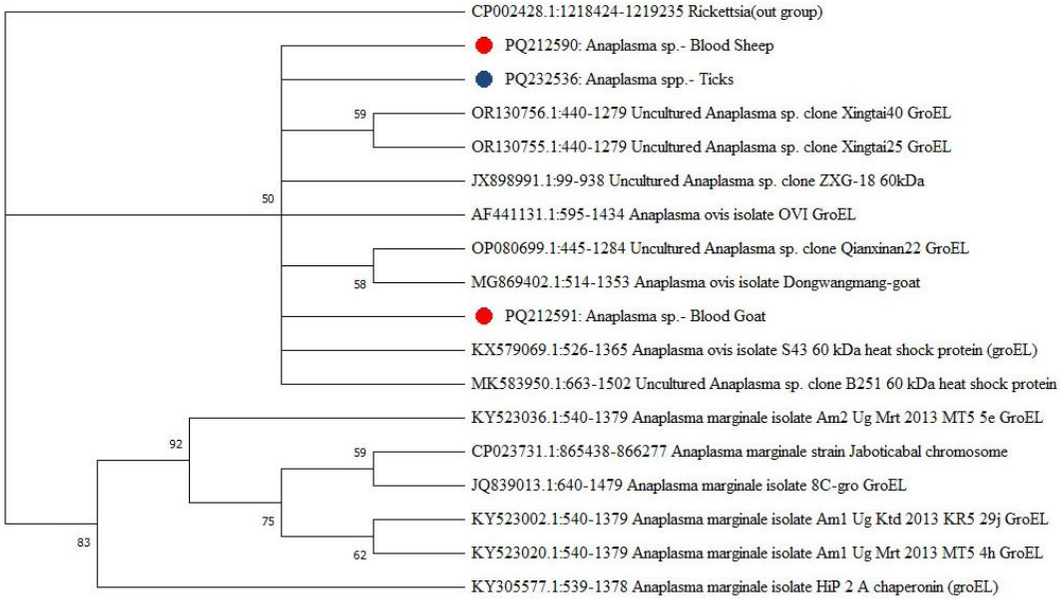

Fig 3. The evolutionary history was inferred using the Neighbor-Joining method. The best resulting tree is shown. Bootstrap values (1,000 replicates), indicating how often related bacteria grouped together, are displayed next to the branches. Sequence divergence was measured using the Tamura 3-parameter method, yielding the number of base changes per site. This analysis included 18 DNA sequences. The first, second, and third codon positions, as well as the noncoding regions, were checked. Unclear parts of the sequences were removed when comparing pairs, through pairwise deletion. The final dataset comprised 858 positions. Evolutionary analysis was performed using MEGA version 1. All newly obtained DNA sequences have been deposited on Genbank (Accession Numbers PQ212590-PQ232536-PQ212591). The epidemiological data are available from the corresponding author upon request.

RESULTS

In this study, a total of 562 sheep and 357 goat blood samples were evaluated for the presence of *Anaplasma* spp. using PCR. The results indicated that in female sheep, 120 out of 385 samples (31.17%; 95% CI: 26.75% - 35.96%) were infected with *Anaplasma*, while in rams, 41 out of 177 samples (23.16%; 95% CI: 17.56% - 29.9%) showed infection (Table 2). For goats, 68 out of 265 female samples (35%; 95% CI: 25.66% - 31.24%) and 14 out of 92 male samples (15.22%; 95% CI: 9.29% - 23.94%) indicated bacterial contamination. The highest infection rates were found in animals aged 3 to 6 years, with 121 out of 348 samples (34.77%; 95% CI: 29.96% - 39.92%), which was similarly observed in goats, where 54 out of 202 samples (26.73%; 95% CI: 21.1% - 33.23%) showed infection (Table 2). This might be attributed to the larger sample size. In this study, seasonal assessments of sheep and goat blood samples to identify *Anaplasma* revealed that the highest infection rates occurred in summer and spring, while the lowest rates were observed in autumn and winter (Table 2). The region was divided into three sections: north, south, and central. In both sheep and goat populations, the highest infection rates were found in the northern part of the province, while the lowest rates were recorded in the central region.

In this study, a total of 426 tick samples from sheep and goats belonging to the genera *Hyalomma* and *Rhipicephalus* were collected from various locations (Table 3). After identification using diagnostic keys, the species *Rhipicephalus sanguineus*, *Rhipicephalus turanicus*, *Hyalomma asiaticum*, *Hyalomma aegyptium*, and *Hyalomma anatolicum* were recognized. The ticks were then categorized into groups of five by gender and species and assessed for the presence of *Anaplasma* bacteria. The results indicated that *Rhipicephalus sanguineus* exhibited the highest infection rate, with females showing greater susceptibility to the bacteria than males (Table 4). Out of 84 tick pools (54 males and 30 females), 21 pools tested positive for the bacteria, including 15 male pools (15 out of 51; 29.41%; 95% CI: 18.71% - 43%) and 6 female pools (6 out of 30; 20%; 95% CI: 9.51% - 37.31%).

DISCUSSION

Climate and the environmental changes have caused the tick vectors to expand, raising the risk of TBD (Tick-borne disease), with medical and veterinary importance (21). Livestock are very vulnerable to various TBD, leading to severe effects such as anemia, weight loss, reduced productivity, abortion and death. Sheep and goat, as the most common small ruminants, are primarily impacted by anaplasmosis (22). The subtropical environment of Iran supports a large number of small ruminants that are cultivated for human dietary purposes.

This study utilized the PCR method to amplify the *groEL* gene from blood samples of goats and sheep in the West Azerbaijan province, revealing a 28.65% infection rate in goats and 22.97% in sheep. Additionally, ticks collected from these animals showed a 25.92% infection rate. Compared to the current study's findings, some research has indicated higher *Anaplasma* prevalence in sheep or goats in western Iran, particularly in Kurdistan (78.8%) (21), while others reported lower rates in East Azerbaijan (7.05%) (23) and Kurdistan (6.49%) (24). In some studies, the occurrence of *Anaplasma* infection was comparable to our findings; for example, a microscopic analysis of goats in Dezful revealed a prevalence of 33% (25). Additionally, a separate study in Ahvaz, southwestern Iran, revealed a 33.6% occurrence of *Anaplasma* detected through microscopy (26). Lower molecular incidence of *Anaplasma* was found in sheep in Algeria (12.7%) (27) and 14.3% in goats and sheep in China (7), which is inconsistent with our results. These research findings emphasize the critical function of PCR-based tests in detecting genuine cases and carrier conditions. The variations in prevalence across studies may be due to differences in sampling time, climate diversity (which affects tick vector distribution) and detection method.

In Iran, 46 distinct tick species, categorized into 10 genera, have been documented, with 23 of these species found to harbor parasitic, viral, or bacterial pathogens (28). In this study, 264 ticks were collected from the examined samples in West Azerbaijan province, with *Rhipicephalus sanguineus* (35% male, 22.22% female) and *Hyalomma asiaticum* (16.66% male, 0% female) as the most and least prevalent vectors in this province, respectively. Previously, *Rhipicephalus sanguineus* was found in other provinces, including Kurdistan (13), Alborz (20), Sistan & Baluchestan, Kerman (22), Mazandaran, Hormozgan, Lorestan and Guilan (30). The second most common tick vector identified in this study, *H. aegyptium* (40% male, 33.33% female), has been found in Iran's Tortoise region, particularly in West Azer-

Table 2. Age, gender, region, and season-wise inspection of hosts for blood collection.

		Animal	
		Sheep (No.562)	**Goat (No.357)**
Gender			
	Female	120/385	68/265
		120 (n=385; 31.17%; 95%Cl: 26.75 %-35.96%)	68 (n=265; 35%; 25.66 %Cl: 20.78%-31.24%)
	Male	41/177	14/92
		41 (n=177; 23.16 %; 95%Cl: 17.56 %-29.9%)	14 (n=92; 15.22%; 95%Cl: 9.29%-23.94%)
Age group (Years old)			
	< 2	23/106	17/78
		23 (n=106; 21.7%; 95%Cl: 14.92%-30.46%)	17 (n=78; 21.79 %; 95%Cl: 14.07%-32.16%)
	3-6	121/348	54/202
		121 (n=348; 34.77%; 95%Cl: 29.96 %-39.92%)	54 (n=202; 26.73%; 95%Cl: 21.1%-33.23%)
	> 6	17/108	11/77
		17 (n=108; 15.74%; 95%Cl: 10.07%-23.77%)	11 (n=77; 14.29%; 95%Cl: 8.17%-23.8%)
Season			
	Spring	79/152	41/97
		79 (n=152; 51.97%; 95%Cl: 44.08%-59.77%)	41 (n=97; 42.27%; 95%Cl: 32.92%-52.21%)
	Summer	73/143	37/92
		73 (n=143; 51.05%; 95%Cl: 42.94%-59.11%)	37 (n=92; 40.22%; 95%Cl: 30.79%-50.44%)
	Autumn	5/134	3/87
		5 (n=134; 3.73%; 95%Cl: 1.16%-8.44%)	3 (n=87; 3.45%; 95%Cl: 1.18%-9.66%)
	Winter	4/133	1/81
		4 (n=133; 3.01%; 95%Cl: 1.18%-7.48%)	1 (n=81; 1.23%; 95%Cl: 0.22%-6.66%)
Region			
	North	89/236	58/142
		89 (n=236; 37.71%; 95%Cl: 31.77%-44.04%)	58 (n=142; 40.85%; 95%Cl: 18.12%-56.71%)
	Center	9/117	3/122
		9 (n=117; 7.69%; 95%Cl: 4.01%-13.97%)	3 (n=122; 2.46%; 95%Cl: 0.84 %-6.98%)
	South	63/209	21/192
		63 (n=209; 30.14%; 95%Cl: 24.32%-36.67%)	21 (n=192; 10.94%; 95%Cl: 7.27%-16.14%)
	Total	161/562	82/357
		161 (n=562; 28.65%; 95%Cl: 25.07%-32.52%)	82 (n=357; 22.97%; 95%Cl: 18.91 %-27.61%)

Table 3. Number of ticks collected from different body parts of specified hosts.

Host Animals			**Body Parts**						
Animal type	**Examined**	**Infested**	**Sternum**	**Belly**	**Head and neck region**	**Anal region**	**Udder**	**Legs**	**Total collection**
Goats	135	63	26	31	40	28	38	26	189
Sheep	192	79	28	40	52	29	49	39	237
Overall	264	187	54	71	92	57	87	65	426

baijan (25). In nearby provinces, *Hyalomma* ticks are shown to be prevalent in Kurdistan, Zanjan, and East Azerbaijan, while *Rhipicephalus* ticks are common in Kurdestan and East Azarbayjan. Notably, there is no data on tick infestation in Zanjan province. Additionally, *Rhipicephalus* ticks (primarily *R. bursa* and *R. sanguinus*) are the most frequently isolated ticks from small ruminants in Iran, according to published literature (29, 30), which confirms our findings. In field studies in Iraq, sheep and goats examined showed infestations of *R. bursa*, *R. turanicus*, *Heamophilus parva* and *Hyalomma* species (28). Ticks of

Table 4. Identification of *Anaplasma* spp. from ticks taken from sheep and goats.

Ticks					
Number of ticks	**Tick species**	**No.**	**Pool**	**Genus**	**groEL (95%Cl)**
146	*Rhipicephalus sanguineus*	103	21	Male	7/20 (35%)
					7 (n=20; 35%; 95%Cl: 18.12%-56.71%)
		43	9	Female	2/9 (22.22%)
					2 (n=9; 22.22%; 95%Cl: 6.32%-54.74%)
63	*Rhipicephalus turanicus*	37	7	Male	2/7 (28.57%)
					2 (n=7; 28.57%; 95%Cl: 8.22%-64.11%)
		26	5	Female	1/5 (20%)
					1 (n=5; 20%; 95%Cl: 3.62%-62.45%)
56	*Hyalomma asiaticum*	33	6	Male	1/6 (16.66%)
					1 (n=6; 16.67%; 95%Cl: 3.01%-56.35%)
		23	4	Female	0/4 (0.0%)
					0 (n=4; 0%; 95%Cl: 0%-48.99%)
87	*Hyalomma aegyptium*	56	11	Male	4/10 (40%)
					4 (n=10; 40%; 95%Cl: 16.82%-68.73%)
		31	6	Female	2/6 (33.33%)
					2 (n=6; 33.33%; 95%Cl: 9.68%-70%)
74	*Hyalomma anatolicum*	45	9	Male	1/8 (12.50%)
					1 (n=8; 12.5%; 95%Cl: 2.24%-47.09%)
		29	6	Female	0/6 (0.0%)
					0 (n=6; 0%; 95%Cl: 0%-39.03%)
		274	54	Male	15/51 (29.41%)
					15 (n=51; 29.41%; 95%Cl: 18.71%-43%)
		152	30	Female	6/30 (20%)
					6 (n=30; 20%; 95%Cl: 9.51%-37.31%)
Total		426	84		21/84 (25.92%)
					21 (n=84; 25.93%; 95%Cl: 17.63%-36.41%)

the genera *Haemophysalis*, *Hyalomma*, *Boophilus*, *Dermacentor*, *Rhipicephalus*, and *Argas* are widespread throughout Turkey. Turkey shares boundaries with Iran (499 Km) in the east (29). Given the significant prevalence of *Rhipicephalus sanguineus* in West Azerbaijan province, additional preventive measures should be implemented for this tick species (31).

It should be noted that tick samples in the present study were pooled for molecular screening purposes; therefore, the detected prevalence reflects pooled positivity rather than individual tick infection rates, which may have led to an underestimation of the true infection frequency.

The first molecular identification of *Anaplasma* in Iran origins traces back to 2014 (30), when the parasitic agent was identified in *Rhipicephalus sanguineus*/*Ixodes ricinus* ticks using nested-PCR. A recent study in Kurdistan examined 26 *Rhipicephalus sanguineus sensu lato (s.l.)* (8 males, 18 females) for *Anaplasma* DNA, revealing an infection rate of 78.8%, which is significantly higher than our results (21). Additionally, only two cases of *A. ovis* infection were identified in *R. sanguineus* tick isolates by Hosseini Chegeni and Tavakoli (2020) through sequencing, from three provinces in Iran (Hormozgan, Lorestan, Guilan).

According to our findings, the highest frequency of *Anaplasma* infection was observed among female small ruminants, with 31.17% of sheep and 35% of goats. Moreover, the highest prevalence of *Anaplasma* (34.71% in sheep & 26.73% in goats) was observed in animals aged 3-6 years. Female animals may be more susceptible to stress and weakened immune responses during breastfeeding due to nutritional deficiencies and physical strain, increasing the likelihood of infections in this group (32). In

addition, females with reproductive cycles experience greater hormonal fluctuations, increasing their susceptibility to infection (33). Similar studies in Iran, Rahravani et al. (2023), Belkahia et al. (2014) in Tunisia, and Niaz et al. (2021) and Naeem et al. (2023) in Pakistan supported the present study's conclusions, although research in Hamadan province, western Iran, revealed higher infection levels within male populations (13, 32).

Anaplasma prevalence was higher during the spring months (34-36). To explain this observation, it is possible that there is an increased activity of vectors, which probably corresponds to the period of infestation with local tick species, like *Rhipicephalus sanguineus* and *R. turanicus* (37). These findings are in contrast to those of Atif et al. (2023), who reported the most incidence of their highest incidence during the summer. An interesting point to consider is that mechanical transmission has been implicated in disease spread, and the lack of common tick vectors points to a role for this form of transmission. Thus, differences in disease occurrence vary with geographical location and breeding microenvironments (35). The study region's typical cold winters and short, arid summers yield little seasonal variation that likely modifies the incidence of disease.

Phylogenetic analysis of *groEL* in this study revealed three previously uncharacterized *Anaplasma* isolates that grouped into a single clade. The observed variation in the *Anaplasma groEL* gene compared with entries in GenBank could be due to the site's geographical conditions at the time of bacterial collection. Geographical and climatic conditions that determine the diversity and abundance of the tick population may also influence the virulence of Anaplasma strains. This way, detailed research will set the regional genetic diversity of *Anaplasma* in Iran and its relationship to its virulence. Given the limited number of DNA sequences included, further studies with more samples are needed to provide stronger evidence for evolutionary analysis. Other variants of *groEL* may circulate among small ruminant populations in Iran.

Interestingly, the highest number of ticks as well as the highest infestation prevalence were recorded in the head and neck region compared with other body regions of sheep and goats. These findings are consistent with reports from other parts of the world, where the ear has been identified as the most common site of tick attachment (28). While several explanations may account for this distribution pattern, tick attachment and spatial distribution have been shown to be influenced by multiple factors, including host selection pressure, intraspecific variation, interspecific competition, and attraction to expired respiratory gases. The relative contribution of these factors warrants further investigation (31).

This study provides the first evidence of five tick species in the genera *Rhipicephalus* and *Hyalomma* in the Sistan region. Notably, livestock and canids are the main hosts for most of these ticks, while humans and wild animals serve as incidental or accidental hosts (10). Interestingly, several species belonging to the genera *Hyalomma* and *Rhipicephalus* have been reported to infest livestock hosts, including cattle, goats, sheep, and dogs, across different altitudinal zones and climatic conditions in Iran (21, 30) as well as in Nepal, Pakistan, and Turkey (13, 35). These countries share broadly similar environmental conditions and internal ecological factors, including high livestock density, communal grazing practices, and the presence of comparable alternative hosts such as canids, birds, and other animals, which may collectively facilitate the widespread distribution of these tick genera. The presence of ticks in the study region suggests the potential for accidental cross-transmission among domestic animals, wildlife, and birds. This is further supported by the higher prevalence observed in the northern areas of West Azerbaijan, which may be attributed to close contact between livestock and herbivorous wildlife such as deer, rabbits, and other free-ranging animals. Ticks most likely have abundant host populations in these areas, thereby increasing their transmission and survival rates. Shrubs and grassland can serve as habitats for rodents and other small mammals. Under these conditions, domestic livestock in the study region and wild herbivores often share common grazing grounds and vegetation, which may provide suitable habitats for the larval, nymphal, and adult stages of ticks. In addition, local farmers frequently collect grasses and fodder from surrounding areas and transport them to livestock holdings, a practice that may further enhance tick survival and facilitate their transmission. This finding aligns with other studies (38, 39) showing that host activity significantly influences tick distribution in the environment. The low winter temperatures and relatively high summer temperatures suggest that climate and geographic factors determine where ticks are found.

CONCLUSION

The present study on the prevalence and distribution of ticks in small ruminants of northwestern Iran. It initially identifies and describes five tick species found in West Azerbaijan. Ticks typically favor the head and neck regions, where thin skin can lead to itching, swelling, and significant skin damage. Consequently, the government should prioritize urgent tick treatment and awareness programs, particularly in agricultural areas. Future research should emphasize the epidemiological significance of *Rhipicephalus sanguineus* ticks as prevalent and highly infected vectors in the region. In the present cross-sectional study, we assessed the prevalence of *Anaplasma* infection among small ruminants in West Azerbaijan Province, northwestern Iran. Accurate diagnosis of anaplasmosis in grazing has remained challenging in this region. Although larger sample sizes are required to draw definitive conclusions, our findings indicate that *Anaplasma* infection is widely distributed among SR in West Azerbaijan Province. These results contribute to improved understanding of anaplasmosis among the general public, veterinarians, healthcare providers, and health authorities, and may support the development of more effective surveillance and control strategies. Further studies across all areas of the province are recommended to explore additional risk factors and better understand *Anaplasma* infection dynamics in the region.

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