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**Research Article**

# Prevalence of *Anaplasma* and *Ehrlichia* Infection with Molecular Characterization of *Anaplasma* Species in Cattle from Northeastern Anatolia, Türkiye

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**Abstract**

This study aimed to molecularly identify and characterize *Anaplasma* species and detect *Ehrlichia* species in cattle from Kars, Ardahan, and Iğdır provinces. Blood samples from 1000 clinically healthy cattle were analyzed using PCR and Reverse Line Blotting (RLB) techniques. The prevalence of *Anaplasma* and *Ehrlichia* spp. and their association with age, sex, and breed were evaluated using the "prevalence" package (version 0.2.0) and Pearson's chi-square test. The results revealed that only *Anaplasma* species (36.6%) were detected, whereas *Ehrlichia* spp. were not found in any of the samples. The species distribution was as follows: *A. marginale* (17.9%), *A. phagocytophilum* (14.0%), and *A. bovis* (4.2%). Mixed infections were observed in 1.37% of the cases. *Anaplasma marginale* was most frequently detected in Kars (23.60%) and Iğdır (10.66%), while *A. phagocytophilum* was most prevalent in Ardahan (23.28%). Sequence analysis of *A. marginale*, *A. phagocytophilum*, and *A. bovis* isolates showed 100% identity with previously published sequences in GenBank. In conclusion, the detection of *A. marginale*, *A. phagocytophilum*, and *A. bovis* in cattle demonstrates that the ecological and epidemiological conditions in Northeastern Anatolia are favorable for the circulation of these pathogens. The high prevalence of *A. marginale* underscores its potential impact on regional livestock health. *Anaplasma phagocytophilum* may represent a threat not only to cattle but also to public health in the region.

**Keywords:** *Anaplasma*, Cattle, *Ehrlichia*, Molecular detection, Prevalence

## Introduction

Ticks are considered the second most important arthropod vectors after mosquitoes, surpassing other hematophagous arthropods in their ability to transmit a wide range of pathogens. They are known to host at least 83 viral, 31 bacterial, and 32 protozoan species [1]. *Anaplasma* and *Ehrlichia* species are obligate intracellular, tick-borne rickettsial microorganisms belonging to the family Anaplasmataceae [2]. These pathogens pose significant health threats to domestic animals, wildlife, and human populations worldwide [3-5]. These obligate intracellular bacteria are transmitted by ticks and cause a variety of clinical disorders in ruminants, canines, and humans, often resulting in significant economic losses and public health concerns [5,6]. Among *Anaplasma* species, *A. phagocytophilum* and *A. marginale* are the primary pathogens affecting cattle, leading to febrile illnesses characterized by hemolytic anemia, decreased milk yield, respiratory signs, and reproductive disorders. In contrast, *A. bovis* and *A. centrale* are considered less pathogenic and typically result in subclinical infections [7]. Bovine ehrlichiosis is caused by several species of *Ehrlichia*, primarily transmitted by various species of hard ticks. The common species affecting large ruminants include *Ehrlichia bovis*, *E. ondiri*, *E. chaffeensis*, and *E. ruminantium* [8,9]. Clinical signs include irregular fever, ear drooping, turning movements, and lymphadenitis. Some studies have reported high mortality occurring within a few hours in the peracute stage and within 36–48 hours in the acute stage, although the disease often remains subclinical [10].

Although there is a serological study reporting a 52.1% seroprevalence of *Anaplasma marginale* in cattle in the Kars region [11], no molecular epidemiological data are

currently available for the provinces of Kars, Ardahan, and Iğdır. In contrast, several molecular studies on *Anaplasma* species in cattle have been conducted in other regions of Türkiye [7,12-15]. Given the growing importance of molecular identification of pathogens that infect veterinary animals, as emphasized in recent studies [16], such investigations are essential for understanding epidemiology and improving disease control strategies. The combination of favorable climatic conditions, geographic features, and widespread pasture-based livestock production in these provinces creates a suitable ecological niche for tick proliferation, suggesting a high potential for the transmission of tick-borne pathogens.

Therefore, the aim of this study was to molecularly identify the presence of *Anaplasma* and *Ehrlichia* species in cattle across the provinces of Kars, Ardahan, and Iğdır, to genetically characterize the positive samples obtained, and to update regional prevalence data. This study also acknowledges its regional scope, which may limit broader generalization of the findings, yet provides essential baseline data for future large-scale epidemiological investigations.

## Material and Methods

### Ethical Statement

All procedures involving animals were approved by the Kafkas University Local Ethics Committee on Animal Experiments (Decision no: KAÜ-HADYEK 2022/099), and sampling was conducted with permission from the Provincial Directorate of Agriculture and Forestry.

### Study Region and Sample Collection

This study was conducted in the northeastern Anatolia region of Türkiye, specifically in the provinces of Kars, Ardahan, and Iğdır. Between 2022 and 2023, a total of 1.000 blood samples were collected from cattle raised on randomly selected farms engaged in bovine husbandry across various districts of each province *(Fig. 1)*. Epidemiological data, including the animals' age, sex, breed, and geographic location, were also recorded.

**Fig 1.** The geographic distribution of blood samples collected from 64 villages across the provinces of Kars, Ardahan, and Iğdır. Each point represents a sampling location in a specific village (generated using QGIS)

### Inclusion and Exclusion Criteria

Clinically healthy cattle without recent antibiotic treatment were included in the study. Samples with insufficient epidemiological data or poor sample quality were excluded.

### Blood Sample Collection

A total of 1.000 blood samples were collected from clinically healthy or asymptomatic cattle. Approximately 5 mL of blood was drawn aseptically from either the jugular vein or coccygeal vein using standard techniques and transferred into EDTA-containing tubes. The samples were transported to the laboratory at 4°C and stored at -20°C until DNA extraction was performed.

### DNA Extraction, PCR Amplification, and Reverse Line Blotting (RLB) for the Detection of *Anaplasma* and *Ehrlichia* Species

Genomic DNA was extracted from blood samples using a commercial kit (EcoPURE Blood Genomic DNA Kit, Cat. No: E1075-50x, Türkiye) according to the manufacturer's instructions. The extracted DNA samples were stored at −20°C until molecular analyses were performed.

Reverse Line Blotting (RLB) is a two-step technique. In the first step, target gene amplification is performed by PCR, and in the second step, species identification is achieved through hybridization with species-specific oligonucleotide probes.

The variable V1 region (492-498 bp) of the 16S rRNA gene from *Anaplasma* and *Ehrlichia* species was amplified in the first PCR step using primers 16S8FE and BGA1B [17].

To reduce non-specific amplification, a touchdown PCR protocol was employed. The program began with an initial denaturation at 94°C for 5 min, followed by cycles consisting of denaturation at 94°C for 15 sec, annealing at 60°C for 15 sec, and extension at 72°C for 30 sec. These steps were repeated in two cycles each at progressively decreasing annealing temperatures of 60, 58, 56, 54, 52, and 50°C. Subsequently, 40 additional cycles were performed with denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min. A final extension step was carried out at 72°C for 5 min.

A positive control DNA and distilled water were used as negative controls in the PCR reactions. Each PCR reaction was prepared in a final volume of 25 µL, containing 2 µL of DNA template, 12.5 µL of 2X PCR Master Mix (EcoTaq), 1 µL of each primer (10 pmol), and 8.5 µL of nuclease-free water. Thermal cycling was performed under appropriate conditions as described above.

Five microliters of the PCR product were electrophoresed on a 1.5% agarose gel stained with ethidium bromide and visualized under UV transillumination. The remaining PCR amplicons were stored at -20°C until hybridization for Reverse Line Blotting (RLB). Probes containing an N-terminal N-(trifluoracetamidohexyl-cyanoethyl, N,N-diisopropylphosphoramidite [TFA])-C6 amino linker were synthesized by Macrogen (Korea) and used at concentrations ranging from 200 to 900 pmol per 150 µL hybridization mixture *(Table 1)*. The preparation of the Biodyne C membrane, hybridization procedures, and post-hybridization washing steps were performed as previously described [18]. Chemiluminescent signal detection was carried out using the ChemiDoc™ MP Imaging System (Bio-Rad, USA), and the presence of positive signals was interpreted based on the appearance of black dots at specific probe positions.

### Sequencing and Phylogenetic Analysis of Positive Samples

Selected PCR- and RLB-positive samples were subjected to nucleotide sequencing of the 16S SSU rRNA gene [22,23] and the 16S rRNA major surface protein-1b (msp1b) gene [24] for species confirmation and genetic characterization. Sanger sequencing of the purified amplicons was performed by Medsantek Inc. (Istanbul, Türkiye). The quality scores of the obtained chromatograms were assessed using Geneious® version 9.1.8 software.

The forward and reverse nucleotide sequences obtained from each primer were aligned with reference sequences available in GenBank using the MAFFT algorithm (version 7.526), and consensus sequences were generated. The consensus sequences obtained in this study were submitted to GenBank, and corresponding accession numbers were obtained. Additionally, the consensus sequences of the positive samples were compared with similar pathogen sequences deposited in GenBank using the BLAST (Basic Local Alignment Search Tool) algorithm to assess nucleotide similarity.

Anaplasma 16S rRNA gene sequences obtained from the NCBI database were aligned using MAFFT version 7.526, and the best-fitting substitution models were selected for each aligned dataset using JModelTest version 2.1.10, based on the Akaike Information Criterion (AIC). The TPM1uf+G model was selected for Anaplasma bovis while the GTR+G model was identified as the best fit for A. marginale and A. phagocytophilum. Phylogenetic trees were reconstructed using the Maximum Likelihood (ML) method implemented in IQ-TREE multicore version 2.4.0, with 1000 bootstrap replicates to assess branch support. The resulting trees were rooted using appropriate outgroup sequences and visualized using FigTree version 1.4.4.

### Statistical Analysis

All statistical analyses in this study were performed using the R programming language [25]. For data processing and analysis, the epiR package (version 2.0.62) was employed [26], which facilitates the calculation of core epidemiological metrics such as sensitivity, specificity, and prevalence. The Wilson method [27] was used to calculate confidence intervals for prevalence and diagnostic accuracy estimates. This method was preferred over the traditional Wald method, as the latter tends to be unreliable for small sample sizes or extreme proportion values. The Wilson method, derived from the binomial distribution, provides more accurate and asymmetric interval estimates. Associations between *Anaplasma/Ehrlichia* positivity and variables such as province, age group, breed, and sex were assessed using the Pearson chi-square ($\chi^2$) test. This significance refers specifically to the comparison of prevalence rates among the three provinces (Kars, Ardahan, and Iğdır). A P-value less than 0.05 was considered statistically significant.

## Results

This study was conducted by collecting blood samples from a total of 1.000 cattle across 64 villages in the provinces of Kars, Ardahan, and Iğdır, located in the Northeastern Anatolia Region of Türkiye. The sampling distribution was as follows: 483 cattle from 25 villages in Kars, 292 cattle from 25 villages in Ardahan, and 225 cattle from 14 villages in Iğdır.

### Molecular Detection Results

PCR analysis using the primers 16S8FE and BGA1B identified 42 positive samples out of 1000 cattle,

***Table 1.*** *Oligonucleotide Probes Used in Reverse Line Blotting (RLB)*

| Probe | Sequences (5'-3') | Reference |
|---|---|---|
| Catchall (*Anaplasma* spp.+*Ehrlichia* spp.) | Amino-GGG GGA AAG ATT TAT CGC TA | [19] |
| *Anaplasma marginale* | Amino-GAC CGT ATA CGC AGC TTG | [19] |
| *Anaplasma centrale* | Amino-TCG AAC GGA CCA TAC GC | [19] |
| *Anaplasma bovis* | Amino-GTA GCT TGC TAT GRG AAC A | [20] |
| *Anaplasma (E.) phagocytophylum* | Amino-TTG CTA TRR AGA ATA RTT AGT GG | [21] |
| *E. ruminantium* | Amino-AGT ATC TGT TAG TGG CAG | [19] |
| *E. chaffeensis* | Amino-CC TTT TGG TTA TAA ATA ATT GTT | [17] |

corresponding to a positivity rate of 4.2%. Following Reverse Line Blotting (RLB) analysis, the overall detection rate increased to 36.6%.

Among the identified species, *Anaplasma marginale* was detected in 17.9% of the samples, followed by *A. phagocytophilum* in 14.0%, and *A. bovis* in 4.2%. Additionally, mixed infections involving two or more species were identified in 1.37% of the samples. No *Ehrlichia* spp. were detected in any of the analyzed samples.

### Regional Distribution of *Anaplasma* Species

The prevalence of *Anaplasma* species detected in this study exhibited regional variation. The highest prevalence was observed in Kars province (19.5%), followed by Ardahan (12.6%) and Iğdır (4.5%). In addition, the detection of mixed infections in 1.37% of the samples suggests the co-circulation of multiple *Anaplasma* species within the same geographic area *(Table 2)*.

Based on the data obtained in this study, no statistically significant association was found between *Anaplasma* species and the variables of age, breed, or sex overall (P>0.05). However, when stratified by province, a significant association was detected between age and *Anaplasma* species in Kars, and between sex and *Anaplasma* species in Iğdır (P<0.05). In contrast, breed was not significantly associated with *Anaplasma* positivity in any of the provinces *(Table 3, Table 4, Table 5)*.

### DNA Sequencing and Phylogenetic Analysis

To achieve molecular characterization, genotyping, and phylogenetic analysis of the *Anaplasma* species identified in this study, DNA sequencing was performed on selected PCR-positive samples. The *Anaplasma marginale, A. phagocytophilum,* and *A. bovis* species identified by Reverse Line Blotting (RLB) were subjected to nucleotide sequencing of the 16S SSU rRNA variable region and the major surface protein 1b (msp1b) gene. The resulting consensus sequences were submitted to GenBank and assigned the following accession numbers: *A. marginale* - PV569600, *A. bovis* - PV569601, *A. phagocytophilum* - PV569602.

Phylogenetic trees were constructed using the 16S SSU rRNA and msp1b gene sequences of these species: *Fig. 2: A. marginale, Fig. 3: A. phagocytophilum, Fig. 4: A. bovis.*

The Maximum Likelihood (ML) method was applied using IQ-TREE (multicore version 2.4.0), with 1000 bootstrap replicates to assess node reliability. Tree visualization was performed with FigTree version 1.4.4.

## Discussion

In this study, molecular analyses were performed on blood samples collected from a total of 1,000 cattle in the provinces of Kars, Ardahan, Iğdır, and DNA belonging to *Anaplasma marginale*, *Anaplasma bovis*, and *Anaplasma phagocytophilum* was detected. No *Ehrlichia* species were identified in any of the samples. The application of the Reverse Line Blotting (RLB) technique, combined with

***Table 2.*** *Distribution of Anaplasma species detected in cattle from Kars, Ardahan, and Iğdır provinces*

| Province | *Anaplasma* Species | Positive Samples | N | %Prev (95% CI) | P | $\chi^2$ | Mean |
|---|---|---|---|---|---|---|---|
| Kars | *A.bovis* | 22 | 483 | 4.55 (3.02-6.79) | 0.0000000241 | 35.08 | P<0.05 |
| | *A. marginale* | 114 | | 23.60 (20.03-27.58) | | | |
| | *A. phagocytophlum* | 55 | | 11.38 (8.85-14.52) | | | |
| | *A. bovis/A. marginale* | 3 | | 0.62 (0.21-1.81) | | | |
| | *A. bovis/A. phagocytophilum* | 1 | | 0.20 (0.01-1.16) | | | |
| Ardahan | *A. bovis* | 16 | 292 | 5.47 (3.40-8.71) | | | |
| | *A. marginale* | 41 | | 14.04 (10.52-18.49) | | | |
| | *A. phagocytophlum* | 68 | | 23.28 (18.80-28.46) | | | |
| | *A. bovis/A.marginale* | 1 | | 0.34 (0.01-1.91) | | | |
| | *A.bovis/A. phagocytophilum* | 0 | | 0 (0-1.29) | | | |
| Iğdır | *A. bovis* | 4 | 225 | 1.77 (0.69-4.48) | | | |
| | *A. marginale* | 24 | | 10.66 (7.27-15.38) | | | |
| | *A. phagocytophlum* | 17 | | 7.55 (4.77-11.76) | | | |
| | *A. bovis/A. marginale* | 0 | | 0 (0-1.67) | | | |
| | *A. bovis/A. phagocytophilum* | 0 | | 0 (0-1.67) | | | |

***N:*** *Number of sample,* ***CI:*** *Confidence intervals,* ***Prev:*** *Prevalence, Statistical significance was defined as P<0.05*
*The value "0.0000000241" represents a prevalence proportion, not a p-value*

***Table 3.*** *Distribution of Anaplasma species by sex in cattle from Kars, Ardahan, and Iğdır provinces*

| Province | Species | Sex | | | | | | P | $\chi^2$ | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Female | | | Male | | | | | |
| | | Positive Samples | N | %Prev (95% CI) | Positive Samples | N | %Prev (95% CI) | | | |
| Kars | *A. bovis* | 22 | 462 | 4.76 (3.16-7.10) | 0 | 21 | 0 (0-15.46) | 0.070 | 3.27 | P>0.05 |
| | *A. marginale* | 112 | | 24.24 (20.55-28.35) | 2 | | 9.52 (2.65-28.91 | | | |
| | *A. phagocytophlum* | 53 | | 11.47 (8.87-14.70) | 2 | | 9.52 (2.65-28.91 | | | |
| | *A. bovis/A. marginale* | 3 | | 0.64 (0.22-1.89) | 0 | | 0 (0-15.46) | | | |
| | *A. bovis/A. phagocytophilum* | 1 | | 0.21 (0.01-1.21) | 0 | | 0 (0-15.46) | | | |
| Ardahan | *A. bovis* | 15 | 277 | 5.41 (3.30-8.74) | 1 | 15 | 6.66 (0.34-29.81) | 0.603 | 0.27 | P>0.05 |
| | *A. marginale* | 38 | | 13.71 (10.16-18.26) | 3 | | 20.00 (7.04-45.18) | | | |
| | *A. phagocytophlum* | 67 | | 24.18 (19.52-29.56) | 1 | | 6.66 (0.34-29.81) | | | |
| | *A. bovis/A. marginale* | 1 | | 0.31 (0.01-2.01) | 0 | | 0 (0-20.38) | | | |
| | *A. bovis/A. phagocytophilum* | 0 | | 0 (0-1.36) | 0 | | 0 (0-20.38) | | | |
| Iğdır | *A. bovis* | 4 | 180 | 2.22 (0.86-5.57) | 0 | 45 | 0 (0-7.86) | 0.007 | 7.34 | P<0.05 |
| | *A. marginale* | 22 | | 12.22 (8.21-17.81) | 2 | | 4.44 (1.22-14.82) | | | |
| | *A. phagocytophlum* | 17 | | 9.44 (5.98-14.60) | 0 | | 0 (0-7.86) | | | |
| | *A. bovis/A.marginale* | 0 | | 0 (0-2.08) | 0 | | 0 (0-7.86) | | | |
| | *A. bovis/A. phagocytophilum* | 0 | | 0 (0-2.08) | 0 | | 0 (0-7.86) | | | |

***N:*** *Number of sample,* ***CI:*** *Confidence intervals,* ***Prev:*** *Prevalence, Statistical significance was defined as P<0.05*
*The values 0.070, 0.603, and 0.007 correspond to prevalence proportions rather than p-values.*

***Table 4.*** *Distribution of Anaplasma species by age group in cattle from Kars, Ardahan, and Iğdır*

| Province | Species | Age | | | | | | | | | P | $\chi^2$ | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0-12 Months | | | 12-24 Months | | | 24+ Months | | | | | |
| | | Positive Samples | N | %Prev (95% CI) | Positive Samples | N | %Prev (95% CI) | Positive Samples | N | %Prev (95% CI) | | | |
| Kars | *A. bovis* | 0 | 0 | 0 (0-0) | 0 | 51 | 0 (0-7.00) | 22 | 432 | 5.09 (3.38-7.58) | 0.00002 | 18.08 | P<0.05 |
| | *A. marginale* | 0 | | 0 (0-0) | 4 | | 7.84 (3.09-18.49) | 110 | | 25.46 (21.58-29.77) | | | |
| | *A. phagocytophlum* | 0 | | 0 (0-0) | 2 | | 3.92 (1.08-13.21) | 53 | | 12.26 (9.50-15.69) | | | |
| | *A. bovis/A. marginale* | 0 | | 0 (0-0) | 0 | | 0 (0-7.00) | 3 | | 0.69 (0.23-2.02) | | | |
| | *A. bovis/A. phagocytophilum* | 0 | | 0 (0-0) | 0 | | 0 (0-7.00) | 1 | | 0.23 (0.01-1.29) | | | |
| Ardahan | *A. bovis* | 0 | 2 | 0 (0-65.76) | 1 | 41 | 2.43 (0.12-12.59) | 15 | 249 | 6.02 (3.68-9.70) | 0.152 | 3.76 | P>0.05 |
| | *A. marginale* | 0 | | 0 (0-65.76) | 5 | | 12.19 (5.32-25.54) | 36 | | 14.45 (10.62-19.36) | | | |
| | *A. phagocytophlum* | 1 | | 50.00 (2.56-97.43) | 6 | | 14,63 (6.88-28.44) | 61 | | 24.49 (19.57-30.20) | | | |

***Table 4.*** *Continue*

| Province | Species | Age | | | | | | | | | P | $\chi^2$ | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0-12 Months | | | 12-24 Months | | | 24+ Months | | | | | |
| | | Positive Samples | N | %Prev (95% CI) | Positive Samples | N | %Prev (95% CI) | Positive Samples | N | %Prev (95% CI) | | | |
| Ardahan | *A. bovis/A. marginale* | 0 | 2 | 0 (0-65.76) | 0 | 41 | 0 (0-8.56) | 1 | 249 | 0.40 (0.02-2.23) | 0.152 | 3.76 | P>0.05 |
| | *A. bovis/A. phagocytophilum* | 0 | | 0 (0-65.76) | 0 | | 0 (0-8.56) | 0 | | 0 (0-1.51) | | | |
| Iğdır | *A. bovis* | 0 | 18 | 0 (0-17.58) | 1 | 51 | 1.96 (0.10-10.30) | 3 | 156 | 1.92 (0.65-5.50) | 0.145 | 3.86 | P>0.05 |
| | *A. marginale* | 0 | | 0 (0-17.58) | 4 | | 7.84 (3.09-18.49) | 20 | | 12.82 (8.45-18.97) | | | |
| | *A. phagocytophlum* | 1 | | 5.55 (0.28-25.75) | 3 | | 5.88 (2.02-15.92) | 13 | | 8.33 (4.93-13.73) | | | |
| | *A. bovis/A. marginale* | 0 | | 0 (0-17.58) | 0 | | 0 (0-7.00) | 0 | | 0 (0-2.40) | | | |
| | *A. bovis/A. phagocytophilum* | 0 | | 0 (0-17.58) | 0 | | 0 (0-7.00) | 0 | | 0 (0-2.40) | | | |

***N:*** *Number of sample,* ***CI:*** *Confidence intervals,* ***Prev:*** *Prevalence, Statistical significance was defined as P<0.05*
*The values 0.00002, 0.152, and 0.145 correspond to prevalence proportions rather than p-values.*

***Table 5.*** *Distribution of Anaplasma species by cattle breed in Kars, Ardahan, and Iğdır provinces*

| Province | Species | Breed | | | | | | P | $\chi^2$ | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Dual-Purpose | | | Dairy | | | | | |
| | | Positive Samples | N | %Prev (95% CI) | Positive Samples | N | %Prev (95% CI) | | | |
| Kars | *A. bovis* | 20 | 463 | 4.31 (2.81-6.57) | 2 | 20 | 10.00 (2.78-30.10) | 0.507 | 0.44 | P>0.05 |
| | *A. marginale* | 111 | | 23.97 (20.30-28.06) | 3 | | 15.00 (5.23-36.04) | | | |
| | *A. phagocytophlum* | 51 | | 11.01 (8.47-14.19) | 4 | | 20.00 (8.06-41.60) | | | |
| | *A. bovis/A. marginale* | 2 | | 0.43 (0.11-1.56) | 1 | | 5.00 (0.25-23.61) | | | |
| | *A. bovis/A. phagocytophilum* | 1 | | 0.21 (0.01-1.21) | 0 | | 0 (0-16.11) | | | |
| Ardahan | *A. bovis* | 16 | 289 | 5.53 (3.43-8.80) | 0 | 3 | 0 (0-56.14) | 0.810 | 0.058 | P>0.05 |
| | *A. marginale* | 40 | | 13.84 (10.33-18.29) | 1 | | 33.33 (1.70-79.23) | | | |
| | *A. phagocytophlum* | 67 | | 23.18 (18.68-28.38) | 1 | | 33.33 (1.70-79.23) | | | |
| | *A. bovis/A. marginale* | 1 | | 0.34 (0.01-1.93) | 0 | | 0 (0-56.14) | | | |
| | *A. bovis/A. phagocytophilum* | 0 | | 0 (0-1.31) | 0 | | 0 (0-56.14) | | | |
| Igdır | *A. bovis* | 4 | 198 | 2.02 (0.78-5.07) | 0 | 27 | 0 (0-12.45) | 1.000 | 0.00 | P>0.05 |
| | *A. marginale* | 20 | | 10.10 (6.63-15.08) | 4 | | 14.81 (5.91-32.47) | | | |
| | *A. phagocytophlum* | 16 | | 8.08 (5.03-12.72) | 1 | | 3.70 (0.18-18.28) | | | |
| | *A. bovis/A. marginale* | 0 | | 0 (0-1.90) | 0 | | 0 (0-12.45) | | | |
| | *A. bovis/A. phagocytophilum* | 0 | | 0 (0-1.90) | 0 | | 0 (0-12.45) | | | |

***N:*** *Number of sample,* ***CI:*** *Confidence intervals,* ***Prev:*** *Prevalence, Statistical significance was defined as P<0.05*
*The values 0.507, 0.810, and 1.000 correspond to prevalence proportions rather than p-values.*

sequencing, enabled more sensitive detection and accurate species differentiation compared to conventional PCR.

In recent years, numerous studies on vector-borne diseases conducted worldwide have demonstrated that the prevalence of such infections varies considerably across geographical regions. Molecular investigations targeting

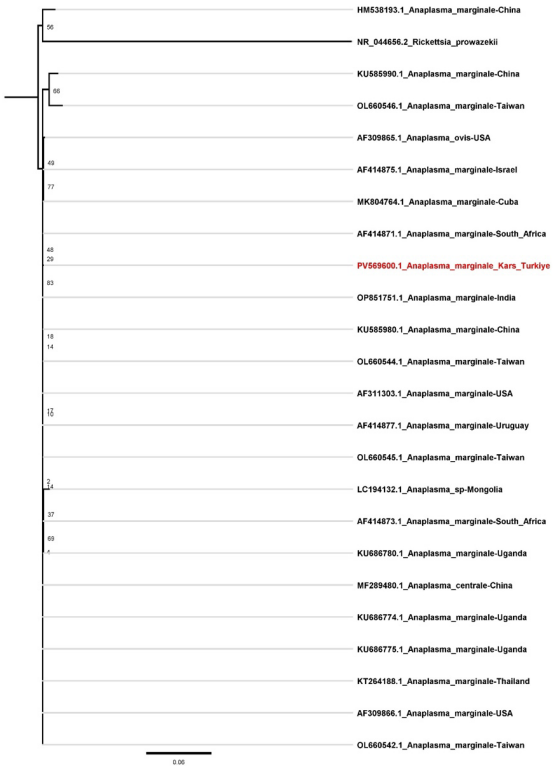


**Fig 2.** Maximum Likelihood phylogenetic tree of *Anaplasma marginale* constructed using IQ-TREE software based on the nucleotide sequences of the 16S rRNA major surface protein-1b (msp1b) gene. The tree is based on a multiple sequence alignment of partial msp1b gene sequences (265 bp) of *A. marginale* (GenBank accession no: PV569600)


**Fig 3.** Maximum Likelihood phylogenetic tree of *Anaplasma phagocytophilum* constructed using IQ-TREE software based on the nucleotide sequences of the 16S SSU rRNA gene. The tree is derived from a multiple sequence alignment of partial 16S SSU rRNA gene sequences (641 bp) of *A. phagocytophilum* (GenBank accession no: PV569602)


**Fig 4.** Maximum Likelihood phylogenetic tree of *Anaplasma bovis* constructed using IQ-TREE software based on the nucleotide sequences of the 16S SSU rRNA gene. The tree is based on a multiple sequence alignment of partial 16S SSU rRNA gene sequences (345 bp) of *A. bovis* (GenBank accession no: PV569601)

*Anaplasma* species have revealed prevalence rates ranging from 1.7% in Kyrgyzstan [28], 3.2% in China [29], and 20.8% in Thailand [30], to as high as 36% in Ecuador [31] and 87.3% in Colombia [32]. In Türkiye, reported prevalence rates range between 0% and 30.8% [33].

The growing importance of molecular identification of pathogens in veterinary medicine has been emphasized in previous studies [16], and our results support the need for continued molecular surveillance to better understand epidemiological patterns and improve disease control strategies. In the present study, the overall prevalence of *Anaplasma* species was found to be 36.6%, which is relatively high compared to several previous reports. This variation may be attributed to differences in ecological and geographical conditions, vector distribution, host susceptibility, and importantly, the sensitivity of the molecular detection techniques employed.

Geographical studies on bovine anaplasmosis in Türkiye have demonstrated that the distribution and dynamics of *Anaplasma* species vary significantly across regions [7,15,33-35]. In the Black Sea Region, *A. phagocytophilum* has been reported with a prevalence of 30.8%, while *A. marginale* and *A. bovis* were detected at rates ranging from 2.8% to 18.8% and 0.7% to 1%, respectively [34,35]. A study from the Eastern Anatolia Region, specifically in Malatya, found

*A. marginale* at a prevalence of 32.5%, along with the detection of other *Anaplasma* species [7]. In the Aegean Region, *A. phagocytophilum* was detected at a prevalence of 5% [15]. Furthermore, a large-scale study covering 16 provinces of Türkiye reported prevalence rates of *A. marginale* (10.5%), *A. phagocytophilum* (13.8%), *A. bovis* (0.5%), and other *Anaplasma* species (2.9%) in the Central and Southeastern Anatolia regions [25]. In the current study, *A. marginale* showed the highest prevalence in Kars (23.6%) and Iğdır (10.66%), while *A. phagocytophilum* was most frequently detected in Ardahan (23.28%). These differences may be attributed to local ecological conditions, the density and species composition of tick populations, livestock management practices, and other environmental factors. The unique environmental characteristics of each province likely influence tick activity and pathogen transmission, thereby contributing to regional variation in prevalence. Such geographic disparities underscore the need for region-specific epidemiological assessments and tailored control strategies.

Ticks responsible for the transmission of *Anaplasma* species are widely distributed across Türkiye and are considered among the most important arthropod vectors. These include genera such as *Ixodes, Haemaphysalis, Dermacentor, Rhipicephalus (Boophilus), Hyalomma,* and *Ornithodorus* [36]. Several studies conducted in this region have documented tick infestations in cattle, sheep, and dogs [17,37-39]. *Dermacentor marginatus* has been reported as a predominant species in both cattle and sheep, with prevalence rates ranging from 18.8% to 66.31% [17,37,38]. Additionally, the presence of *Haemaphysalis parva* (14%-77.27%), *Haemaphysalis punctata* (0.21%-3.0%), *Rhipicephalus bursa* (0.2%-14.62%), *Dermacentor reticulatus* (0.39%-12.5%), and, to a limited extent, *Ixodes ricinus* (0.2%) has been recorded in the same region [17,39,40]. The findings of the present study regarding the presence of *Anaplasma* species in cattle support the hypothesis that the tick species prevalent in Kars, Ardahan, and Iğdır, particularly *Dermacentor marginatus*, play a significant role in the transmission of *Anaplasma* pathogens. The high prevalence of this tick species underscores its potential contribution to the spread of tick-borne diseases in the region.

*Anaplasma marginale* is recognized as the most prevalent agent of bovine anaplasmosis worldwide, with its biological transmission primarily associated with tick vectors, particularly species from the *Rhipicephalus* and *Dermacentor* genera [41]. In the present study, *A. marginale* was detected in 17.9% of the cattle samples, indicating a relatively high prevalence in the region. These findings suggest that the widespread distribution of *Rhipicephalus* and *Dermacentor* ticks in the study area may contribute significantly to the transmission dynamics of this pathogen. Consequently, effective control of bovine anaplasmosis in the region requires the implementation of integrated tick management strategies tailored to local vector ecology.

In addition to tick vectors, the potential role of other arthropods in the transmission of *Anaplasma marginale* should not be overlooked. Blood-feeding flies such as *Tabanus* spp.[42] and *Stomoxys calcitrans* [43] have been implicated as possible mechanical vectors. These flies may transmit the pathogen by mechanically transferring infected blood between animals. Although ticks remain the principal biological vectors of *A. marginale,* the involvement of hematophagous flies highlights the complexity of its epidemiology. While clinical and field studies consistently demonstrate the effectiveness of tick control strategies, managing fly populations is also essential to reduce the risk of mechanical transmission. Therefore, integrated vector management approaches targeting both ticks and biting flies are recommended for comprehensive disease control.

Although *Ixodes* species are widely recognized as the principal vectors of *A. phagocytophilum* [44], there is ongoing speculation regarding the involvement of other tick species in the transmission of this pathogen [45]. In the present study, the prevalence of *A. phagocytophilum* was found to be 14.00%. Despite the relatively low abundance of *Ixodes* ticks in the study area, the observed infection rate was notably high. This finding raises the possibility that other tick genera may also contribute to the transmission cycle of *A. phagocytophilum* [46]. Previous studies [12,36,47-49] have also supported this hypothesis, suggesting that the presence of *A. phagocytophilum* may not be restricted solely to transmission by *Ixodes ricinus*. These findings underscore the need to consider local tick biodiversity in epidemiological surveillance and control programs. However, further studies are required to confirm the role of alternative vectors. In addition, the results of this study indicate that *A. phagocytophilum* may pose a significant public health risk, not only to cattle but also to humans, particularly those involved in animal husbandry or individuals at increased risk of tick exposure. As a known zoonotic pathogen, its presence in livestock highlights the importance of integrated control strategies to mitigate potential transmission to humans.

*Anaplasma bovis* is generally associated with subclinical infections; however, it can cause severe disease in immunocompromised animals [50]. In the present study, the highest prevalence of *A. bovis* was recorded in Ardahan province (5.47%), while the lowest was observed in Iğdır (1.77%). These regional differences may be attributed to variations in tick population density and environmental factors. The relatively high prevalence detected in Kars province suggests that local tick species

may act as effective biological vectors for *A. bovis* in this region. Furthermore, the widespread occurrence of subclinical infections implies that many animals may act as asymptomatic carriers, complicating the diagnosis of *A. bovis* in the field and potentially contributing to the silent spread of the pathogen within herds.

The absence of *Ehrlichia* species detection in this study may be attributed to the lack of specific tick vectors belonging to the Ixodid family, particularly *Amblyomma* species, which were not observed in the study region. According to the literature, certain *Ehrlichia* species rely heavily on these tick genera for biological transmission [51] . The absence of such vectors in the current sampling area may explain the failure to detect *Ehrlichia* DNA in the cattle population. This finding suggests that the composition and diversity of the local tick fauna, along with environmental factors influencing vector ecology, may significantly impact the transmission dynamics of *Ehrlichia* spp. Therefore, more comprehensive investigations are needed to clarify the potential presence and epidemiological relevance of *Ehrlichia* species in this region.

The provinces of Kars, Ardahan, and Iğdır are located in the Eastern and Northeastern regions of Türkiye and are characterized by significant ecological diversity. Climatic variations and the presence of extensive pasturelands in these areas play a pivotal role in shaping agricultural and livestock activities. However, these geographical and environmental features also influence the distribution and density of vector populations, particularly ticks, which are responsible for the transmission of various vector-borne diseases. The ecology of each region determines the conditions favorable for tick proliferation, thereby affecting disease dynamics. As such, the geographical and ecological characteristics of each province should be taken into account when designing regional disease control strategies. The climatic and topographic differences among these three provinces likely contribute to the observed variation in the prevalence and transmission routes of *Anaplasma* species. Consequently, the density and distribution potential of tick populations in these regions are critical factors that directly influence the spread of *Anaplasma* spp., highlighting the need for locally tailored control measures to effectively manage these pathogens.

This study has certain limitations. First, it was restricted to three provinces in Northeastern Anatolia, which may limit the generalization of the findings to other regions. Second, sequencing was performed only on a subset of positive samples, meaning that the full genetic diversity of *Anaplasma* spp. in the study area may not have been fully captured. Despite these limitations, the results provide valuable baseline data for future large-scale epidemiological studies.

In conclusion, this study demonstrated the presence of *A. marginale, A. phagocytophilum,* and *A. bovis* in cattle from Northeastern Anatolia, while no *Ehrlichia* spp. were detected. The findings highlight the role of ecological and epidemiological conditions in shaping pathogen distribution and underscore the importance of region-specific control strategies. Although limited to three provinces and partial sequencing, the study provides valuable baseline data for future epidemiological investigations and contributes to the understanding of tick-borne pathogen dynamics in Türkiye.

## Declarations

**Availability of Data and Materials:** The data and materials used in this study are available upon request from the corresponding author (N. Aydın).

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**Authors Contributions:** Conceptualization, NA, BS, ZV, GTT, NO, MEI, MY, and RT; funding acquisition, NA, ZV, GTT, and RT; methodology, NA, MEI, MY, and RT; investigation, NA, BS, ZV, GTT, NO, MEI, MY, and RT; formal analysis, NA, BS, ZV, GTT, NO, MEI, MY, and RT; writing-original draft preparation, NA; writing-review & editing, ZV, BS, GTT, NO, and NA; visualization, MEI, MY, and NA. All authors have reviewed and approved the final version of the manuscript for publication.

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