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Molecular Survey of *Anaplasma phagocytophilum* and Related Strains in Sheep and Goats from Sivas; with a High Prevalence of *Anaplasma phagocytophilum*-like 1

Sivas Koyun ve Keçilerinde Anaplasma phagocytophilum ve Suşlarının Moleküler Yöntemlerle Araştırılması; Anaplasma phagocytophilum-like-1'in Yüksek Prevalansı

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ABSTRACT

Objective: This study aimed to investigate *Anaplasma phagocytophilum* and related strains (*A. phagocytophilum*-like 1 and like 2) in sheep and goats for the first time in Sivas province with molecular techniques.

Methods: The study material was composed of 247 animal (159 sheep and 88 goats) blood samples from four districts of Sivas province (Sivas City Center, Kangal, Koyulhisar, and Yıldızeli). *A. phagocytophilum* and related strains were screened with polymerase chain reaction (PCR), PCR-RFLP, and DNA sequence analysis.

Results: *A. phagocytophilum* related strains were found in 44.93% (111/247) of the small ruminants using PCR. The infection rate was 45.91% (73/159) in sheep and 43.18% (38/88) in goats. In this study, 110 samples were positive for only *A. phagocytophilum*-like 1, while *A. phagocytophilum*-like 1 and like 2 were mix-infection in one sample. *A. phagocytophilum* was not detected in sheep or goats. Two randomly selected PCR products were sequenced in both directions, and the consensus sequences were deposited on the GenBank under accession numbers: ON598644 and ON598645. Nucleotide similarity of 99.34-100% was determined between *A. phagocytophilum*-like 1 isolates obtained in this study and those of *A. phagocytophilum*-like 1 isolates present in the GenBank database.

Conclusion: This study provides the first molecular data on *A. phagocytophilum*-like 1 and like 2 in Sivas province. Considering the high positive rate of the *A. phagocytophilum*-like 1 in sheep and goats, there is a paucity of data on clinical symptoms and vector species of the pathogen. Therefore, further studies are needed to investigate the vector tick species and clinical symptoms of the pathogen in the host.

Keywords: *A. phagocytophilum, A. phagocytophilum*-like 1 and 2, sheep, goat, Sivas

ÖZ

Amaç: Bu çalışmada, Sivas ilinde ilk kez moleküler teknikler kullanılarak koyun ve keçilerde *Anaplasma phagocytophilum* ve ilişkili suşların (*A. phagocytophilum*-like 1 ve like 2) araştırılması amaçlanmıştır.

Yöntemler: Çalışma materyali olarak Sivas ilinin dört farklı ilçesinde (Sivas şehir merkezi, Kangal, Koyulhisar ve Yıldızeli) 247 hayvana (159 koyun ve 88 keçi) ait kan örneği kullanılmıştır. Çalışmada *A. phagocytophilum* ve ilişkili suşlar polimeraz zincir reaksiyonu (PCR), PCR-RFLP ve DNA dizi analizi kullanılarak araştırılmıştır.

Bulgular: Çalışmada küçük ruminantların %44,93'ünün *A. phagocytophilum* ve ilişkili suşlar ile enfekte olduğu PCR ile belirlendi. Koyun örneklerindeki enfeksiyon oranı %45,91 (73/159) iken keçi örneklerinde ise %43,18 (38/88) idi. Bu çalışmada 110 örneğin sadece *A. phagocytophilum*-like 1 ile enfekte olduğu tespit edilirken, bir örnekte ise *A. phagocytophilum*-like 1 ve like 2 tespit edildi. Koyun ve keçi örneklerinde *A. phagocytophilum* tespit edilmedi. Tesadüfi olarak seçilen iki örneğin DNA dizi analizi yapıldı ve elde edilen konsensus sekanslar GenBank'a ON598644 ve ON598645 erişim numaraları ile yüklendi. Çalışmada tespit edilen *A. phagocytophilum*-like 1 izolatları GenBank'ta bulunan *A. phagocytophilum*-like 1 izolatlarıyla nükleotid benzerliği yönünden karşılaştırıldı ve %99,34-100 oranında benzerlik tespit edildi.

Sonuç: Bu çalışma, Sivas ilinde *A. phagocytophilum*-like 1 ve like 2'nin varlığı ile ilgili ilk moleküler bilgiyi sunmaktadır. Koyun ve keçi örneklerinde *A. phagocytophilum*-like 1'nin yaygın olarak bulunmasına rağmen patojenin klinik semptomları ve vektörleri

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Türkiye PARAZİTOLOJİ Dergisi

hakkında bilgi eksikliği bulunmaktadır. Bu nedenle bu türlerin klinik semptomlarının ve vektör türlerinin belirlenmesi amacıyla daha ileri çalışmalara ihtiyaç bulunmaktadır.

Anahtar Kelimeler: *A. phagocytophilum, A. phagocytophilum*-like 1 ve 2, koyun, keçi, Sivas

INTRODUCTION

Species in the genus *Anaplasma* (family: Anaplasmataceae, order: Rickettsiales) are rickettsial pathogens, and the genus comprises seven *Anaplasma* species; *Anaplasma ovis* (*A. ovis*), *A. bovis, A. centrale, A. playts, A. phagocytophilum, A. marginale,* and *A. capra* (1-3). These species are obligate intracellular pathogens and invade and proliferate in the host cell of tick and vertebrate hosts (1,4).

Anaplasma species are transmitted to the host via biological and mechanical vectors (1,5,6). While biological transmission occurs by different Ixodid tick species, mechanical transmission can occur via blood-feeding arthropods and blood-contaminated fomites (1,5). Cellular tropism, geographical distribution, host range, vector species, and clinical symptoms may change according to *Anaplasma* species (5,7). *Anaplasma phagocytophilum* invades neutrophil granulocytes of the hosts and the species causes infection in a wide range of vertebrates such as cattle, horses, dogs, sheep, goats, and also humans (1,5,8). Hyperthermia, leukopenia, thrombocytopenia, depression, lack of appetite, reduced milk production, and abortion are associated with *A. phagocytophilum* infection in ruminants (5,8,9).

In recent years, with the increment in the use of molecular diagnostic techniques, studies on the genetic diversity of pathogens, such as *Anaplasma* species, have increased (2,3,6,10-12). The molecular studies revealed that high genetic diversity is present in *A. phagocytophilum* (7,8). Furthermore, two different *Anaplasma* strains, which are genetically related to *A. phagocytophilum* (*A. phagocytophilum*-like 1 and like 2), were documented in different parts of the world on the basis of different gene sequences, such as *16S rRNA, groEL*, and *gltA* (2,10,13,14). *A. phagocytophilum*-like 1 was detected in cattle, sika deer (*Cervus nippon)*, and different tick species from Japan (13,15-17). Recently, in China, another *A. phagocytophilum*-like 2 was identified in *Hyalomma asiaticum* obtained from cattle and sheep (14). To date, *A. phagocytophilum*-like 1 and like 2 have been documented in sheep, goats, cattle, and various tick species from different parts of the world such as Japan, China, Tunisia, South Korea, Italy, Turkey, and Kyrgyzstan (2,10,11,13,18-23).

In Turkey, various *Anaplasma* species, such as *A. phagocytophilum, A. marginale, A. centrale, A. bovis*, *A.ovis*, and *A. capra,* have been well documented in cattle, sheep, goats, and tick species (3,12,24-26). But very few studies are available on *A. phagocytophilum* related strains in Turkey (21,22). In these studies, *A. phagocytophilum*-like 1 was found in sheep, goat, and cattle (21,22) whereas *A. phagocytophilum*-like 2 was detected in cattle in Turkey (21). Climate, geographical features, and vegetation of Sivas province supply an appropriate habitat for vector species such as ticks and blood-sucking flies which are mechanic and biological vectors of *Anaplasma* species to maintain their presence. But there is limited information on the presence and distribution of *Anaplasma* species in Sivas province (3,25,27). According to the literature reviews, there is no data on *A. phagocytophilum* and related strains in sheep and goats in the city. The purpose of this study was to investigate presence of *A. phagocytophilum* and related strains in sheep and goats for the first time in different district of Sivas province using conventional polymerase chain reaction (PCR), PCR-RFLP, and DNA sequencing analysis.

METHODS

Study Area and Samples

Turkey has a unique geographical location that lies between Asia and Europe. This location supplies a natural bridge for transfer of different pathogens between the continents of Europe and Asia (28). Turkey has seven geographic regions, and these regions are named Eastern Anatolia, Southeastern Anatolia, Mediterranean, Aegean, Marmara, Black Sea, and Central Anatolia, respectively (Figure 1). Sivas is the second-largest city in Turkey and is located in the Central Anatolia region. The city lies at the intersection of Central Anatolia, Eastern Anatolia, and the Black Sea regions of Turkey (Figure 1).

The study material was consisted of 159 sheep and 88 goats blood samples from four districts of Sivas province (Sivas City Center, Kangal, Koyulhisar, and Yıldızeli) (Table 1). Sheep and goats sampled in this study were apparently healthy.

Table 1. Prevalence and distribution of *A. phagocytophilum* and related strains according to sampling area

Host	Distinct	No. of tested animals	No. of positive animals (%)
Sheep	Sivas city center	33	17 (51.51%)
	Koyulhisar	35	14 (40.00%)
	Yıldızeli	44	24 (54.54%)
	Kangal	47	18 (38.29%)
Sheep total		159	73 (45.91%)
Goat	Sivas city center	12	8 (66.66%)
	Koyulhisar	25	7 (28.00%)
	Yıldızeli	32	17 (53.12%)
	Kangal	19	6 (31.57%)
Goat total		88	38 (43.18%)

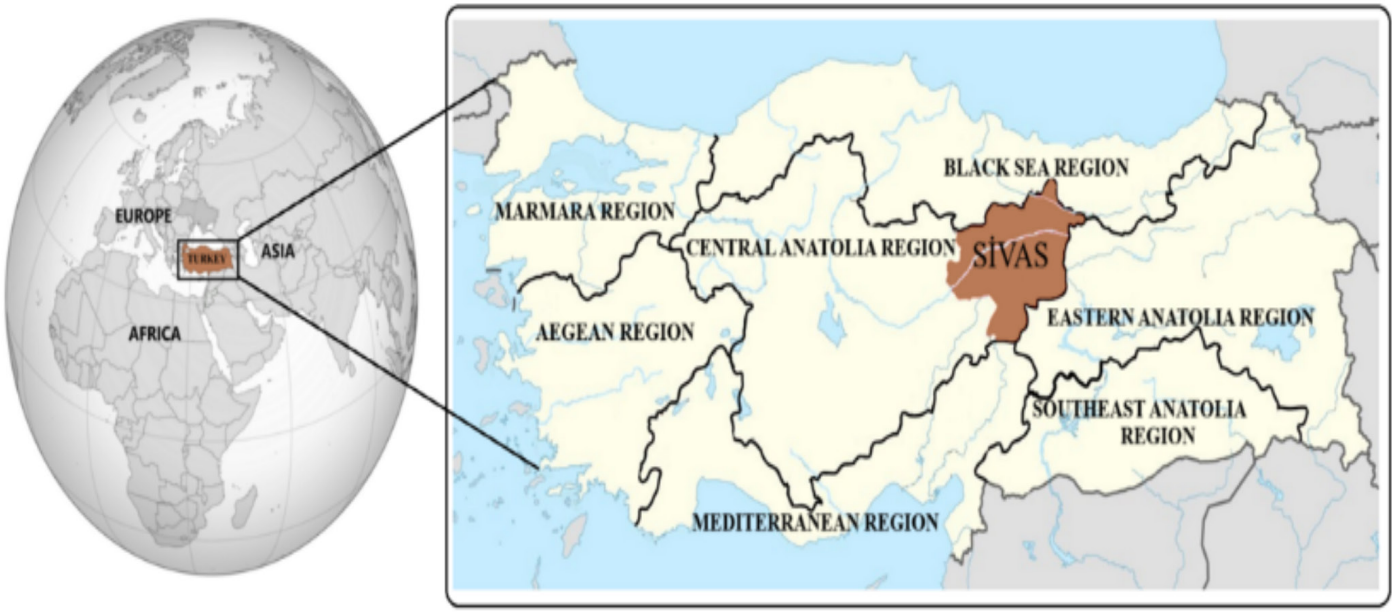

Figure 1. Location of Turkey and Sivas province

Genomic DNA Extraction and Detection of *A. phagocytophilum* and Related Strains (*A. phagocytophilum*-like 1 and 2) with PCR Analyses

Genomic DNA was extracted from the EDTA-treated blood samples using a PureLink Genomic DNA kit (Cat. no.: K1820-02, Invitrogen, Carlsbad, USA) following the manufacturer's instructions. DNA of *A. phagocytophilum* and related strains (*A. phagocytophilum*-like 1 and 2) were screened using forward SSAP2F and reverse SSAP2R primers amplifying 641-642 bp parts of the *16S rRNA* gene in PCR (29). PCR was performed in a final volume of 25 µL, including DNase-RNase-free sterile water (Cat no.: 129114, Qiagen®, Germany), 10×PCR buffer (Thermo Scientific™, Lithuanian), $MgCl_2$ (25 mM) (Thermo Scientific™, Lithuanian), 200 µM of each dNTP (Cat.No.: R0181, Thermo Scientific™, Lithuanian), 1.25 U of Taq DNA polymerase (Cat. no.: EP0402, Thermo Scientific™, Lithuanian), 1 µL (10 pmol/µL) of each of the primers, and 2.5 µL template DNA. PCR assay was utilized by described protocol of Kawahara et al. (29). DNase-RNase-free sterile water (Qiagen ®, Germany) and genomic DNA of *A. phagocytophilum* (GenBank accession no: MW672121) were used as negative and positive controls in the PCR assay. The PCR products were screened by electrophoresis in 1.5% agarose gel stained with ethidium bromide at 90 V for 60 minutes. PCR products of positive reactions were stored at -20 °C for PCR-RFLP analysis.

Determination of *A. phagocytophilum* and Related Strains (like 1 and like 2) with Restriction Fragment Length Polymorphism (RFLP)

Anaplasma phagocytophilum and *A. phagocytophilum*-like strains were determined with PCR-RFLP based on *16S rRNA* following the described protocol of Ben Said et al. (10). Briefly, target amplicons were digested with XcmI (New England Biolabs®, UK) and BsaI (New England Biolabs®, UK) restriction enzymes. The XcmI restriction enzyme was used for discrimination of *A. phagocytophilum* and *A. phagocytophilum*-related strains. The XcmI enzyme digests the *A. phagocytophilum,* and the expected band profiles in this process are 297 and 344 bp, but the enzyme does not cut *A. phagocytophilum* related strains. The BsaI restriction enzyme was used to discriminate *A. phagocytophilum*-like 1 and like 2. *A. phagocytophilum*-like 2 is digested with BsaI enzyme, and the expected band profiles are 219 and 422/423 bp. In the case of co-infections with *A. phagocytophilum*-like 1 and 2, band profiles of 219, 422/423, and 641/642 bp are expected in BsaI restriction (10,21). Restriction fragments were visualized after the electrophoresis process at 100V for 60 min in 2% agarose gel stained with ethidium bromide.

Sequencing and Phylogenetic Analyses of *A. phagocytophilum*-like 1

To confirm PCR-RLFP result, randomly selected two PCR products were sequenced with SSAP2F and SSAP2R primers (29). Sequencing in both directions was done using an ABI 3730XL analyzer (Applied Biosystems, Foster City, CA) and a BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Foster City, CA).

The sequences were edited and assembled using MEGA-X software (30). Sequences were aligned to additional reference sequences obtained from GenBank using MUSCLE algorithm in MEGA-X software (30). The consensus sequences were compared for similarity to the sequences present in the GenBank database using BLAST. All consensus sequences were deposited to the GenBank and their accession numbers were obtained. The phylogenetic tree was interfered with maximum likelihood analysis in MEGA-X (30). The best-fit model for maximum likelihood was considered as the Kimura-2 parameter model (31) using the Find Best-Fit Substitution Model in MEGA-X (30). Bootstrap values were performed with 1,000 replicates.

Ethics statement: The Sivas Cumhuriyet University Animal Experiments Local Ethics Committee (approval number: 12.07.2021-573).

Statistical Analysis

Statistical analyses among various parameters were performed using the chi-square test. $P \leq 0.05$ was accepted to be statistically significant.

RESULTS

Overall, 111 (44.93%) of the 247 animals were found to be positive for *A. phagocytophilum* and related strains with conventional PCR in different parts of Sivas province. The PCR positivity in sheep and goat samples were 45.91% (73/159) and 43.18% (38/88), respectively. Yıldızeli was the sampling area where *A. phagocytophilum* related strains were most common in sheep samples, while Sivas city center was in goat samples (Table 1). There were no statistically significant differences ($p \geq 0.05$) between sheep and goats, and between sampling areas in terms of *A. phagocytophilum* related strains.

According to PCR-RFLP analysis, 110 samples were positive for only *A. phagocytophilum*-like 1, whereas one sheep sample obtained from Sivas city center was found mix-infected with *A. phagocytophilum*-like 1 and *A. phagocytophilum*-like 2. *A. phagocytophilum* was not found in sheep and goat samples in this study (Figure 2).

To confirm RFLP results, randomly selected two positive PCR products were sequenced and aligned with *A. phagocytophilum* and related strains sequences present in the GenBank. The consensus sequences uploaded to the GenBank under accession numbers ON598644 (*A. phagocytophilum*-like 1 sheep isolate) and ON598645 (*A. phagocytophilum*-like 1 goat isolate). The sequences obtained in this work shared 100% similarities with each other. In addition, these sequences were 99.34-100% similar to *A. phagocytophilum*-like 1 sequences available in GenBank. Our sequences were 100% identical to the goat isolate from Turkey (accession number: JF807994), cattle isolate from Kyrgyzstan (accession number: MW811655), and goat isolate from China (accession number: MN922957). *A. phagocytophilum*-like 2 positive sample was not sequenced due to being co-infected with *A. phagocytophilum*-like 1.

The phylogenetic analysis of *16S rRNA* clustered our samples with *A. phagocytophilum*-like 1 isolates from several countries including Turkey in ML tree. Phylogenetic analyses also revealed close relationship between *A. phagocytophilum*-like 1 and *A. phagocytophilum*-like 2 clusteres and the position of *A. phagocytophilum* cluster as a sister taxon to the *A. phagocytophilum*-like clusters. (Figure 3). Nucleotide differences between *A. phagocytophilum* and *A. phagocytophilum*-related strains (like 1 and like 2) according to partial sequences of the *16S rRNA* gene were shown in Table 2.

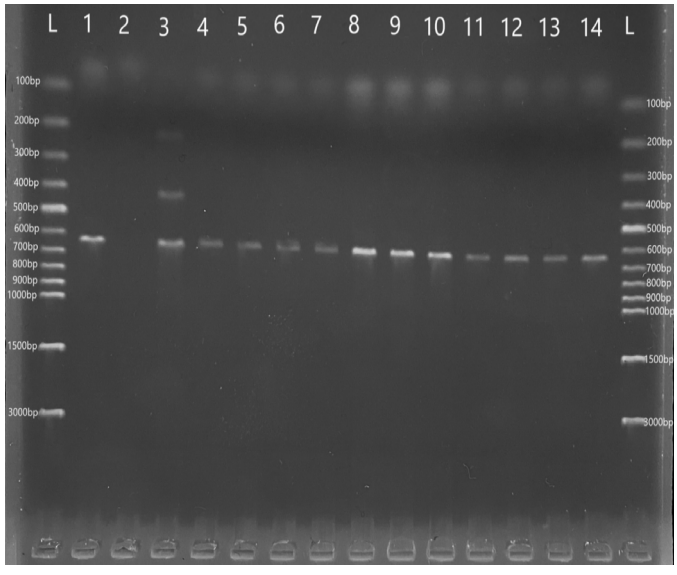

Figure 2. RFLP band profiles of *16S rRNA* PCR positive amplicons digested with restriction enzymes. L. Ladder. 1. *A. phagocytophilum*-like 1 positive control, 2. Negative control, 3. Mixed-infection with *A. phagocytophilum*-like 1 and like 2, 4-14. *A. phagocytophilum*-like 1 positive samples
PCR: Polymerase chain reaction

DISCUSSION

Turkey is one of the important countries for sheep and goat breeding in the world, and approximately 45 million sheep and 11.5 million goats are present in the country. There are 836,673 sheep and 64,359 goats in Sivas and the city is known to be one of the important small ruminant breeding centers in Turkey (38). *A. phagocytophilum* is the etiological agent of tick-borne fever or pasture fever in sheep and goats and the pathogen has world-wide distribution (5,6,8). In Turkey, *A. phagocytophilum* has been detected in sheep and goats (24,26,39,40). Recently, studies have indicated that there are two strains genetically related to the *A. phagocytophilum* (like 1 and like 2), and that these strains circulate in different hosts including sheep and goats in around the world (10,11,18,22). There is a paucity of information on the presence and prevalence of *A. phagocytophilum* related strains in sheep and goats in Turkey. In this study, *A. phagocytophilum* and related strains were researched among sheep and goats in different parts of Sivas province with conventional PCR, PCR-RFLP, and DNA sequencing analysis.

The microscopic, serological, and molecular techniques have been used for the detection of *Anaplasma* species in hosts (1,5). Molecular techniques have been more preferred than other identification techniques because molecular techniques have grater specificity (10,12,25,40). In this study, *A. phagocytophilum* and related strains were investigated with PCR. *A. phagocytophilum* related strains were detected in 44.93% (111/247) of the sheep and goats. In the current study, the infection rates were slightly higher in sheep samples (45.91%) than in goat samples (43.18%). In sheep, the prevalence of *A. phagocytophilum* related strains was higher than the prevalence in Tunisia (7.7%) (18), Kyrgyzstan (6.9%) (unpublished data), Turkey (27.0%) (22), Italy (45.61%) (11), and China (35.1%) (37) and was lower than in Tunisia (100%) (11). The prevalence of these strains in goat samples was lower than in Tunisia (47.5-87.6%) (18,11) and Italy (44.6%) (11) but higher than in Turkey (26.3%) (22) and China (26.4%) (37). The prevalence of the tick-borne pathogens, like *A. phagocytophilum* related strains, may change the management systems of animals (barn or pasture), sampling season, the climate of the sampling areas, age of the animals, and mostly the presence and distribution of tick species in the sampling areas (5,20,41). Moreover, studies have indicated that even the prevalence of tick-borne pathogens in samples collected from several parts of a province may be different (6,11,18,22). In this work, it was determined that *A. phagocytophilum* related strains were most prevalent in Yıldızeli and Sivas city center in sheep and goat samples, respectively (Table 1). We speculated that this result could be related with higher presence and wider distribution of competent tick species, that might act as vectors of *A. phagocytophilum* related strains, in Yıldızeli and Sivas city centers than in other sampling areas.

Table 2. Nucleotide differences among 16S rRNA sequences from *Anaplasma phagacytophilum* and *A. phagocytophilum* related strains

	GenBank accession numbers	Country	Host	Nucleotide positions																			References
				829	841	894	976	997	1095	1097	1099	1106	1124	1134	1223	1225	1226	1228	1245	1254	1277	1392	1402	
***Anaplasma phagacytophilum*-like 1**	ON598644	Turkey	Sheep	A	T	A	G	A	G	A	T	C	G	T	T	T	C	C	G	A	C	G	C	This study
	ON598645	Turkey	Goat	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	This study
	MT338494	Turkey	Cattle	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	21
	JF807994	Turkey	Goat	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	24
	MG869519	China	Sheep	*	*	*	A	*	*	*	-	*	*	C	*	*	*	*	*	*	*	*	*	32
	OL678405	China	Goat	*	*	*	A	*	*	*	-	*	*	C	*	*	*	*	*	*	*	C	*	Unpublished data
	OM540435	Kyrgyzstan	Sheep	*	*	*	*	*	*	*	-	*	*	C	*	*	*	*	*	*	*	*	*	Unpublished data
	MW672121	Kyrgyzstan	Cattle	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	2
	MT881655	Turkey	Sheep	-	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	22
	MN922957	China	Goat	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	Unpublished data
	MZ477262	China	Dog	*	*	*	A	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	Unpublished data
	KM285229	Tunisia	Goat	*	*	C	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	18
Anaplasma phagacytophilum	DQ458805	China	*Niviventer confucianus*	*	*	*	*	*	*	T	A	*	A	*	*	*	*	T	*	*	*	*	T	33
	GQ412337	China	*Apodemus agrarius*	*	*	*	*	*	A	T	A	*	A	*	*	*	*	*	*	*	*	*	*	34
	DQ342324	China	*Apodemus agrarius*	*	*	*	*	*	A	T	A	*	A	*	*	*	*	*	*	*	*	*	*	35
	KC470064	China	*Rattus norvegicus*	*	*	*	*	*	*	T	A	*	A	*	*	*	*	T	*	*	*	*	T	36
	HQ872465	China	Goat	*	*	*	*	*	*	T	A	*	A	*	*	*	*	*	*	G	*	*	*	Unpublished data
***Anaplasma phagacytophilum* like 2 *Anaplasma phagacytophilum*-like**	KM285228	Tunisia	Goat	*	C	*	*	G	*	*	*	T	*	C	C	C	T	*	*	*	T	*	*	18
	MH292917	Tunisia	*Rhipicephalus turanicus*	*	*	*	*	G	*	*	*	T	*	C	C	C	T	*	*	*	T	*	*	20
	MK358050	China	Cattle	*	*	*	*	G	*	*	*	T	*	C	C	C	T	*	A	*	T	*	*	Unpublished data
	MT338504	Turkey	Cattle	*	*	*	*	G	A	*	*	T	*	C	C	C	*	*	A	*	T	*	*	21
	MT338503	Turkey	Cattle	*	*	*	*	G	A	*	*	T	*	C	C	C	*	*	A	*	T	*	*	21
	KX272643	China	Goat	*	*	*	*	G	*	*	*	T	*	C	C	C	*	*	A	*	T	*	*	37
	KX702980	Tunisia	Sheep	*	*	*	*	G	*	*	*	T	*	C	C	C	T	*	A	*	T	*	*	10

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

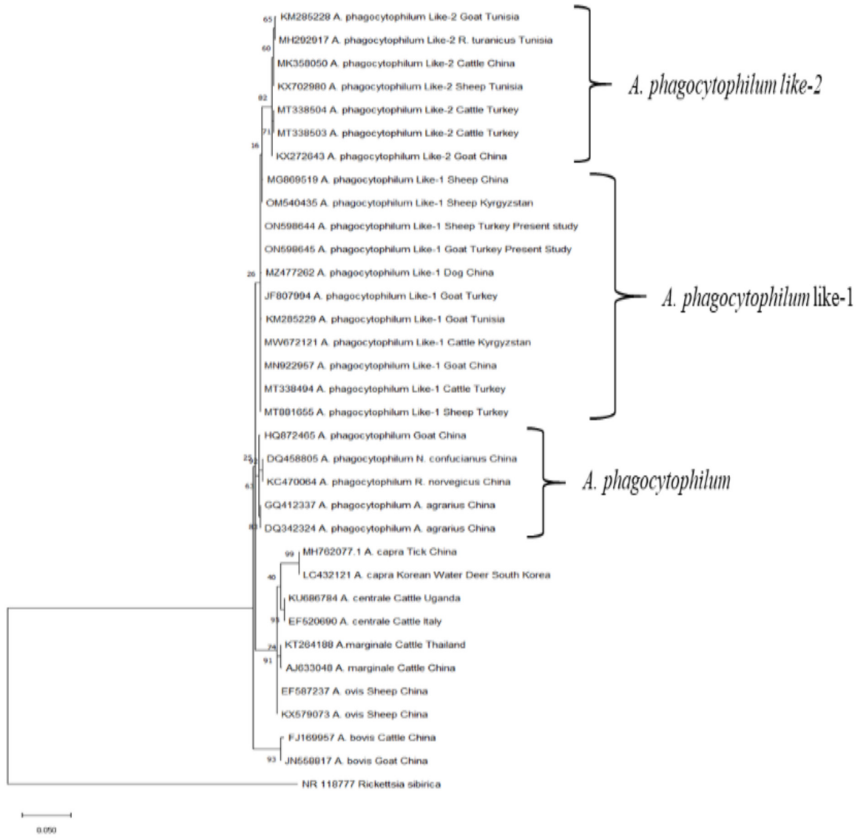

Figure 3. Phylogenetic tree based on *16S rRNA* sequences of *A. phagocytophilum, A. phagocytophilum* related strains, and other *Anaplasma* species using the maximum likelihood method. Numbers at the nodes represent the bootstrap values with 1.000 replicates. The evolutionary history was inferred by using the maximum likelihood method and Kimura 2-parameter model (31). Scale bar represents 0.05 substitutions per nucleotide position. *Rickettsia sibirica* (accession number: NR118777) was used as an outgroup in the tree. Evolutionary analyses were conducted in MEGA-X (30)

Based on PCR-RFLP results, *A. phagocytophilum*-like 1 was found in 110 samples. Mixed infections with *A. phagocytophilum*-like 1 and like 2 were detected in one sheep sample. *A. phagocytophilum* was not found in sheep and goat samples in this study. *A. phagocytophilum*-like 1 and like 2 were evaluated as non-pathogenic species in farm animals because there are no clinical symptoms in infected animals (10,11,18,21). The sheep and goats infected with these strains did not show clinical symptoms associated with anaplasmosis, and this was compatible with previous studies. But it is known that *Anaplasma* species can cause subclinical infections (5,8), and these two strains have been newly discovered and there are lack of information about them (2,10,13,14,17). Therefore, it is thought that experimental studies are needed to investigate the clinical infection of two strains in animals and their economic impact on small ruminant production industries. In addition, it is necessary to investigate the vectors species and the zoonotic potential of these novel strains.

DNA sequence analysis has been used in studies for different objectives, such as confirmation of PCR results, determination of phylogenetic positions and genetic diversity of pathogens, and also the assignation of novel genotypes or species (2,3,10,13,14,22). DNA sequence analysis was used in this study verifying PCR-RFLP results. The consensus sequences, obtained in this study after DNA sequence analysis, shared 99.34-100% nucleotide similarity with the available *A. phagocytophilum*-like 1 sequences in GenBank. Furthermore, our sequences were showed 100% nucleotide similarity with *A. phagocytophilum*-like 1 isolates from Turkey (JF807994), Kyrgyzstan (MW811655), and China (MN922957). The phylogenetic tree based on partial sequences of *16S rRNA* gene also indicated that our sample clustered in *A. phagocytophilum*-like 1 group (Figure 3). In addition, the nucleotide differences, which are present in the *16S rRNA* gene between *A. phagocytophilum* and related strains, were also determined in this study (Table 2). According to the nucleotide difference table, *A. phagocytophilum* is genetically more similar to *A. phagocytophilum*-like 1 than *A. phagocytophilum*-like 2.

CONCLUSION

This study provides the first molecular data on the distribution and prevalence of *A. phagocytophilum*-like 1 and like 2 in sheep and goats in Sivas province. *A. phagocytophilum*-like 2 was reported for the first time in sheep for in Turkey with the current study. Furthermore, molecular studies have revealed that genetically related strains with *A. phagocytophilum* have a high prevalence in farm animals. But there is still a lack of information on the clinic manifestation, potential vectors, and zoonotic potential of *A. phagocytophilum* related strains.

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* Ethics

Ethics Committee Approval: The Sivas Cumhuriyet University Animal Experiments Local Ethics Committee (approval number: 12.07.2021-573).

Informed Consent: In the study, with the permission of the animal owners, samples were collected and used in the study.

Peer-review: Internally peer-reviewed.

* Authorship Contributions

Concept: U.E., K.A., Design: U.E., K.A., Data Collection or Processing: U.E., Ö.F.Ş., K.A., Analysis or Interpretation: U.E., Ö.F.Ş., K.A., Literature Search: U.E., Ö.F.Ş., K.A., Writing: U.E., Ö.F.Ş., K.A.

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