



# The first molecular detection of *Anaplasma capra* in domestic ruminants in the central part of Turkey, with genetic diversity and genotyping of *Anaplasma capra*

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## Abstract

Tick-borne diseases have been an increasing threat to human and animal health all over the world. Anaplasmosis is one of the emerging tick-borne diseases and has zoonotic potential. A new novel species, which was detected in China in 2010–2012 and provisionally named *Anaplasma capra* in 2015, causes zoonotic infections and infects many different animal species. In this study, we investigated the presence of *A. capra* in domestic ruminants from Turkey. A total of 468 blood samples (cattle, sheep, and goat) were examined by the *gltA* gene-specific nested polymerase chain reaction, revealing the presence of *A. capra* in six samples (1.28%): one of them from cattle (0.41%) and the other five from sheep (3.22%). According to DNA sequences results of the *gltA* gene, *A. capra* isolates identified in the present study were shown high nucleotide similarity with *A. capra* isolates detected from different hosts. However, the nucleotide differences were detected in the same nucleotide positions between *A. capra* isolates. For this reason, we thought that at least two different *A. capra* genotypes could be circulating in the world. As a result, it is seen that *A. capra*, which was determined to be a new species with zoonotic potential, was revealed in European and Asian countries and in different hosts. In order to raise awareness about human anaplasmosis infections, it is important to reveal the prevalence of the species in the world. The emergence of *A. capra* in Turkey reveals the need for a re-evaluation of the human and animal health risk analysis in terms of anaplasmosis.

**Keywords** *Anaplasma capra* · Cattle · Sheep · *gltA* · Turkey

## Introduction

Tick-borne diseases (TBDs) pose a global risk to human and animal health (de la Fuente et al. 2017; Madison-Antenucci et al. 2020). With the development of molecular diagnostic methods and their widespread use in the researches, many new species and/or genotypes have been found in humans and animals (Altay et al. 2007; Liu et al. 2012; Aktas et al. 2021; Aktas and Çolak 2021; Altay et al. 2022). *Anaplasma capra* is a newly identified species and has a zoonotic character (Li et al. 2015). The novel species within the *Anaplasma* genus was detected for the first time in goats in China (2010–2012) (Zhou et al. 2010; Liu et al. 2012), and it was provisionally named as “*Anaplasma capra*” in 2015

(Li et al. 2015). However, in a study conducted on serows in Japan in 2009 based phylogenetic analyses of *16S SSU rRNA* DNA sequences shown that some isolates collected in a different clade from known *Anaplasma* species as *A. capra* (Sato et al. 2009). After the first detection of *A. capra* in goats in China, its presence has been detected in Asia (China, Japan, South Korea, Malaysia, Iranian, and Kyrgyzstan) (Sato et al. 2009; Koh et al. 2018; Wei et al. 2020; Miranda et al. 2021; Staji et al. 2021; Altay et al. 2022) and Europe (France and Spain) (Jouglin et al. 2019; Remesar et al. 2021). On the other hand, *A. capra* has been found to infect humans (Li et al. 2015), goat, sheep, cattle, dog, and wild animals (e.g., deer, takin, Persian onegar, muntjac, serow) (Sato et al. 2009; Liu et al. 2012; Peng et al. 2018; Yang et al. 2018a, b; Jouglin et al. 2019; Shi et al. 2019; Seo et al. 2020; Staji et al. 2021; Altay et al. 2022). *A. capra* has been also detected in Ixodid tick species such as *Ixodes persulcatus*, *Dermacentor everstianus*, *Haemaphysalis longicornis*, *H. qinghaiensis*, and *Rhipicephalus microplus*, but potential biological vectors of *A. capra* have not yet been

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proven (Fang et al. 2015; Li et al. 2015; Yang et al. 2016; Qin et al. 2018; Guo et al. 2019; Han et al. 2019). Although *A. capra* was originally thought to be a common species in China, ongoing researches in this area highlight the global importance of this species in influencing human and animal health (Jouglin et al. 2019; Miranda et al. 2021; Remesar et al. 2021; Staji et al. 2021; Altay et al. 2022).

Turkey's climate and geographical features, vegetation, and wildlife provide a suitable environment for ticks to continue their existence (Dumanli et al. 2012). Although the presence and prevalence of TBDs vary among the geographical regions, these diseases affect human and animal health in almost every region of the country (Dumanli et al. 2016; Inci et al. 2016). It has been reported that 19 TBDs, including parasitic, bacterial, and viral, have been detected in humans and animals in Turkey (Inci et al. 2016). Anaplasmosis is one of the important infections affecting human and animal health in Turkey. *A. marginale*, *A. centrale*, *A. bovis*, *A. ovis*, *A. platys*, and *A. phagocytophilum* species that cause anaplasmosis have been mostly reported from different hosts in Turkey, using serological (IFA) and molecular techniques (PCR, nested-PCR, PCR-RFLP, RLB, and DNA sequencing) (Gunes et al. 2011; Dumanli et al. 2012; Altay et al. 2014, 2020; Aktas et al. 2015; Aktas and Özübek 2018; Aktas and Çolak 2021; Aktas and Ozubek 2021); in these studies, two different *A. phagocytophilum*-related strain-like-1 and -2, three *A. marginale* genotypes (B, C, and G genotypes), and 13 *A. ovis* genotypes were detected (Aktas and Özübek 2018; Aktas and Çolak 2021; Aktas and Ozubek 2021; Aktas et al. 2021). But a study showing the presence of *A. capra* is not available in Turkey.

Determining the regional presence and prevalence of new species/genotypes and vectors will contribute to the

understanding of epidemiology of vector-borne diseases such as anaplasmosis, which threatens human and animal health, and to the development and application of control methods worldwide. The aim of the study was to investigate that presence of *A. capra* in domestic ruminants from Turkey by citrate synthase (*gltA*) gene which has great variation between *Ehrlichia* species-specific nested polymerase chain reaction. The phylogenetic position of *A. capra* Turkey isolate among the world isolates was determined.

## Materials and methods

### Study area and blood sampling

Turkey has a subtropical climate and is located on the Eurasian continent divided into seven distinct geographical regions (Eastern Anatolia, Southeastern Anatolia, Mediterranean, Aegean, Marmara, Black Sea, and Central Anatolia) with some of its lands in Europe and Asia. Turkey's geographical location provides a natural bridge for the transmission of many diseases among the continents of Africa, Asia, and Europe (Inci et al. 2016). Sivas is located in the Central Anatolia region and with a surface area of 28,488 km<sup>2</sup>; it is Turkey's second largest province in terms of land. Most of the lands of Sivas enter the Kizilirmak, and some of them enter the Yesilirmak and Firat basins (Anonymous, 2019). Sivas is at the intersection of Central Anatolia, Eastern Anatolia, and Black Sea regions (Fig. 1). While these features add richness and diversity to Sivas in terms of vegetation, they provide a suitable environment for the existence of different vectors in the different areas of the province (Dumanli et al. 2012; Inci et al. 2016). This study was conducted on



**Fig. 1** Location of Turkey and Sivas province

cattle, sheep, and goats from Sivas (Center, Zara, Yildizeli, Koyulhisar, and Ulas). A total of 468 blood samples (241 cattle, 155 sheep, and 72 goat) were taken into collection tubes with EDTA. Randomly selected animals were clinically healthy and grazing on pasturelands during sampling periods. The animals were aged older than 1. The blood samples were stored at  $-20^{\circ}\text{C}$  until DNA extraction.

### DNA extraction and *Anaplasma capra-gltA*-nested PCR

Total genomic DNA was obtained from EDTA-treated blood samples using PureLink Genomic DNA kit (Cat. No.: K1820-02, Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. Positive (*A. capra*-positive cattle blood sample, Accession number: MW672115) and negative (DNase-RNase-free sterile water, Cat No.: 129114, Qiagen®, Germany) controls were used to control false positive or negative results during the DNA extraction and nested PCR. The extracted total genomic DNA sample was diluted with 200  $\mu\text{L}$  DNA elution buffer and was stored at  $-20^{\circ}\text{C}$ , until using PCR.

To survey the presence of *A. capra* in cattle, sheep, and goats from Turkey, the DNA of 468 blood samples were screened for the *gltA* gene by nested PCR, with the primers listed in Table 1. The PCR conditions were performed as described by Li et al. (2015) and Yang et al. (2016). PCR products were stained with ethidium bromide following electrophoresis in 1.5% agarose gel. The results visualized by UV transillumination. To avoid the risk of contamination, DNA extraction, PCR, and gel electrophoresis were performed in different compartments.

### Sequencing of the *gltA* gene and phylogenetic analyses

The nested PCR products of all positive samples were purified from agarose gel using PCR Clean-Up & Gel Extraction Kit (GeneDireX®, Cat.No.: NA006-0300), according to the manufacturer's instructions. Sequencing in both directions was performed using ABI 3730XL analyzer (Applied Biosystems, Foster City, CA) using BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Foster City,

CA). Nested PCR inner primer pairs listed Table 1 used for sequencing of *A. capra* the *gltA* gene.

The consensus sequences were obtained using the MUSCLE algorithm (Edgar 2004) in MEGA-X software (Kumar et al. 2018), and compared for similarity with published sequences in GenBank by a BLAST search. The sequences done in this study have been submitted and deposited in the GenBank database. Phylogenetic analyses of the *gltA* gene of *A. capra*-positive samples detected in this study and the other *Anaplasma* species DNA sequences obtained from GenBank were carried out (Fig. 2). Phylogenetic trees were constructed using maximum likelihood analysis (ML) in Mega X (Kumar et al. 2018). The best-fit model for ML was evaluated as the Kimura-2 parameter model (Kimura 1980) using the Find Best-Fit Substitution Model in Mega X (Kumar et al. 2018). Bootstrap values were performed with 1000 replicates (Fig. 2).

### Ethics statement

The ethics committee report including permissions were obtained from the Sivas Cumhuriyet University Animal Experiments Local Ethics Committee (Approval number: 65202830-050.04.04-573).

### Results

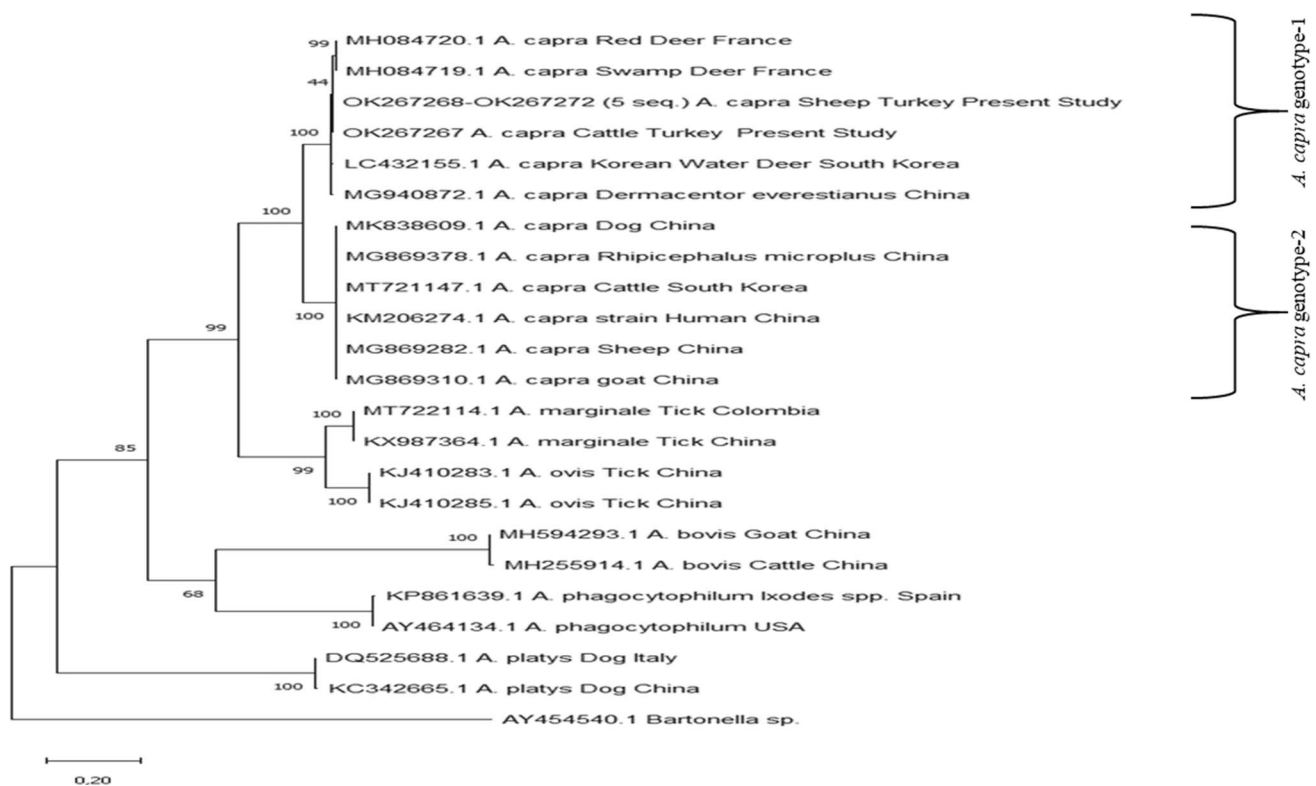
A total of 468 blood samples were analyzed with nested PCR to investigate the presence of *A. capra* in Turkey. In the analysis of the blood samples for the determination of the *A. capra*, positive results were obtained in a total of six samples (1.28%). The positive rate was 0.41% (1/241) in cattle and 3.22% (5/155) in sheep, respectively. No positive result was detected in goats (Table 2).

The six positive *A. capra*-nested PCR products were sequenced and aligned with *A. capra* sequences present in the GenBank and deposited in the GenBank. The GenBank accession numbers of *A. capra gltA* gene detected in this study are as follows: OK267267 (cattle) and OK267268-OK267272 (sheep).

BLAST analysis showed that DNA sequence of *A. capra* obtained in this study was found to have 88.39–99.44% similarity to the isolates of *A. capra* obtained from different host

**Table 1** Primers used in the amplification of the *Anaplasma capra* the *gltA* gene

Target gene	Primer name	Primer sequence (5'-3')	Amplicon size (bp)	Reference
<i>gltA</i>	Outer-f	GCGATTTTAGAGTGYGGAGATTG	1031	Li et al. 2015
	Outer-r	TACAATACCGGAGTAAAAGTCAA		
	Inner-f	TCATCTCCTGTTGCACGGTGCCC	594	Yang et al. 2016
	Inner-r	CTCTGAATGAACATGCCACCCCT		



**Fig. 2** Phylogenetic tree based on the *gltA* sequences of *A. capra* (OK267267-OK267272) using the maximum likelihood method. Numbers at the nodes represent the bootstrap values with 1000 replicates. The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model (Kimura

1980). Scale bar represents 0.20 substitutions per nucleotide position. *Bartonella* sp. (Accession number: AY454540) was used as an out-group in the tree. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018)

**Table 2** *Anaplasma capra* the *gltA* gene-specific nested PCR results of blood samples from cattle, sheep and goats in Turkey

Animal species	Number of animals	Number of Positive (%)	Genotype*
Cattle	241	1 (0.41)	<i>A. capra</i> genotype-1
Sheep	155	5 (3.22)	<i>A. capra</i> genotype-1
Goat	72	-	
Total	468	6 (1.28)	

\*Genotypes were determined according to DNA sequences of the *gltA* gene of *A. capra* isolate obtained from different hosts present in GenBank and obtained from the current study

species in France, South Korea, and China. The *A. capra* DNA sequences detected in this study and *A. capra* isolates present in GenBank were aligned. Our DNA sequences results of *A. capra gltA* gene have high nucleotide similarity with *A. capra* isolates identified in red deer and swamp deer (France), Korean water deer (South Korea), and *Dermacentor everestianus* (China) (Amer et al. 2019; Han et al. 2019; Jouglin et al. 2019). The phylogenetic analysis of our *A. capra* DNA sequencing result with *A. capra* isolates which are obtained from different hosts present in GenBank

revealed 59 nucleotide differences in the position of the same nucleotide of the *gltA* gene of *A. capra* (Table 3). For this reason, we thought that at least two different *A. capra* genotypes circulates in the world. According to the *gltA* gene sequences result, *A. capra* isolates obtained from sheep and cattle in the present study (Accession numbers: OK267267-OK267272), red deer and swamp deer in France (Accession numbers: MH084719- MH084720), Korean water deer in South Korea (Accession number: LC432155), and *Dermacentor everestianus* in China (Accession number: MG940872) could be named *A. capra* genotype-1, thanks to nucleotide similarity in the same nucleotide positions compared with other *A. capra* isolate (Amer et al. 2019; Han et al. 2019; Jouglin et al. 2019). *A. capra* isolates identified from dog in China (Accession number: MK838609), *Rhipicephalus microplus* in China (Accession number: MG869378), cattle in South Korea (Accession number: MT721147), human in China (Accession number: KM206274), sheep in China (Accession number: MG869282), and goat in China (Accession number: MG869310) could be named *A. capra* genotype-2 due to nucleotide differences in the same nucleotide positions compared with the *A. capra* genotype-1 of the *gltA* gene sequences (Li et al. 2015; Guo et al. 2018; Shi et al.





our results are important in terms of showing that *A. capra* could be a worldwide species. Additionally, in this study, all samples were collected from clinically healthy animals. The certain clinic form in animals of *A. capra* is unclear, and subclinical anaplasmosis persisting more than 3 years may be the lifetime in animals, and the persisted infected animals may serve as reservoirs to vectors (Kocan et al. 2010; Brown and Barbet 2016). With this result, it can be concluded that new perspectives should be developed in the evaluations to be made in terms of anaplasmosis in animals.

The *16S SSU rRNA*, *gltA*, *groEL*, and *msp4* genes have been used for detection and description of *A. capra* isolates from other *Ehrlichia* species (Peng et al. 2018, 2020; Yang et al. 2018a, b; Shi et al. 2019; Wei et al. 2020; Altay et al. 2022). The *gltA* gene has a great genetic variation and may be preferred for both phylogenetic analysis and identification research of *Anaplasma* species (Inokuma et al. 2001). In this study, we used the *gltA* gene for phylogenetic analysis and identification of *A. capra* in Turkey. A total of six *A. capra* *gltA* gene partial sequences, one of which from cattle and the others from sheep, were obtained. The DNA sequence results we obtained for *A. capra* agreed with those for *A. capra* obtained from different host species with 88.39–99.44% similarity. Our samples showed high nucleotide sequence similarity with *A. capra*, which were detected in red deer and swamp deer from France (Jouglin et al. 2019), Korean water deer from South Korea (Amer et al. 2021), and *Dermacentor everestianus* from China (Han et al. 2019). But according to alignment results of the *gltA* gene sequences, nucleotide differences were detected between our and the abovementioned *A. capra* isolates and *A. capra* isolates obtained from dogs, cattle, sheep, goats, humans, and tick (Li et al. 2015; Guo et al. 2018; Shi et al. 2019; Miranda et al. 2021) in the same nucleotide positions of the *gltA* gene (Table 3). Moreover, the phylogenetic tree also revealed that *A. capra* isolates were gathered into two groups among themselves (Fig. 2). Therefore, we assert that at least two different *A. capra* genotypes (*A. capra* genotype-1 and *A. capra* genotype-2) have circulated among suitable host species.

In conclusion, *A. capra* was reported for the first time in Turkey using molecular identification techniques with the present study. This species is known to be human pathogenic novel *Anaplasma* genotype and can cause severe disease symptoms in humans. But there is paucity of information about distribution, genetic diversity, suitable vectors species, and host specificity of *A. capra* in the world. For this reason, the determination of distribution, genetic diversity, vector, and host specificity of *A. capra* isolates is important to protect both human and animal health and to constitute control strategies against the pathogen.

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**Author contribution** Kursat Altay (DVM, PhD, Prof.): Conceptualization, field work, methodology, validation, formal analysis, supervision, writing—original draft preparation, reviewing, and editing

Ufuk EROL (DVM, PhD, Assist. Prof.): Conceptualization, field work, methodology, validation, data curation, formal analysis, writing—original draft preparation

Omer Faruk SAHİN (DVM, Res. Assist.): Field work, data curation, methodology, formal analysis

**Data availability** All data generated or analyzed during this study are included in this manuscript.

**Code availability** Not applicable.

## Declarations

**Ethics approval** All procedures performed in studies involving animals were in accordance with the ethical standards approved by the Sivas Cumhuriyet University Animal Experiments Local Ethics Committee (Approval number: 65202830–050.04.04–573).

**Consent to participate** The consent of all animal owners was sorted before this study was carried out.

**Consent for publication** All authors have seen and approved the final version of the manuscript being submitted. They warrant that the article is the authors' original work, has not received prior publication, and is not under consideration for publication elsewhere.

**Conflict of interest** The authors declare no competing interests.

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