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RESEARCH

The detection and phylogenetic analysis of *Anaplasma phagocytophilum*-like 1, *A. ovis* and *A. capra* in sheep: *A. capra* divides into two genogroups

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Abstract

In this study, the presence, prevalence, and genotypes of *Anaplasma phagocytophilum, A. ovis*, and *A. capra* in sheep were investigated based on 16 S SSU rRNA, *groEL*, and *gtlA* gene-specific polymerase chain reaction (PCR), respectively. The sequences of the genes were used for detection of the phylogenetic position of the species. Additionally, a restriction fragment length polymorphism (RFLP) were carried out for discrimination of *A. phagocytophilum* and related variants (*A. phagocytophilum*-like 1 and 2). The prevalence of *Anaplasma* spp. was found as 25.8% (101/391), while it was found that *A. ovis*, *A. phagocytophilum*-like 1, and *A. capra* are circulating in the sheep herds in Kyrgyzstan, according to the PCRs, RFLP and the partial DNA sequencing results. The positivity rates of *A. phagocytophilum*-like 1, *A. ovis*, and *A. capra* genotype-1 were 6.9, 22.5, and 5.3%, respectively. A total of 32 (8.2%) sheep were found to be mix infected. Moreover, phylogenetic analyses and sequence comparison with those available in the GenBank showed that *A. capra* formed two distinct genetic groups (*A. capra* genotype-1 and *A. capra* genotype-2). Considering the zoonotic potential of these species, it may be necessary to make changes in the interpretation of anaplasmosis cases in animals and there is a need for further studies to determine the pathogenicity of the species/genotypes circulating in animals.

Keywords *Anaplasma species* · *Anaplasma capra* genotypes · 16S SSU rRNA · *groEL* · *gtlA* · Sheep

Introduction

Anaplasmosis is one of the emerging-tick borne diseases, and the disease affects both human and animal health. The genus *Anaplasma* (order Rickettsiales, family Anaplasmataceae) includes the species of *A. marginale, A. centrale, A. bovis, A. platys, A. ovis, A. capra* and *A. phagocytophilum*, the last three of which cause infection in sheep (Friedhoff 1997; Dumler et al. 2001; Liu et al. 2012).

Anaplasma capra is a tick-borne pathogen discovered for the first time in China in 2012 (Liu et al. 2012). In Northern China, *Anaplasma* organisms identified from asymptomatic goats considered to be pathogenic in humans and were provisionally named as *Anaplasma capra* in 2015 based on the molecular and phylogenetic data (Li et al. 2015; Liu et al. 2012). The clinical manifestation of the species has not been clarified, however, fever, headache, weakness, dizziness, myalgia, chills, rash, eschar, lymphadenopathy, gastrointestinal symptoms, and neck stiffness were observed in humans (Li et al. 2015). After the first detection of *A. capra* in goats in China, its presence has been detected in goats in seven other countries, such as France, Iran, South Korea,

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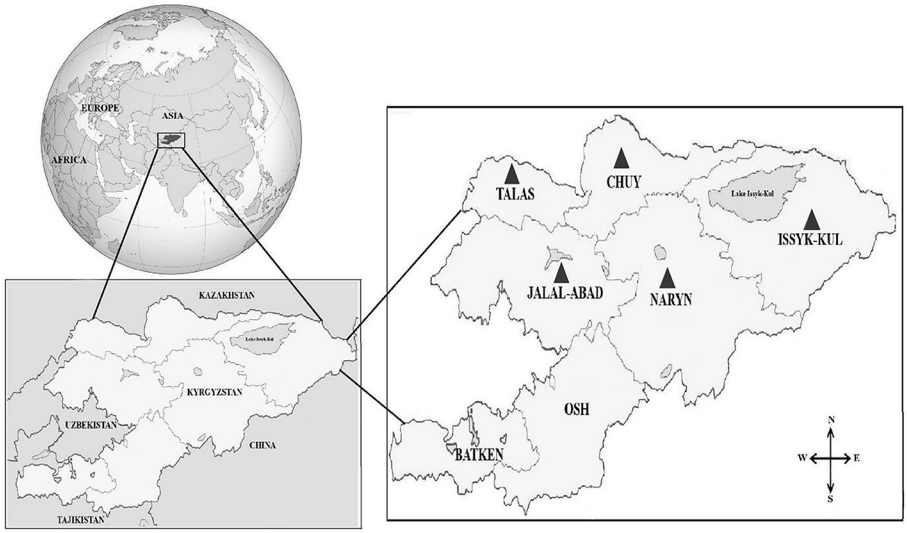

Fig. 1 Location and regions of Kyrgyzstan. Sampling regions were shown with triangles

Kyrgyzstan, Malaysia, Spain, and Türkiye (Koh et al. 2018; Jouglin et al. 2019; Wei et al. 2020; Miranda et al. 2021; Staji et al. 2021; Altay et al. 2022a, b; Remesar et al. 2022). The novel species has been detected in humans, sheep, cattle, dog, wild animals (e.g. Korean water deer (*Hydropotes inermis argyropus*), forest musk deer (*Moschus berezovskii*), takin (*Budorcas taxicolor*), Persian onegar *(Equus hemionus onager*), Reeves's muntjacs (*Muntiacus reevesi*), serows (*Capricornis crispus*), and ixodid tick species such as *Ixodes persulcatus*, *Dermacentor everestianus, Haemaphysalis longicornis*, *H. qinghaiensis*, and *Rhipicephalus microplus* (Li et al. 2015; Fang et al. 2015; Yang et al. 2016; Qin et al. 2018; Guo et al. 2018, 2019; Amer et al. 2019; Han et al. 2019; Lu et al. 2022). Although the existing literature may interpret *A. capra* as a global pathogen, researches that will contribute to the understanding of its epidemiology and genetic diversity are still required, as it is a newly defined species.

Anaplasma phagocytophilum causes human granulocytic anaplasmosis, canine granulocytic anaplasmosis, equine granulocytic anaplasmosis, and tick-borne fever, in humans, dogs, horses, and ruminants, respectively (Karshima et al. 2022). As a result of recent phylogenetic analyses based on sequences of different genes such as 16 S SSU rRNA, *gltA*, and *groEL*, two *A. phagocytophilum*-related variants have been identified in cattle, *Cervus nippon*, and ixodid ticks from Japan, and in *Hyalomma asiaticum* and small ruminants from China. These variants were described as *A. phagocytophilum*-like 1 and *A. phagocytophilum*-like 2, respectively (Ohashi et al. 2005; Kawahara et al. 2006; Jilintai et al. 2009; Yoshimoto et al. 2010; Kang et al. 2014; Yang et al. 2015; Ben Said et al. 2015, 2017).

Anaplasma ovis is the most prevalent *Anaplasma* species of sheep in the world, which also infects goats and wild ruminants (Friedhoff 1997; Dumler et al. 2001). *Anaplasma ovis* is transmitted by *Rhipicephalus bursa* and other ticks in the Old World, while *Dermacentor* species are vectors of *A. ovis* in the western United States (Friedhoff 1997). Although there is some evidence suggesting that *A. ovis* may cause zoonotic infections like *A. phagocytophilum*, these are very limited and need to be clarified. To date, *A. ovis* DNA has only been detected in a symptomatic human patient in Cyprus (Chochlakis et al. 2010) and an asymptomatic person in Iran (Hosseini-Vasoukolaei et al. 2014).

In this study, the presence, prevalence, and genotypes of *A. phagocytophilum, A. ovis*, and *A. capra* were investigated in sheep from Kyrgyzstan based on 16 S SSU rRNA, *groEL*, and *gtlA* gene-specific polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) and sequencing.

Materials and methods

Collection of blood samples and DNA extraction

This study was conducted in five regions (Chuy, Talas, Jalal-Abad, Naryn, Issyk-Kul) of Kyrgyzstan (Fig. 1). Blood samples from sheep were collected between June, 2017 and September, 2018 from 34 sheep flocks. A total of 391 blood samples were taken into collection tubes with EDTA from randomly selected 22 different sheep flocks. Between 9 and 20 blood samples were collected from each flock. The animals were clinically healthy and at least 8 months age sheep and stored at -20 °C, until DNA isolation.

Total genomic DNA was extracted from EDTA-treated blood samples using commercial extraction kit (PureLink Genomic DNA kit, Cat. No.: K1820-02, Invitrogen, Carlsbad, USA), according to the manufacturer's instructions. During the DNA extraction, positive (*A. capra* positive

Table 1 Primers used for amplification of the 16 S SSU rRNA, *groEL*, and *gltA* gene of *A. phagocytophilum* and related variants (*A. phagocytohilum*-like 1 and 2), *Anaplasma ovis and Anaplasma capra*, respectively

Target gene	Primer name	Primer sequence (5'-3')	Species	Reference
16 S SSU rRNA	SSAP2f	GCTGAATGT GGGGATAATTTAT	*A.phagocytophilum* and related variants	Kawahara et al. 2006
	SSAP2r	ATGGCTGCTTCCTTT CGGTTA		
groEL	JH0011	TAAAAGCCAAGGAG-GCTGTG	*A.ovis*	Haigh et al. 2008
	JH0012	TTGCTCTCCTCGAC-CGTTAT		
gltA	Outer-f	GCGATTTTAGAGT-GYGGAGATTG	*A.capra*	Li et al. 2015
	Outer-r	TACAATACCGGAGTA-AAAGTCAA		
	Inner-f	TCATCTCCTGTTG-CACGGTGCCC		Yang et al. 2016
	Inner-r	CTCTGAATGAACAT-GCCCACCCT		

sheep blood sample, Accession number: OK267268, Altay et al. 2022b) and negative (DNase-RNase-free sterile water, Cat No.: 129,114, Qiagen®, Germany) samples were used in order to avoid false positive or negative results. Extracted total DNA samples were diluted with 200 µl DNA elution buffer and stored at -20 °C until use.

Polymerase chain reaction (PCR)

In order to investigate the presence of *A. phagocytophilum* and related variants (*A. phagocytophilum*-like 1 and 2), *A. ovis*, and *A. capra* in sheep from Kyrgyzstan, the DNA of 391 blood samples were screened for 16 S SSU rRNA, *groEL*, and *gltA* genes by PCR, respectively. The primers used in this study are listed in Table 1.

The PCR assays were performed as described before (Kawahara et al. 2006; Haigh et al. 2008; Li et al. 2015; Yang et al. 2016), and the genomic DNA of *A. phagocytophilum* (GenBank accession no: JF807995, Altay et al. 2014), A. *ovis* (HE580282, Altay et al. 2014)d *capra* (MW672115, Altay et al. 2022a) were used as the positive controls, and DNase-RNase-free sterile water (Cat No.: 129,114, Qiagen®, Germany) was used as the negative control in the PCRs.

PCR products were loaded on 1.6% agarose gel containing ethidium bromide and visualized under UV transilluminator. The DNA extraction, PCR, and gel electrophoresis were performed in separate compartments of the laboratory to minimize the risk of contamination.

Discrimination *Anaplasma phagocytophilum* and related variants (*A. phagocytohilum*-like 1 and 2) based restriction of 16 S SSU rRNA genes with XcmI and BsaI

A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed to discriminate between *A. phagocytophilum*, *A. phagocytophilum*-like 1 and 2. After a 641/642 bp of the 16 S SSU rRNA gene of *A. phagocytophilum* and/or related variants (like 1 and 2) were amplified with SSAP2f and SSAP2r primers, the PCR products were digested with *Xcm*I and *Bsa*I enzymes as previously described (Ben Said et al. 2017; Aktas and Colak 2021).

The expected RFLP band profiles of *A. phagocytophilum* digested with *Xcm*I are 344 and 297 bp. *Xcm*I does not cut *A. phagocytophilum*-like 1 and 2. On the other hand, the expected RFLP band profiles of *A. phagocytophilum*-like 2 digested with *Bsa*I are 219 and 422/423 bp. *Bsa*I does not cut *A. phagocytophilum* and *A. phagocytophilum*-like 1. In the *A. phagocytophilum*-like 1 and 2 mix infections, band profiles of 219, 422/423 and 641/642 bp are expected in *Bsa*I restriction (Ben Said et al. 2017; Aktas and Colak 2021). The confirmation of RFLP results were carried out with the sequence analysis.

Sequencing and phylogenetic analysis

The 21 of *A. capra*, three of *A. phagocytophilum*-like 1, and two of *A. ovis* PCR positive samples were sequenced. To perform sequence analysis, the PCR products were purified from agarose gel using a commercial gel extraction kit (PCR Clean-Up & Gel Extraction Kit, GeneDireX®, Cat. No.: NA006-0300), according to the manufacturer's recommendations. The SSAP2f/r and the inner primer pairs listed in Table 1 used for sequencing of 16 S SSU rRNA gene of *A. phagocytophlum*-like 1 and *gltA* gene of *A. capra*, respectively. A part of 16 S SSU rRNA gene of *A. ovis* were sequenced using one set of primers (16S8FE and B-GA1B) which is specific 492–498 bp fragment of the 16 S SSU rRNA gene, spanning the V1 region of *Anaplasma* and *Ehrlichia* species (Schouls et al. 1999).

Sequencing was performed using ABI 3730XL analyzer (Applied Biosystems, Foster City, CA) and BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Foster City, CA).

The consensus sequences in this study were determined using the MUSCLE algorithm of MEGA-X software (Kumar et al. 2018). These consensus sequences were compared with sequences present in the GenBank to determine nucleotide similarities with the BLAST algorithm. The

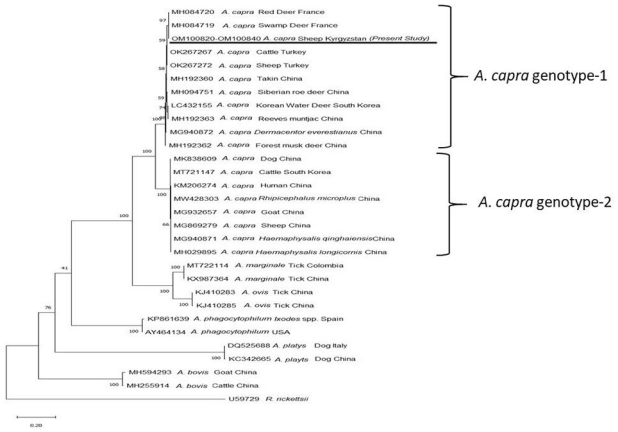

Fig. 2 Phylogenetic tree based on the *gltA* gene sequences of *A. capra* (OM100820-OM100840) using the maximum likelihood method. Numbers at the nodes represent the bootstrap values with 1000 replicates. The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model (Kimura 1980). Scale bar represents 0.20 substitutions per nucleotide position. *Rickettsia ricketsii* (Accession number: U59729) was used as an outgroup in the tree. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018)

Table 2 Distribution and frequency of *Anaplasma* species detected in sheep from Kyrgyzstan (n:391)

	Number of positive samples	*Anaplasma* species		
		***A. phagocytophilum*-like 1**	***A.ovis***	***A.capra***
	3	+	-	-
	62	-	+	-
	4	-	-	+
	15	+	+	-
	8	-	+	+
	6	+	-	+
	3	+	+	+
Total	101 (25.8%)	27 (6.9%)	88 (22.5%)	21 (5.3%)

sequences from this study were submitted to the GenBank database and their accession numbers were obtained.

Phylogenetic analyzes of the sequences identified in this study were performed using other *gltA* and 16 S SSU rRNA nucleotide sequences of *Anaplasma* species available in the GenBank. The phylogenetic tree was carried out with maximum likelihood analysis in Mega X (Kumar et al. 2018). The best-fit model for maximum likelihood was considered as the Kimura-2 parameter model for *gltA* and 16 S SSU rRNA genes (Kimura 1980) using the Find Best-Fit Substitution Model in Mega X (Kumar et al. 2018). Bootstrap values were performed with 1,000 replicates (Fig. 2).

Results

Prevalence and distribution of *Anaplasma* spp. in sheep

The result of PCR and RFLP of 391 samples revealed the presence of *A. phagocytophilum*-like 1, *A. ovis* and *A. capra* in sheep in Kyrgyzstan. The prevalence and frequency of *A. phagocytophilum*-like 1, *A. ovis and A. capra* is shown in Table 2. Overall prevalence of *Anaplasma* species in sheep was found to be 25.8% (101/391) by three different species-specific PCR. The most abundant species was *A. ovis* (88/391, 22.5%) followed by *A. phagocytophilum*-like 1 (27/391, 6.9%) and *A. capra* (21/391, 5.3%). Only one *Anaplasma* species was found in 69 sheep, whereas mixed infections with two or three species were detected in 32 sheep in this study. A total of 15 sheep were infected with both *A. phagocytophilum*-like 1 and *A. ovis*, eight sheep were infected with both *A. ovis* and *A. capra* whereas six sheep were infected with both *A. phagocytophilum*-like 1 and *A. capra* and three sheep were infected with the three species.

Discrimination of *Anaplasma phagocytophilum* and related variants *A. phagocytophilum*-like 1 and 2

In this study, *A. phagocytophilum* or related variants were detected in 27 samples by PCR (Table 2). All of the 27 PCR products were analyzed with RFLP using *Xcm*I and *Bsa*I restriction enzymes. *A. phagocytophilum*-like 1 was detected in all 27 sample by PCR-RFLP, while *A. phagocytophilum* and *A. phagocytophilum*-like 2 were not detected.

To confirm the RFLP results, randomly selected three representative samples were sequenced. These sequences were submitted to the Genbank, and deposited with accession numbers: OM540435-OM540437. The sequences were 99.83–100% similar to *A. phagocytophilum*-like 1 sequences available in the GenBank. The *A. phagocytophilum*-like 1 sequence obtained in this study were 100% identical to those of *A. phagocytophilum*-like 1 detected in sheep from Tunisia (KM285230), cattle from Türkiye (GU223365), goat from China (OL678408) and Sika deer (*Cervus nippon*) from Japan (JM055357).

Analysis of the *gtlA* gene sequences for determination of *A. capra* genotypes

All the positive samples (21 samples) were sequenced and aligned with *A. capra* sequences present in the GenBank and then all the sequences were deposited in the GenBank, as accession numbers: OM100820-OM100840.

Table 3 The homolog rates between *A. capra* sequences obtained from this study and other *A. capra* based the *gltA* gene sequences present in GenBank

Accession number	Host	Country	Homology rates	References
MH084719	Swamp deer	France	100%	Jouglin et al. 2019
MH084720	Red deer	France	100%	Jouglin et al. 2019
MH094751	Siberian roe deer	China	98.70%	Wang et al. 2019
MH192360	Takin	China	98.70%	Yang et al. 2018
MH192362	Forest musk deer	China	98.52%	Yang et al. 2018
MH192363	Reeve's muntjac	China	98.33%	Yang et al. 2018
LC432155	Korean water deer	South Korea	98.56%	Amer et al. 2019
MG940872	*Dermacentor everestianus*	China	98.39%	Han et al. 2019
OK267267	Cattle	Türkiye	99.25%	Altay et al. 2022b
OK267272	Sheep	Türkiye	99.08%	Altay et al. 2022b
MT721147	Cattle	South Korea	86.24%	Miranda et al. 2021
MG932657	Goat	China	86.04%	Peng et al. 2018
KM206274	Human	China	86.03%	Li et al. 2015
MG869279	Sheep	China	86.03%	Guo et al. 2018
MH940871	*Haemaphysalis qinghaiensis*	China	86.03%	Han et al. 2019
MH029895	*Haemaphysalis longicornis*	China	86.03%	Qin et al. 2018
MW428303	*Rhipicephalus microplus*	China	86.03%	Lu et al. 2022
MK838609	Dog	China	86.01%	Shi et al. 2019

The *gltA* gene sequences of 21 positive samples obtained in this study showed a complete consensus. However, BLAST analysis showed that the *gtlA* sequences of *A. capra* obtained in this study were found 86.01–100% similar to the 174 *A. capra* sequences present in the GenBank. There was a high homology (98.33–100%) between sequences obtained in this study and 27 *gltA* sequences of *A. capra* present in the GenBank. In contrast, a low homology was determined (86.01–86.24%) with 147 sequences present in GenBank. Detailed information about nucleotide similarity rates between *A. capra* genotypes was given in Table 3. Additionally, the sequence alignment results showed that only 0–7 nucleotides differences emerged between sequences obtained from the present study and the sequences from red deer, swamp deer (*Rucervus duvaucelii*), Siberian roe deer (*Capreolus pygargus*), takin, Reeve's muntjac, Forest musk deer, *D. everestianus*, Korean water deer, cattle, and sheep, while 68–70 nucleotides differences were observed between the sequences from dog, cattle, sheep, goat, human, *H. qinghaiensis H. longicornis*, and *R. microplus* (Fig. 3).

Phylogenetic analysis

The phylogenetic analysis based on the *gltA* gene revealed that *A. capra* was separated into two clusters, and *A. capra* identified in this study clustered within red deer, swamp deer, Siberian roe deer, takin, Reeve's muntjac, Forest musk deer, *D. everestianus*, Korean water deer, cattle, and sheep (Fig. 2).

Anaplasma phagocytophilum-like 1 variant isolated in the present study clustered a distinct group with those of previously published sequences of *A. phagocytophilum*-like 1 (Fig. 4).

In this study, we also determined a partial sequence of 16 S SSU rRNA gene of *A. ovis* to validate the PCR results. Two sequences of *A. ovis* were deposited in the GenBank under the accession numbers of OM453952 and OM453953. The BLAST and phylogenetic analysis of the sequences showed that the *A. ovis* sequences obtained in this study were in full compliance with the *A. ovis* sequences present in the Genbank (data not shown).

Discussion

Tick-borne diseases such as anaplasmosis have enormous negative effects on the livestock industry almost all over the world (Kocan et al. 2010). The prevalence of TBDs like anaplasmosis may vary according to multiple factors, including sampling seasons, differences in animal feeding and husbandry, presence and abundance of ticks and other vectors, sampling area (especially climatic and ecological factors), host resistance, and sample processing methods (Torina et al. 2008; Kocan et al. 2010; Belkahia et al. 2014). In this study, the overall prevalence of anaplasmosis in sheep was found to be 25.8% (101/391). The prevalence at the species level of *A. ovis*, *A. phagocytophilum*-like 1 and *A. capra* genotype-1 were determined to be 22.5, 6.9 and, 5.3%, respectively.

Anaplasma capra is a newly described species which has zoonotic character and can infect a wide range of hosts. In this study, we investigated the presence and prevalence of *A. capra* in sheep, and genotypes of the species were documented for the first time. The results (5.3%) in this study were compared with other countries, the prevalence of *A. capra* was lower than that previously found in sheep

Fig. 3 Nucleotide differences in the same positions among the *gltA* sequences from *Anaplasma capra* (594 bp)

GenBank Accession Numbers	Host	Nucleotide Positions[a] (170 175 183 185 187 188 189 199 220 226 232 238 256 259 268 271 274 283 288 298 304 312 313 319 334 335 340 349 352 367 370 380 385 395 405 412 424 431 476 479 482 493 517 538 550 553 559 562 568 573 580 583 586 589 592 602 607 620 637 643 652 655 666 667 670 672 691 697 715 721)	References
OM100820-OM100840	Sheep	T C T G T A T G A G C G T C C G C C C T T G T C G G T G C T A A A G C C T T A G G T A T T T T C A C C C A A A T T A A C G G T T T A T A T A	Present study
MH084719	Swamp deer	* *	Jouglin et al. 2019
MH084720	Red deer	* *	Jouglin et al. 2019
OK267272	Sheep	- - - * * G * * * * * * * * * * * * * * C * C * C G	Altay et al. 2022b
OK267267	Cattle	- - - - - - - - * * * * * * * * * * * * C * C * C G	Altay et al. 2022b
MH094751	Siberian roe deer	* * * * * G * * * * * * C * * * * * * * C * C * * * * * * * * * * * T * - -	Wang et al. 2019
MH192360	Takin	* * * * * G * * * * * * * * * * * * * * C * C * * * * * * * * * * * T * - -	Yang et al. 2018
LC432155	Korean water deer	* * * * * G * * * * * * * * * * * * * * C * C * * * * * * * * * * * T * C -	Amer et al. 2019
MH192363	Reeves' muntjac	* * * * * G * * * * * * * * * * * * * * C * C * * * * * * * * * * * T * - -	Yang et al. 2018
MG940872	*D. everestianus*	* * * * * G * * * * * * * * * * * * * * C * C T * * * * * * * * * * T * C G	Han et al. 2019
MH192362	Forest musk deer	* * * * * G * * * * * * * * * * * * * * C * C T * * * * * * * * * * T * G - -	Yang et al. 2018
MK838609	Dog	C T C T C G C T G A G A G T G A A T T C C C C T A A C A T C G G C A T T C C G A A C G C C C C T G T T T T G G G C G G T A T * C C G C C C G	Shi et al. 2019
MT721147	Cattle	C T C T C G C T G A G A G T G A A T T C C C C T A A C A T C G G C A T T C C G A A C G C C C C T G T T T T G G G C G G T A T * C C G C C C G	Miranda et al. 2021
KM206274	Human	C T C T C G C T G A G A G T G A A T T C C C C T A A C A T C G G C A T T C C G A A C G C C C C T G T T T T G G G C G G T A T C C C G C C C G	Li et al. 2015
MW428303	*R. microplus*	C T C T C G C T G A G A G T G A A T T C C C C T A A C A T C G G C A T T C C G A A C G C C C C T G T T T T G G G C G G T A T C C C G C C C G	Lu et al., 2022
MG932657	Goat	C T C T C G C T G A G A G T G A A T T C C C C T A A C A T C G G C A T T C C G A A C G C C C C T G T T T T G G G C G G T A T C C C G C C C G	Peng et al., 2018
MG869279	Sheep	C T C T C G C T G A G A G T G A A T T C C C C T A A C A T C G G C A T T C C G A A C G C C C C T G T T T T G G G C G G T A T C C C G C C C G	Guo et al., 2018
MG940871	*H. qinghaiensis*	C T C T C G C T G A G A G T G A A T T C C C C T A A C A T C G G C A T T C C G A A C G C C C C T G T T T T G G G C G G T A T C C C G C C C G	Han et al. 2019
MH029895	*H. longicornis*	C T C T C G C T G A G A G T G A A T T C C C C T A A C A T C G G C A T T C C G A A C G C C C C T G T T T T G G G C G G T A T C C C G C C C G	Qin et al., 2018

Abbreviations: Nucleotides C, Cytosine; T, Thymine; G, Guanine; A, Adenine. * Asterisks show the conserved nucleotide positions.

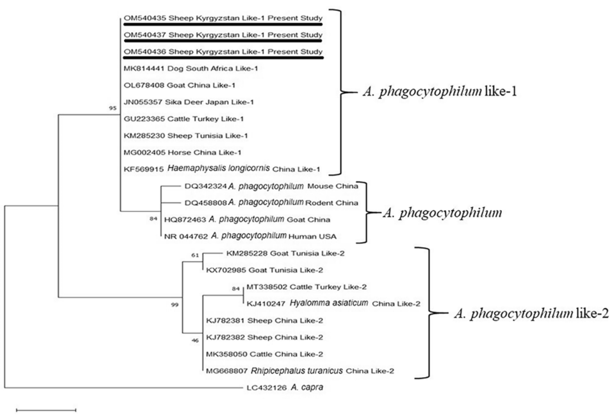

Fig. 4 Phylogenetic tree based on the 16 S SSU rRNA gene sequences of *A. phagocytophilum*- like 1 (OM540435-OM540437) using the maximum likelihood method. Numbers at the nodes represent the bootstrap values with 1000 replicates. The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model (Kimura 1980). Scale bar represents 0.0050 substitutions per nucleotide position. *Anaplasma capra* (Accession number: LC432126) was used as an outgroup in the tree. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018)

(16.3%) and goats (12.3%) from China (Yang et al. 2017), Korean water deer (13.8%) from Korea (Amer et al. 2019), dogs (12.1%) from China (Shi et al. 2019). The *A. capra* prevalence determined in this study was higher than in cattle (0.3%) and goats (0.3%) from Korea (Miranda et al. 2021), deer (swamp and red deer) (4.5%) from France (Jouglin et al. 2019), and cattle (0.3%) from Kyrgyzstan (Altay et al. 2022a), but was close to that found in roe deer (5.8%) from Spain (Remesar et al. 2022). This work was the first to reveal the presence of *A. capra* in Kyrgyzstan sheep, and it will contribute to the understanding of the epidemiology of this species in the world. However, further research is needed to determine its vectors and the pathogenicity of the novel *Anaplasma* species. In this study, all samples were collected from clinically healthy animals and no ticks were collected from sheep in the sampling process. The pathogenicity of *A. capra* is not clear among animal host, and a research conducted by Jouglin et al. (2019) showed that *A. capra* can persist in red deer for four months. The persistently infected animals may serve as reservoirs for vectors, and these animals are important in the epidemiology of the pathogens (Kocan et al. 2010; Brown and Barbet 2016). In this study, animals infected with *A. capra* were clinically healthy, and probably these animals were persistently infected with *A. capra*.

With the increase in the number of the hosts in the countries where *A. capra* is detected by molecular studies, the sequence registration rate in the GenBank of this species also increases. Thus, it is possible to compare different *A. capra* samples genetically. In the present study, 21 *A. capra* PCR positive samples were detected by the *gltA* gene sequences. The phylogenetic and BLAST analyses, including the *A. capra* sequences identified in this study and sequences present in the GenBank revealed that *A. capra* is divided into two different geno-groups (*A. capra* genotype-1 and *A. capra* genotype-2) (Figs. 2 and 3). A relationship between these geno-groups, the host, or the region from which they were isolated, could not be determined. While the similarity rates of 27 *A. capra* samples in the first group and sequences obtained in this study were 98.33–100%, the 147 *A. capra* sequences in the second group differ significantly from this group and the similarity rate decreases to 86.01–86.24%. Although the difference between the two groups was significant, the homology within the groups was quite high (Table 3). *A. capra* genotype-1 and *A. capra* genotype-2 are clearly distinguished from each other according to the *gltA* gene sequences compared to other gene sequences such as 16 S SSU rRNA and *groEL* (Unpublished data). We think that the naming of these two groups can be

used until we reach research results that will provide a further nomenclature.

Recently based molecular studies has documented that *A. phagocytophilum* consists of one species and two related variants (*A. phagocytophilum*-like 1 and 2) (Ben Said et al. 2015, 2017). According to the results of PCR, RFLP and DNA sequence in this study, *A. phagocytophilum*-like 1 was found in 27 samples (6.9%). The prevalence was close to that reported in Tunisian sheep (7%) (Ben Said et al. 2017), but lower than that reported in small ruminants from Türkiye (26.5%) (Aktas et al. 2021). The phylogenetic tree based on 16 S SSU rRNA sequence revealed that samples identified in this study clustered in *A. phagocytophilum*-like 1 group (Fig. 4). Studies in which the presence of *A. phagocytophilum* related variants in farm animals were determined, stated that both variants did not cause clinical symptoms (Ben Said et al. 2015, 2017; Aktas and Colak 2021). In this study, all the animals sampled were clinically healthy, and this result was compatible with the previous studies (Aktas and Colak 2021; Noaman, 2022). When the studies are evaluated together, it can be thought that *A. phagocytophilum* variants do not cause clinical disease in farm animals. However, detailed studies are needed to determine its clinical effect.

Anaplasma ovis is known as the most prevalent *Anaplasma* species in sheep all over the world (Dumler et al. 2001). We also detected that *A. ovis* was the most prevalent species in sheep from Kyrgyzstan (22.5%). When the prevalence studies and the results from this study are evaluated together, it can be seen that *A. ovis* is an endemic species in many countries (Liu et al. 2012; Altay et al. 2014; Belkahia et al. 2014; Noaman and Sazmand 2022). Although *A. ovis* is generally thought to cause mild disease, it has been reported that it causes severe clinical symptoms and even death in the presence of secondary infections or predisposing factors (Friedhoff 1997; Renneker et al. 2013). Therefore, *A. ovis* infection should be taken into consideration more often in sheep flocks.

In conclusion, this study indicated that *Anaplasma* species are widespread in sheep from Kyrgyzstan with having a 25.8% prevalence. The results of this work indicate the presence of *A. phagocytophilum*-like 1, *A. ovis*, and *A. capra* in sheep in Kyrgyzstan for the first time. In the study, we documented that *A. capra* has two different genotypes. We suggest that the naming of these two groups, *A. capra* genotype-1 and *A. capra* genotype-2 can be used until we reach research results that will provide a further nomenclature. All the results show that *Anaplasma* species are important in sheep breeding in Kyrgyzstan, while revealing the necessity of considering genotypes in studies to be carried out on *A. capra*.

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

Code Availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval All procedures performed in studies involving animals were in accordance with the ethical standards approved by Experimentation Ethics Committee of Kyrgyz-Turkish Manas University (30.06.2017/2017–06/02) and the Sivas Cumhuriyet University Animal Experiments Local Ethics Committee (Approval number: 12.07.2021-564).

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.

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