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First Molecular Anaplasma capra Detection in Turkish Goats

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Abstract

Anaplasma capra infection is usually asymptomatic, but it is known to cause zoonotic tick-borne diseases. *A. capra*'s morphological characteristics and the types of cells infected (such as erythrocytes, monocytes, and neutrophil granulocytes) are unknown. Infection with *A. capra* was mostly found in ixodid ticks. There have been no studies on *A. capra* infection in goats, one of Turkey's most common farm animals. Infection with *A. capra* was mostly found in ixodid ticks. There have been no studies on *A. capra* infection in goats, one of Turkey's most common farm animals. They are required to determine their distribution, genetic diversity, vector species, and host specificity. This study aimed to investigate the *A. capra* pathogen in goats in Turkey's Van province. A total of 200 goat blood samples were examined. Goat samples were subjected to partial amplification of the *gltA* gene fragment using a nested polymerase chain reaction. A. capra DNA was detected in 0.5% of goat blood samples. Phylogenetic analysis of a partial *gltA* gene fragment showed that the eastern Turkey isolate, closely grouped with *A. capra* isolates reported from wild and domestic ruminants in France, Turkey, and Kyrgyzstan, formed a distinct clade. This is the first report of *A. capra* in goats in Turkey.

Introduction

Anaplasma capra is a species of proteobacteria from the Anaplasmataceae family and was first identified under this name in 28 of 477 patients with a history of tick bites in northern China in 2015. Humans infected with Anaplasma capra develop an undifferentiated flu-like illness, such as fever, headache, malaise, dizziness and chills (Lie et al. 2015). The effects of *Anaplasma capra* on animal health have not yet been assessed. Infected animals are believed to be asymptomatic (Jouglin et al. 2022). When confirmed, it has been suggested that dogs may exhibit clinical symptoms such as anemia (Shi et al. 2019). This newly identified species poses a significant threat to human and animal health. To be protected from zoonotic diseases, it is necessary to first identify the agent or source of the disease. In addition, there is an important question regarding the knowledge of transmission methods. Ticks are the primary vectors of the *Anaplasma* species. Although *Anaplasma capra* has been detected in several tick species, such as *Rhipicephalus (Boophilus) sp., Rhipicephalus microplus, Dermacentor everstianus, Haemaphysalis longicornis*, *H. qinghaiensis and Ixodes persulcatus*, its vector capacity has not yet been proven. Previous studies have reported this species in ruminants (sheep, goats, cattle, and buffalo), domestic dogs and wild animals (e.g., musk deer, Himalayan goral, and Korean water deer). This species was observed in sheep, cattle, and buffalo raised in Sivas, Turkey (Altay et al. 2022; Sahin et al. 2022; Addo et al. 2023). Recent studies in Turkey have shown that goats can act as a natural reservoir for *Anaplasma phagocytophilum*-like 1 and *A. ovis* (Altay et al. 2014; Zhou et al. 2017; Erol et al. 2022). However, there is no data on *A. capra* infection in goats in Turkey. Surveillance studies in different animal species can provide more information about the organisms in which this *Anaplasma* species is or is not localized. Two genotypic variants of *Anaplasma capra* (genotype I and II) have been postulated (Miranda et al. 2021; Jouglin et al. 2022). However, the geographical distribution of *A. capra* is not limited to Asian countries. Its first European report was from France (Jouglin et al. 2019). So far, the *A. capra* species in

our country have been classified as genotype I, which has been reported to differ from Asian isolates (Altay et al. 2022). In the present study, we aimed to determine the *A. capra* pathogen in goats in the Turkish province of Van using nested-PCR targeting the *gltA* gene (citrate synthase gene) and to characterize *A. capra* strains using PCR and sequence analysis of the *gltA* gene.

Material and Method

Sample Collecting

This study was conducted in Van Province in eastern Turkey (38° 30' 3.96" N, 43° 22' 23.16" E). Blood samples were taken from 15 randomly selected goat herds between April and June 2022. In EDTA collection tubes, 200 blood samples in total were obtained. Goats at least a year old and in clinically healthy condition were used, and samples were kept at -20°C until DNA was isolated.

DNA Extraction and PCR Amplification

Following the steps outlined in the commercial DNA extraction kit (Hybrigen Genomic DNA Isolation Kit, Catalog No.: MG-GDNA-01-250, Turkey), genomic DNA was isolated from blood samples. The final elution was set at 50–100 µl, and genomic DNA extracts were kept at -20°C. *Msp4, gltA, groEL*, and 16S SSU rRNA genes were used to detect and identify *A. capra* isolates from other *Ehrlichia* species (Peng et al. 2018, 2020; Yang et al. 2018; Shi et al. 2019; Wei et al. 2020; Altay et al. 2022). However, the *gltA* gene has a high level of genetic diversity and is used for phylogenetic analysis and *Anaplasma* species identification (Inokuma et al. 2001; Altay et al. 2022). DNA isolates from each animal were investigated in nested PCR with primers targeting the partial citrate synthase (*gltA*) gene region. In the first step of nested PCR analysis, general Outer F (5'- GCG ATT TTA GAG TGY GGA GATTG-3') and Outer R (5'-TAC AAT ACC GGA GTA AAA GTCAA-3') for *Anaplasma* species. In the second step, *A. capra*-specific Inner F (5'-TCA TCT CCT GTT GCA CGG TGCCC-3') and Inner R (5'-CTC TGA ATG AAC ATG CCC ACCCT-3') primers were used, and the amplification procedures were optimized according to the relevant literature (Lie et al. 2015; Yang et al. 2016; Altay et al. 2022). The PCR products obtained at the end of all amplifications were electrophoresed on a 1.5% agarose gel and analyzed using a UV transilluminator. Dr. Kürşat ALTAY and Dr. Ufuk EROL (Sivas Cumhuriyet University, Faculty of Veterinary Medicine, Parasitology Department) provided control DNA samples of *Anaplasm capra*. As a negative control, genomic DNA was extracted from a blood sample obtained in previous studies that were negative for *Anaplasma*, as determined by microscopy and PCR. In addition, as a negative control, double-distilled water was added to each PCR reaction.

DNA Sequencing and phylogenetic analysis

The positive PCR amplicon was purified, and the purified products were sequenced with the appropriate primers to determine the nucleotide sequences. PCR products and primers were neatly packaged and sent

to Ankara's "Sentebiolab Biotechnology" for sequence analysis. Purification and reading of the product were carried out in this laboratory. Sequencing is performed on purified PCR products and plasmids using gel or column as starting material in Sanger sequencing and the ABI Prism 3130 Genetic Analyzer. The Ion Torrent Personal Genome Machine (PGM) device is used for next-generation sequencing. Following careful examination of the chromatogram of the isolate whose bidirectional DNA sequence was determined, the final sequences were obtained by performing pair-wise alignments of forward and reverse sequences with BioEdit Sequence Alignment (Hall 1999) software. Blastn was used to analyze the obtained sequence (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Then, using Mega X (Kumar et al. 2016) and BioEdit software, multiple alignments were made with other isolates registered in GenBank worldwide, and their phylogenies and molecular characterizations were revealed. The resulting sequence was added to GenBank. The phylogenetic relationships of the bacterial strains obtained with other strains in international databases were investigated using Mega X genetic analysis software. The maximum likelihood (ML) method was used to determine the phylogenetic structures of *A. capra* in this context. The 1000 replication bootstrap test determined the dependability of the ML method's trees. As an outgroup *Rickettsia conorii* subsp. *raoultii* strain was used.

Results

To investigate *A. capra* in the Van province of Turkey, 200 blood samples were analyzed using PCR techniques (nested PCR). Only one sample (0.5%) yielded positive results (Fig. 1). Sequencing was performed on the single positive *A. capra*-nested PCR product. BLAST analysis showed that the DNA sequence of *A. capra* obtained in this study (GenBank: OQ703060) had 88.35–99.64% similarity to the isolates of *A. capra* obtained from many countries (GenBank: MH084720, ON783819, OM100840, MG940872, KM206274). Phylogenetic analysis based on *gltA* sequences showed that the isolates were clustered within the *A. capra* clade but separate from other well-defined *Anaplasma marginale, A. ovis, A. platys, A. phagocytophilum, A. bovis, Candidatus A. cinensis*, and *C. A. turritanum.* Of note, within this group, the turkey Van isolate was grouped more closely with the four isolates from Corsica Island and a zoological park in France (Jouglin et al., 2019; 2022), three isolates from Turkey (Altay et al. 2022; Sahin et al. 2022), and one isolate from Kyrgyzstan. We found that the Van isolate was grouped in one of these clusters (clade I) and that within this clade, it grouped with the France, Turkey, and Kyrgyzstan isolates (Middle East and Asian/European lineages) discovered in previous studies. Based on the shorter *gltA* gene fragment (GenBank: OQ703060), "clade I" isolates were separated from the clade of East Asian lineage II isolates with high (94%) support. Clade 2 has created strong bootstrap support by utilizing various Asian isolates. As a result, it also established the "East Asian lineage II" group (Fig. 2).

Discussion

This study reports the first molecular detection of *A. capra* in goats in Turkey. Although its DNA has been found in various Ixodid tick species, ticks' role in transmitting this pathogen is unclear(Li et al. 2015; Yang et al. 2016; Qin et al. 2018; Guo et al. 2019; Han et al. 2019; Altay et al. 2022). As in the rest of the world,

our country's livestock is susceptible to tick infestations owing to its location. Diseases transmitted by ticks are extremely pathogenic, and they may be protozoa, helminths, viruses, bacteria, or rickettsia. Certain factors are zoonotic and can cause dangerous epidemics when ticks prefer humans (Aydın 2021). *A. capra*, which was recently discovered, has a diverse host range. The discovery of this zoonotic pathogen in goats and their persistence in infection suggest that they are reservoirs (Li et al. 2015). The prevalence of *A. capra* in goats ranges from 1.3–18.2% in Asian countries (Yang et al. 2018b; Koh et al. 2018; Seo et al. 2019; Miranda et al. 2021). Only a 13% positive rate for *A. capra* was identified in goats on Corsica Island, France (Jouglin et al. 2022). Besides, in the study conducted in Turkey, 72 goats were negative for *A. capra* (Altay et al. 2022). This study presents a low frequency of *A. capra* (0.5%) in goats in Turkey. An epidemiological study with a larger sample size is needed to predict the prevalence of *A. capra* in Turkey ruminants.

The phylogenetic analysis of the *A. capra gltA* gene allowed this *Anaplasma* species to be divided into two distinct clades. Clade I isolates came from Turkey, France, Kyrgyzstan, China, and South Korea, while Clade II isolates came from China and South Korea. The sequences of *A. capra*'s gltA obtained in this study shared 99.64% of their similarities with isolates found in France (Fallow Deer, MH084720), Turkey (Manda, ON783819; Sheep, OK267270; Cattle, OK267267), and Kyrgyzstan (Sheep, OM100840). The *gltA* gene of *A. capra* strains from ticks, wild deer and sheep in East Asia Lineage II formed a distinct lineage instead of clustering with East Asia Lineage I. *A. capra* strain detected in goats from the Van City of Turkey has formed a distinct lineage, with strain recovered from humans (GenBank: KM206274).

Conclusion

The findings of this study imply that goats in Turkey might harbor the *A. capra* infection. To identify the *A. capra* transmission vectors, more investigation is required. Additionally, according to our phylogenetic analyses, the newly found *A. capra* isolate from Turkey:Van is genetically very similar to isolates from Kyrgyzstan, France, and Turkey that were recently discovered (Jouglin et al. 2019, 2022 and Altay et al. 2022).

Declarations

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Data availability

This paper contains all of the data created or analyzed during this investigation.

Code availability

Not applicable

Author Contributions

Conceptualization: BO; Data curation: BO, MSD; Formal analysis: BO, MSD; Investigation: BO, MSD; Methodology: BO, MSD; Resources: BO, MSD; Software: BO; Supervision: BO; Validation: BO, EAO, SEA, MSD; Visualization: BO, EAO, SEA, MSD; Writing – original draft: BO; Writing – review & editing: EAO, SEA, BO, MSD.

Ethics approval

The approval of the Ethics Committee for this research was obtained from the Animal Experiments Local Ethics Committee of Van Yuzuncu Yil University (dated 23/02/2023 and numbered 05-12).

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figures

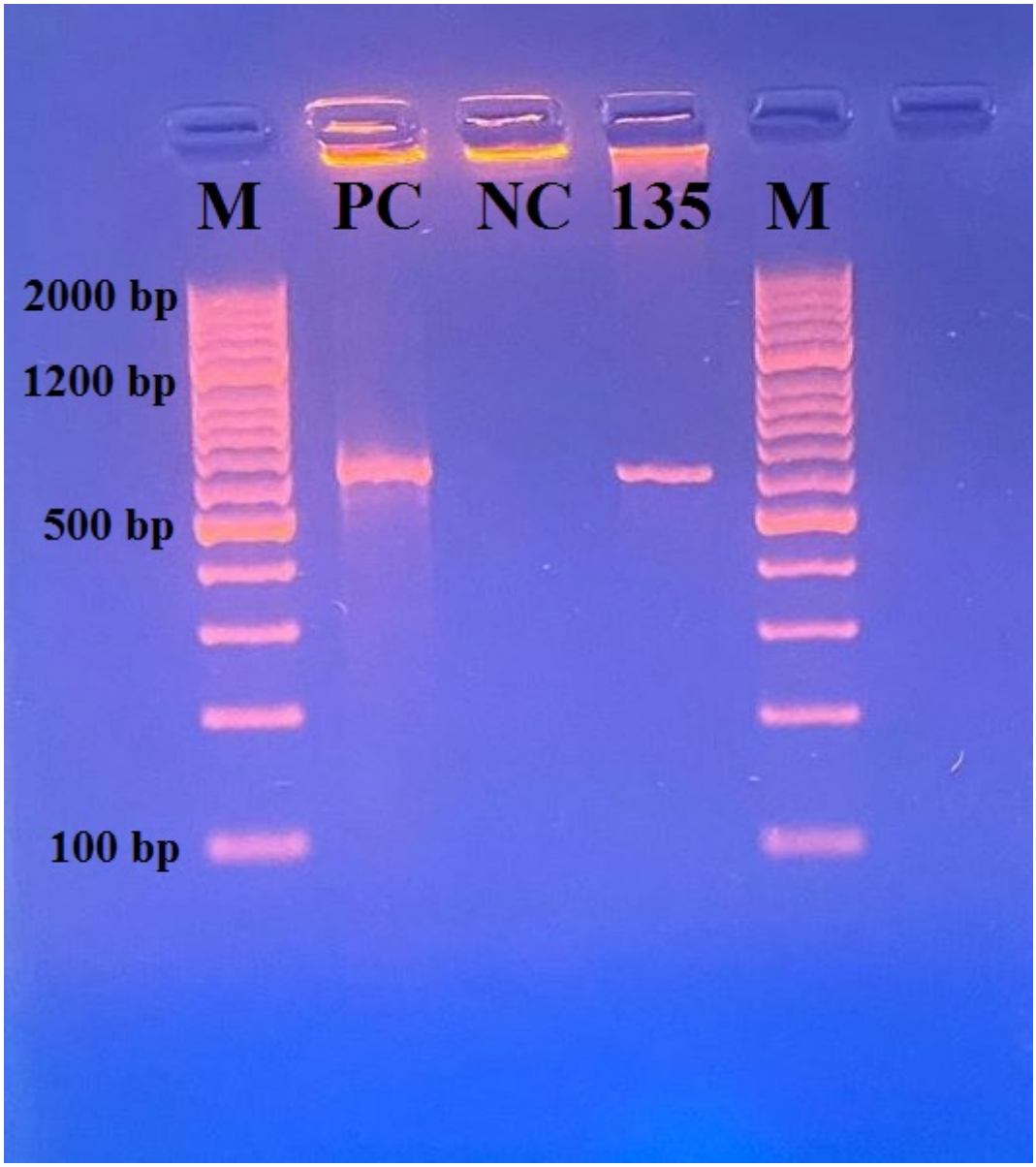


Figure 1

Anaplasma capra positive sample on agarose gel at the 2nd step of the Nested PCR analyses. M: Marker (100bp), 135: Positive sample, PC: Positive control, NC: Negative control

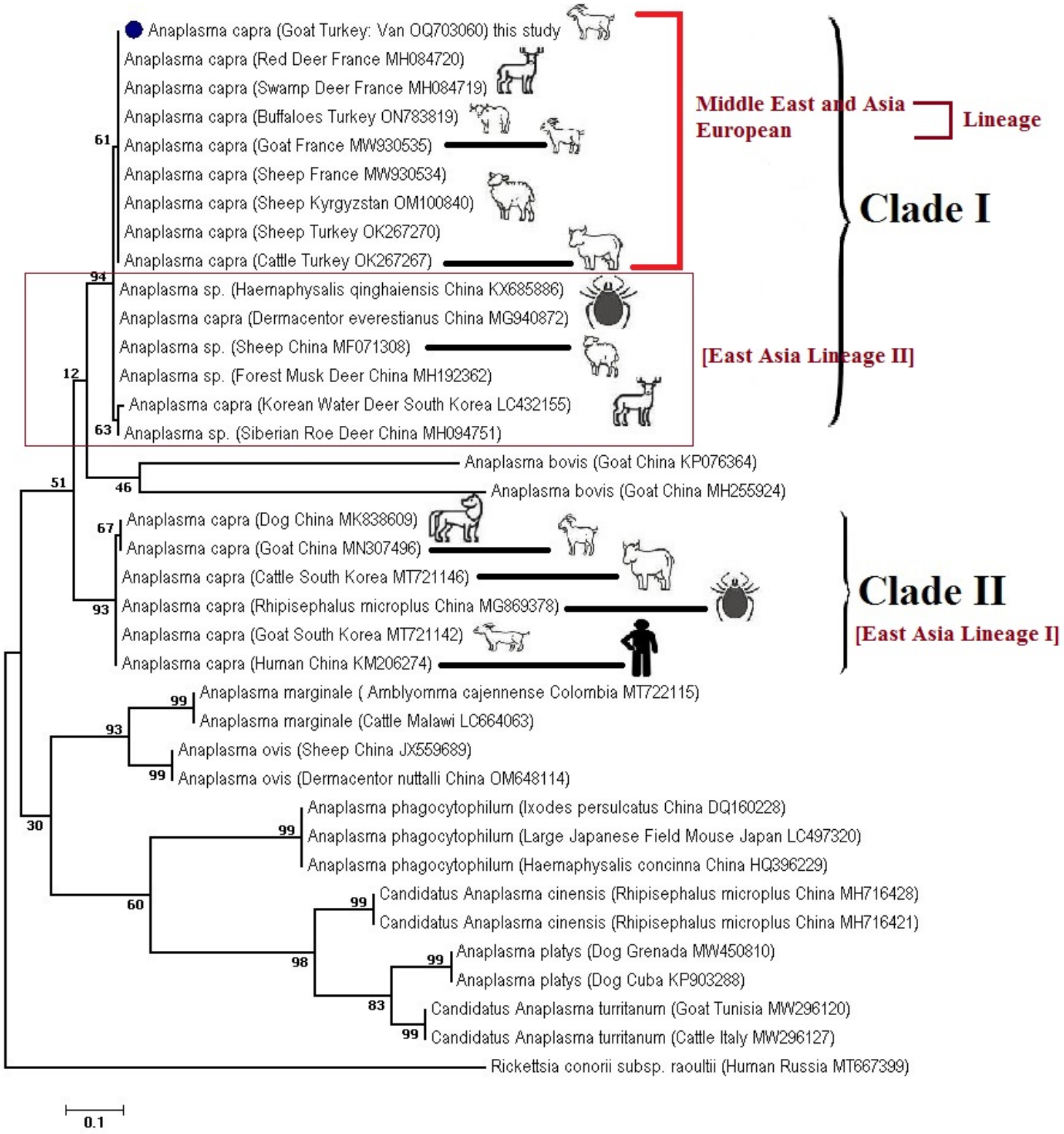


Figure 2

Phylogenetic tree of *A. capra* using gltA gene sequences (1,000 bootstrap replicates) constructed by Maximum Likelihood method and Kimura 2-parameter model. The identification source (host) and country are given after the numbers. Isolates from this study are indicated with a blue round.