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Research Article

Survey of *Anaplasma* spp. in Ticks from Türkiye: First Molecular Evidence for *A. phagocytophilum*-like-1 and 2 Strains

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Abstract

In Türkiye, although there are several studies on *Anaplasma* spp. in vertebrate host, data on the occurrence of *Anaplasma* spp. in ticks are still lacking. This study aims to contribute to control strategies by providing new information on the epidemiology of *Anaplasma* species in Türkiye. A total of 2241 ticks divided into pools in which the presence of *A. ovis* and *A. phagocytophilum* was investigated by molecular methods. Maximum likelihood estimate (MLE) per 1000 ticks with 95% confidence intervals (CI) was used to compute infection rates. Overall MLE of infection rate was determined as 34.1 and 3.61 for *A. ovis* and *A. phagocytophilum*, respectively. While the infection rate for *A. ovis* varied from 9.21 (CI 2.81-21.3) in *Haemaphysalis parva* to 81 (CI 53.8-115) in *Dermacentor marginatus*, *A. phagocytophilum* varied from 2.21 (CI 0.11- 9.51) in *Rhipicephalus bursa* to 95.5 (CI 5.61-359) in *Hae. concinna*. *A. phagocytophilum*-like-1 and 2 strains in Turkish ticks were originally identified using sequencing and phylogenetic analysis based on the 16S rRNA gene. As a result, *A. ovis* and *A. phagocytophilum* could threat animal and human health in the region and strains of *A. phagocytophilum* should be taken into account when making a differential diagnosis for tick-borne diseases.

Keywords: *Anaplasma ovis*, *Anaplasma phagocytophilum* like 1 and 2, PCR, tick, Türkiye.

Introduction

Ticks are significant carriers of numerous diseases that affect both humans and animals, including Türkiye due to suitable climatic conditions and a large-variety animal population. Both domestic and wild animals are significantly impacted by tick-borne diseases (TBDs). Additionally, TBDs threaten human health especially in tropical and subtropical climatic regions including Türkiye [1]. Babesiosis, theileriosis, and anaplasmosis are the three most significant tick-borne illnesses that are known to be endemic in Türkiye [2,3]. Türkiye has a great potential for animal breeding and livestock population comprise about 17 million cattle, 171 thousand water buffalo, 45 million sheep and 12 million goats by year of 2023 according to the Turkish Statistical Institute *(http://www.tuik.gov.tr)*. However, the country's climate makes it a good place for many tick species to maintain their biological diversity [3]. The combination of a high tick species diversity, high livestock and wild animals' populations rise the frequency of TBDs in the country. In addition, the close relationship between animal and human habitats in some parts of Türkiye could increase the risk of human TBDs transmission [3-5].

An important group of tick-borne agents are *Anaplasma* species [6]. *Anaplasma phagocytophilum*, *A. centrale*, *A. marginale*, *A. bovis*, *A. ovis*, *A. platys*, and *A. capra* are all members of the genus *Anaplasma* [7,8]. *Anaplasma ovis* and *A. phagocytophilum* are well known *Anaplasma* species infecting small ruminants. Although *A. ovis* DNA has been detected in one symptomatic human patient in Cyprus [9] and in an asymptomatic person in Iran [10] it is not yet considered a zoonotic as *A. phagocytophilum*. Currently it is considered an important pathogen in small ruminants [11] that causes clinical signs in animals [12] due to some predisposing factors. Several tick species are reported to transmit *A. ovis* including *Rhipicephalus bursa*, *Haemaphysalis sulcata* [13] and *Dermacentor andersoni* [11]. *Anaplasma phagocytophilum* is a zoonotic gram-negative intracellular bacterium transmitted by *Ixodes* spp. ticks. Although it can infect a variety of domestic and wild species, only humans, domestic ruminants, horses, cats, and dogs have been shown to develop clinical infection

named tick-borne fever. The primary risk factors are age, host resistance, and tick contact of the susceptible host after it has left a tick-free area. Main characteristic symptoms are fever, anorexia and loss of weight and yield [7]. In recent years, studies on *A. phagocytophilum* have focused on the genetic diversity of the agent and *A. phagocytophilum*-like 1 and *A. phagocytophilum*-like 2 strains were described in cattle [14,15] small ruminants [4,5,16-20] and ticks [21].

Since TBDs cause significant health and management problems of domestic livestock it is important that an accurate diagnosis and an effective treatment should be performed. Several diagnostic methods can be used to detect and identify *Anaplasma* species in both vectors and hosts. Molecular-based methods have a higher sensitivity and specificity when compared to serological technics and microscopic evaluation of blood smears [14,15].

Several studies have already investigated the prevalence of both *A. ovis* and *A. phagocytophilum* in small ruminants in Türkiye [4,17,18,22]. Recently some molecular studies were conducted to determine *A. phagocytophilum* strains in small ruminants in Türkiye [17,18]. In these studies, *A. phagocytophilum* like-1 isolates were found frequently. Since climatic conditions and animal diversity is suitable for ticks, Black Sea Region was chosen as sampling area. Through species-specific PCR and sequence analysis, this study was conducted to examine *A. ovis* and *A. phagocytophilum* strains in ixodid ticks obtained from sheep and goats in the Black Sea Region of Türkiye.

Materials and Methods

Ethical Statement

Tick samples were collected during a project supported by TUBITAK between 2010-2012 and ethics committee approval was received from the "Firat University Animal Experiments Ethics Committee" (Document No: 16-78, 04.12.2008).

Study Area and Sampling

Black Sea Region of Türkiye constitutes 18% of Türkiye's surface area and represents two different climatic conditions. In general, the region has a humid climate with rainy and a close annual range of temperature in every season. Summers are cool and winters are warm. The mountains in the region prevent passing the humid air to coastal areas. In addition, terrestrial climate features are observed in the interior due to the decrease in the amount of precipitation and the decrease in temperature. Since suitable climatic features, high animal density, managed of animals in the traditional manner and high risk of tick-borne diseases, Black Sea region was selected as sampling area.

Ticks were taken from small ruminants in the Black Sea region of Türkiye's Bolu, Kastamonu, Çorum, Samsun, Tokat, Giresun, and Bayburt provinces over a three-year period *(Fig. 1)* [23]. Out of the 53 locations, a total of 2608 small ruminants (2161 sheep and 447 goats) were screened for the presence of ticks. At least 20-25 animals from each herd were examined for the presence of ticks in the areas under the tail, perineum, scrotum, udder, preputium, inside the ear, under the neck and on the sternum. Ticks were examined under a stereo microscope (Olympus SZX16) and identified according to their morphological characteristics [24].

DNA Extraction and Amplification

The 2241 ixodid ticks were divided into 310 pools *(Table 1)*. The ticks were pooled based on their sex, host, species, province, and degree of blood sucking. Tick counts ranged from 1 to 32 per pool. Each tick pool's total DNA was extracted using a commercial extraction kit in accordance with the manufacturer's instructions (QIAamp DNA Mini Kit, 51306).

The 181 bp region of the 60kDA chaperonin gene (cpn60 or hsp60) was amplified using JH0011

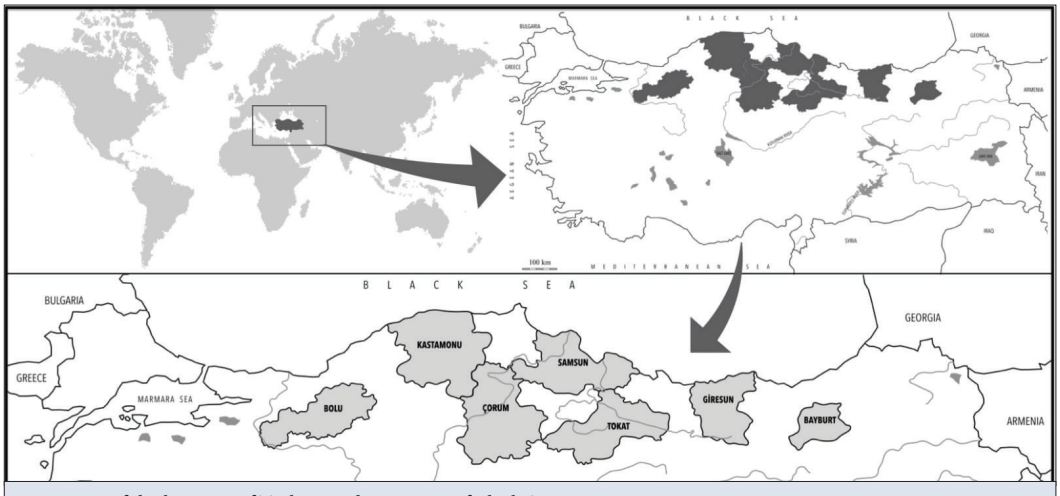

Fig 1. Map of the location of Türkiye and provinces of Black Sea Region

Table 1. *Infection rates for A. ovis and A. phagocytophilum in adult ixodid ticks removed from sheep and goats*

Tick Species	NAT/NAP		Province							Total
			Bolu	Kastamonu	Çorum	Samsun	Tokat	Giresun	Bayburt	NPP, MLE, 95% CI
R. bursa	468/49		1/0/0	99[a]/95[b]/10[c]	24/19/3	187/178/20	68/45/4	132/131/12	0/0/0	9, 20.7, 9.91-37.2
		Ao	-	1[d], 10.6[e], 0.61-46[f]	1, 54.1, 3.11-218	2, 11.5, 1.91-35.1	2, 57.2, 9.61-171	2, 17, 2.81-51.9	-	8, 18.2, 8.31-33.7
		Ap	-	-	-	-	-	1, 7.91, 0.41-34.6	-	1, 2.21, 0.11-9.51
R. turanicus	650/70		94/85/10	1/0/0	337/285/29	178/165/17	113/45/6	78/70/8	0/0/0	30, 58.6, 40.2-81.7
		Ao	2, 26.1, 4.31-78.9	-	15, 71.3, 41.2-113	6, 43.9, 17.5-87.8	2, 52.8, 8.91-157	3, 50.7, 12.7-128	-	28, 53.6, 36.3-75.6
		Ap	-	-	2, 7.11, 1.11-21.9	-	-	1, 15.8, 0.81-68.3	-	3, 4.71, 1.11-12.2
R. sanguineus s.l.	77/6		0/0/0	0/0/0	90/75/5	3/2/1	0/0/0	0/0/0	0/0/0	3, 52.5, 13.1-135
		Ao	-	-	2, 32.3, 5.31-98.1	-	-	-	-	2, 31.2, 5.21-95
		Ap	-	-	1, 14.4, 0.81-62.3	-	-	-	-	1, 14, 0.71-60.5
D. marginatus	367/73		99/83/15	82/77/18	71/63/11	103/93/17	40/16/5	11/6/2	57/30/6	25, 81, 53.8-115
		Ao	1, 12.1, 0.61-52.3	8, 141, 66-251	2, 33.7, 5.61-101	9, 135, 66-236	-	-	5, 339, 123-673	25, 81, 53.8-115
		Ap	-	-	-	-	-	-	-	-
Hae. parva	458/47		153/126/13	6/4/1	73/58/7	156/155/12	54/41/7	0/0/0	190/74/7	4, 9.21, 2.81-21.3
		Ao	-	-	-	4, 30.6, 9.51-70.2	-	-	-	4, 9.21, 2.81-21.3
		Ap	-	-	-	-	-	-	-	-
Hae. punctata	55/13		45/40/8	9/8/3	0/0/0	7/5/1	4/2/1	1/0/0	0/0/0	1, 18.9, 1.01-80.6
		Ao	-	-	-	1, NC	-	-	-	1, 18.9, 1.01-80.6
		Ap	-	-	-	-	-	-	-	-
Hae. sulcata	26/7		13/12/3	2/0/0	0/0/0	4/2/1	16/12/3	4/0/0	0/0/0	1, 41.8, 2.41-173
		Ao	-	-	-	-	-	-	-	-
		Ap	1, 102, 6.01-390	-	-	-	-	-	-	1, 41.8, 2.41-173
Hae. concinna	11/4		5/5/1	1/1/1	2/2/1	4/3/1	0/0/0	1/0/0	2/0/0	1, 95.5, 5.61-359
		Ao	-	-	-	-	-	-	-	-
		Ap	-	-	1, NC	-	-	-	-	1, 95.5, 5.61-359
Hy. marginatum	38/14		10/8/3	16/12/6	10/6/2	1/0/0	12/4/1	13/10/2	0/0/0	-
		Ao	-	-	-	-	-	-	-	-
		Ap	-	-	-	-	-	-	-	-
Hy. excavatum	24/7		0/0/0	2/2/2	25/17/4	0/0/0	5/5/1	1/0/0	0/0/0	1, 41.7, 2.41-171
		Ao	-	-	-	-	-	-	-	-
		Ap	-	1, 500, 38-962	-	-	-	-	-	1, 41.7, 2.41-171
Hy. scupense	9/6		3/3/3	1/1/1	0/0/0	1/0/0	8/5/2	0/0/0	0/0/0	
		Ao	-	-	-	-	-	-	-	-
		Ap	-	-	-	-	-	-	-	-
I. ricinus	58/14		7/6/3	3/2/1	0/0/0	3/1/1	0/0/0	56/49/9	0/0/0	-
		Ao	-	-	-	-	-	-	-	-
		Ap	-	-	-	-	-	-	-	-
Total	2241/310		430/368/59	222/202/42	632/525/62	647/604/71	320/175/30	297/266/33	249/104/13	75, 38.1, 30.1-47.2
		Ao	3, 8.31, 2.01-21.5	9, 49.3, 23.9-87.3	20, 46.1, 28.8-68.8	22, 42.2, 27-62	4, 25.2, 7.81-57.8	5, 20.7, 7.41-44	5, 53.9, 19.6-113	68, 34.1, 26.7-42.8
		Ap	1, 2.71, 0.11-12	1, 5.01, 0.21-21.8	4, 7.81, 2.41-18.1	-	-	2, 7.91, 1.31-24.1	-	8, 3.61, 1.61-6.71

NAT: Number of analyzed tick, NAP: Number of analyzed pool, NPP: Number of positive pool, MLE: Maximum likelihood estimation, CI: Confidence intervals, Ao: Anaplasma ovis, Ap: Anaplasma phagocytophilum, [a] Number of collected tick, [b] Number of analyzed tick, [c] Number of analyzed pool, [d] Number of positive pool, [e] MLE value, [f] CI value, NC: Not calculated (if all pools are positive)

(5'-TAAAAGCCAAGGAGGCTGTG-3') and JH0012 (5'-TTGCTCTCCTCGACCGTTAT-3') primers in order to identify *A. ovis* DNA in ticks [25]. A segment of 492-498 bp in the hypervariable V1 region of the 16SrRNA gene was amplified using primers 16S8FE (5'-GGAATTCAGAGTTGGATCMTGGYTCAG-3')andBGA1B-new (5'-CGGGATCCCGAGTTTGCCGGGRTTYTTCT-3') in order to analyze the sequencing of *A. ovis* [26].

A nested PCR was performed for amplification of *A. phagocytophilum* 16SrRNA gene. Primers EC12A (5′-TGATCCTGGCTCAGAACGAACG-3′) and EC9 (5′-TACCTTGTTACGACTT-3′) were utilized for the initial amplification, which amplifies 1462 bp for all *Anaplasma* and *Ehrlichia* species. Additionally, SSAP2f (5′-GCTGAATGTGGGGGGATAATTTAT-3′) and SSAP2r (5′-ATGGCTGCTTCCTTTCGGTTA-3′) were utilized to amplify 641 bp of *A. phagocytophilum* [27].

Sequencing and Phylogenetic Analyses

Sequence analysing was performed (Macrogen, South Korea) after purification of PCR products by a commercial kit (Qiagen, Hilden, Germany, 28004). The *A. phagocytophilum* (MH636805, MH643970, MH715976, and MT498084-MT498088) and *A. ovis* (MH636802) partial 16S rRNA gene sequences found in this investigation have been added to GenBank. To compare each sequence to the other sequences in the NCBI database *(http://www.ncbi.nlm.nih.gov/nuccore),* BLAST similarity searches were performed. The MAFFT Multiple Sequence Alignment Software Version 7 used the neighbor-joining approach to create a phylogenetic tree from the sequences of the 186 rRNA genes of *Anaplasma* species [28].

Calculation of Infection Rates in Tick Pools

Using the algorithm MLE_IR by Gu et al.[29], the ratio of infected tick numbers in positive tick pools was estimated by calculating the maximum likelihood estimation (MLE) of infection rates with 95% confidence intervals (CI) per 1000 ticks. Because it requires no additional data and produces more accurate results in small pool sizes, the MLE_IR algorithm was chosen.

Results

Prevalence and Distribution of *Anaplasma* spp. by PCR

In all, 2241 ixodid ticks from 12 species and 5 genera were gathered and split up into 310 pools. *Table 1* shows the prevalence of *A. ovis* and *A. phagocytophilum* infections in ixodid ticks by tick species and province. Out of the 310 pools, 75 (23.25%) were found positive to *A. ovis* and/or *A. phagocytophilum*, and the overall MLE of infection rate was 38.1 (CI 30.1-47.2).

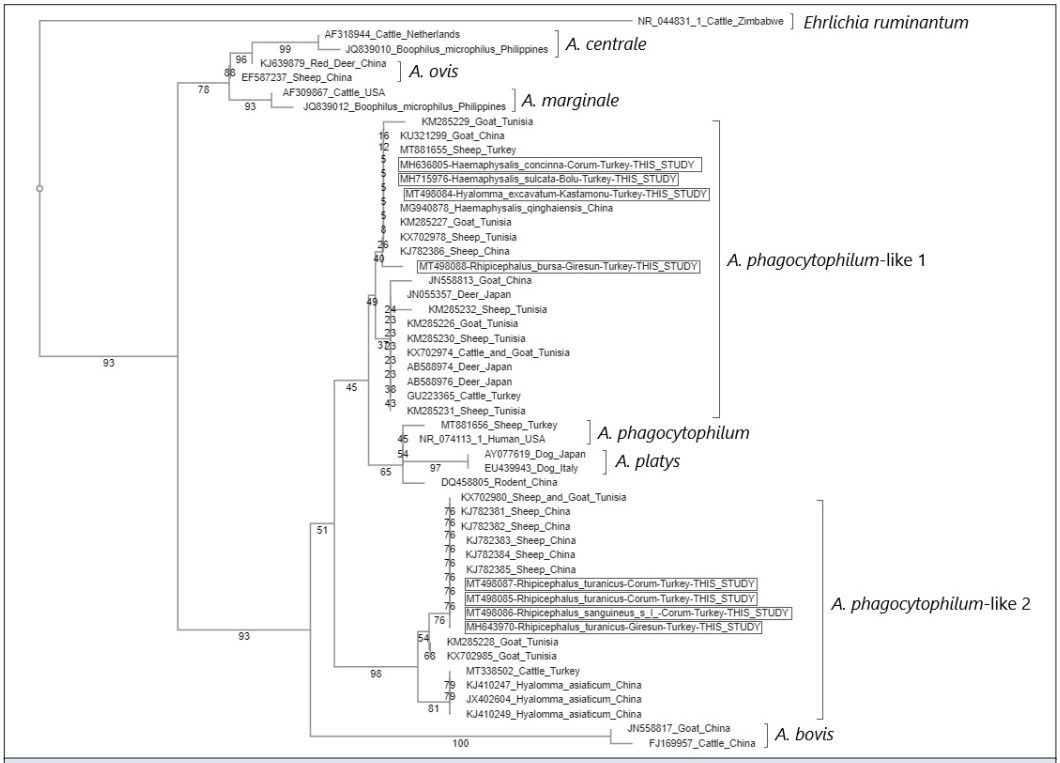

Fig 2. Neighbor-joining analysis of the 16S rRNA gene of the *Anaplasma* species were determined in this study and those present in the GenBank database. Numbers above the branch demonstrate bootstrap support from 1000 replications. The tree was constructed by using the MAFFT Multiple Sequence Alignment Software Version 7. The sequences were given as GenBank accession number, the strain or isolate name, host or vector and country. Sequences described in this study are with outer frame

Table 2. *Homology and genetic distance (%) between 16S rRNA sequences (598–599 bp) from Anaplasma phagocytophilum-like 1 and 2 detected in ticks from Türkiye (*) and other Anaplasma phagocytophilum-like 1 and 2 isolates found in GenBank. The numbers represent the nucleotide identity rates found between the sequences*

Genotypes	**Host or vector**	**Genetic variant**	**Country**	**GenBank**		**Genotype Row**															
						1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
A. phagocytophilum-like 1	*Hae. concinna*	H107	Türkiye	MH636805*	**1**	100	100	100	99.82	99.82	100	100	99.65	98.57	98.57	98.59	98.59	98.59	98.74	98.56	98.41
A. phagocytophilum-like 1	*Hae. sulcata*	H5	Türkiye	MH715976*	**2**		100	100	99.82	99.83	100	100	99.66	98.57	98.57	98.27	98.30	98.32	98.61	98.61	98.47
A. phagocytophilum-like 1	*Hy. excavatum*	H101	Türkiye	MT498084*	**3**			100	99.82	99.82	100	100	99.64	98.57	98.57	98.57	98.57	98.57	98.74	98.56	98.56
A. phagocytophilum-like 1	*R. bursa*	H256	Türkiye	MT498088*	**4**				100	99.64	99.82	99.82	99.47	98.39	98.39	98.40	98.40	98.40	98.56	98.38	98.23
A. phagocytophilum-like 1	Goat	ApGGo4	Tunisia	KM285229	**5**					100	99.67	99.83	99.50	98.38	98.38	98.38	98.41	98.33	98.33	98.33	98.16
A. phagocytophilum-like 1	Sheep	Akseki26	Türkiye	MT881655	**6**						100	99.83	99.50	98.56	98.56	98.56	98.59	98.33	98.33	98.33	98.16
A. phagocytophilum-like 1	*Hae. qinghaiensis*	13QHF23	China	MG940878	**7**							100	99.67	98.56	98.56	98.56	98.59	98.50	98.50	98.50	98.33
A. phagocytophilum-like 1	Cattle	GS29	Türkiye	GU223365	**8**								100	98.57	98.57	98.59	98.62	98.58	98.50	98.50	98.03
A. phagocytophilum-like 2	*R. turanicus*	H141	Türkiye	MT498087*	**9**									100	100	100	100	100	99.82	99.64	99.64
A. phagocytophilum-like 2	*R. turanicus*	H102	Türkiye	MT498085*	**10**										100	100	100	100	99.82	99.64	99.64
A. phagocytophilum-like 2	*R. sanguineus*	H134	Türkiye	MT498086*	**11**											100	100	100	99.82	99.64	99.31
A. phagocytophilum-like 2	*R. turanicus*	H243	Türkiye	MH643970*	**12**												100	100	99.65	99.65	99.32
A. phagocytophilum-like 2	Sheep	YC38	China	KJ782381	**13**													100	99.67	99.67	98.91
A. phagocytophilum-like 2	Goat	ApGGo3	Tunisia	KM285228	**14**														100	99.33	99.17
A. phagocytophilum-like 2	Cattle	S9	Türkiye	MT338502	**15**															100	99.83
A. phagocytophilum-like 2	*Hy. asiaticum*	BL099-6	China	KJ410247	**16**																100

Anaplasma ovis was detected in 68 out of the 310 tick pools (21.93%) from all cities belonging to six tick species *(R. bursa*, *R. turanicus*, *R. sanguineus* s.l., *D. marginatus*, *Hae. parva* and *Hae. punctata*) with an overall MLE of infection rate of 34.1. While highest MLE of infection rate for *A. ovis* was in *D. marginatus* and in Bayburt city, lowest values were detected in *Hae. parva* and in Bolu city. *Anaplasma ovis* DNA has not been detected in *Hyalomma marginatum*, *Hy. scupense* and *I. ricinus*. Under accession number MH636802, one typical sample sequence for *A. ovis* may be found in the GenBank, EMBL, and DDBJ databases.

Anaplasma phagocytophilum was detected in the six tick species *R. bursa, R turanicus, R. sanguineus* s.l., *Hae. Sulcata, Hae. concinna* and *Hy. excavatum*, from Bolu, Kastamonu, Çorum and Giresun cities. *Anaplasma phagocytophilum* DNA was detected in eight of the 310 (2.58%) tick pools, resulting in an overall MLE of infection rate of 3.61. While MLE of infection rate was highest in Giresun city, lowest value was recorded in Bolu city. The MLE of infection rate for *A. phagocytophilum* differed by tick species, ranging from 2.21 in *R. bursa* to 95.5 in *Hae. concinna*. The bacterium DNA has not been detected in *D. marginatus, Hae. parva, Hae. punctata, Hy. marginatum, Hy. scupense* and *I. ricinus* ticks nor from Samsun, Tokat and Bayburt cities. Nucleotide sequences of all positive samples for *A. phagocytophilum* are available under accession numbers of, MH636805, MH643970, MH715976 and MT498084 - MT498088.

Molecular and Phylogenetic Analyses

All PCR positive samples in terms of *A. phagocytophilum* were sequenced to validate PCR results and to determine the variants. Phylogenetic analysis revealed that *A. phagocytophilum* variants determined with this study clustered in two different clades. The *Hae. concinna*, *Hae. sulcate*, *Hy. excavatum*, and *R. bursa* variations H107, H5, H101, and H256, respectively, formed a unique group with the *A. phagocytophilum*-like 1 cluster seen in ruminants and *Haemaphysalis qinghaiensis*. But the H141, H102, H134, and H243 variations from *R. turanicus* and *R. sanguineus* grouped together with the *A. phagocytophilum*-like 2 cluster found in ruminants and *Hyalomma asiaticum (Fig. 2)*. This study's isolates of *Anaplasma phagocytophilum*-like 1 and 2 shared 99.47-100% and 99.31-100% identity with other isolates of *A. phagocytophilum*-like 1 and 2 that are listed in GenBank *(Table 2)*.

Discussion

Rhipicephalus bursa is the main vector for *A. ovis*. However, it was reported that other tick species including *Dermacentor* spp., *Rhipicephalus* spp. and *Hyalomma* spp. can transmit *A. ovis* [11]. In this study, *A. ovis* DNA was detected in *R. bursa*, *R. turanicus*, *R. sanguineus* s.l., *D. marginatus*, *Hae. parva* and *Hae. punctata*. However, the detection of DNA of a pathogen in a tick species is not enough to consider it as a competent vector in the transmission of this pathogen to a host [30]. *Anaplasma ovis* was detected in 68 out of the 310 tick pools (MLE 34.1, CI 26.7-42.8) from all the surveyed cities. Our findings are consistent with earlier research showing that *A. ovis* is highly prevalent in different Turkish locations. Furthermore, a recent study showed that *A. ovis* has low prevalence (0.41% CI 0.02-2.01) in *R. bursa* ticks collected from humans in Türkiye [31]. *Anaplasma ovis* DNA was detected in *R. sanguineus* s.l. [32], *Hae. punctata* [33], *R. bursa* [31] and *D. marginatus* [32] with this study and our findings agree with those from previous studies. *Anaplasma ovis* is a prevalent tick-borne agent in small ruminants globally and although it produces generally mild infections, some cases have severe pathology. In this study we describe high tick infestations with *A. ovis*, therefore it can result with high animal infection rates in the region. Our results indicate that *A. ovis* is present in which is in accordance with several previous studies and further emphasize the possible high risk of *A. ovis* transmission to both animals and humans in the region.

Anaplasma phagocytophilum, a tick-borne rickettsial microorganism, causes granulocytic anaplasmosis or tick-borne fever in horses, dogs and other animals. Furthermore, it is the agent of human granulocytic anaplasmosis (HGA) [6]. Its presence was already shown in domestic animals [17,18,22] and ticks [31] in Türkiye. Infection rates of *A. phagocytophilum* were very low in ticks when compared to *A. ovis*. Similarly, other Turkish and foreign studies found lowest prevalence of *A. phagocytophilum* in hosts [22] and ticks [34,36] compared to *A. ovis*. Although *Ixodes* spp. is considered the main vector for *A. phagocytophilum* [37], there is a speculation about other potential tick vectors of *A. phagocytophilum* [38]. In the present study *A. phagocytophilum* was detected in *R. bursa*, *R. turanicus*, *R. sanguineus* s.l., *Hae. sulcata*, *Hae. concinna* and *Hy. excavatum*. Previous studies have shown the presence of *A. phagocytophilum* DNA in *I. ricinus* [4], *Hy. marginatum, Hy. excavatum* [40] and in *Hae. sulcata* [31] in Türkiye. Furthermore, *A. phagocytophilum* DNA has also been amplified from *I. ricinus* [36,41], *Ixodes persulcatus* [38], *Hy. marginatum* and *Hyalomma lusitanicum* [34], *Hyalomma anatolicum* [35], *Hae. concinna* [36,38], *Hae. longicornis* [42], *Hae. punctata* [33], *Dermacentor* spp. [36,38] and *R. sanguineus* s.l. [43-45]. Interestingly, *A. phagocytophilum* has not been detected in any of the *I. ricinus* ticks in the present study. This may be due to low sample number of *I. ricinus* from the studied area. Furthermore, this species is frequently found along the Black Sea coast, and while the ticks sampled in this study were representative of the Black Sea region, they

were primarily from inland. Primers SSAP2F and SSAP2r are commonly used for the detection of *A. phagocytopilum* in ruminants [19,22] and ixodid ticks [39]. However, they are also known to detect other strains genetically related to *A. phagocytophilum* such as *Anaplasma* sp. Japan [46] and *Anaplasma* sp. China [19], finally designed as *A. phagocytophilum*-like 1 and *A. phagocytophilum*-like 2 [16,47]. Therefore, all the positive samples were sequenced, and it has been shown that *A. phagocytophilum*-like 1 and 2 variants circulate in ticks in the region. In the previous studies carried out in Türkiye, *A. phagocytophilum*-like 1 and *A. phagocytophilum*-like 2 strains were detected in cattle [14] and small ruminants [4,17,18]. On the other hand, this is the first account of *A. phagocytophilum* – like 1 and 2 strains in ticks in the nation. Similar to our study, *A. phagocytophilum*-like 2 strain was detected in *R. turanicus* collected from small ruminants in Tunisia [48]. It is concluded that there may be other main vectors for *A. phagocytophilum* except *I. ricinus* and we suggest futher studies about *A. phagocytophilum* variants in ixodid ticks. Even though the zoonotic potential of these variants has not yet been established, we think that the distinction between *A. phagocytophilum*-like 1 and 2 in human and animal cases would be helpful in this respect.

In the studies on *A. phagocytophilum* variants in Türkiye, the presence and distribution of *A. phagocytophilum* - like 1 and 2 were investigated in cattle, sheep, goats and buffaloes [14,17,18,49]. This study provided important data on the presence of these variants in ticks in Türkiye. The data obtained in this study suggest that these variants should be taken into consideration in the differential diagnosis of tick-borne infections in the Black Sea region, where tick contact is intensively seen in humans and animals. Presence and distribution of *Anaplasma* species is influenced by several factors including climate condition and tick diversity of the region [50]. In this investigation, *A. phagocytophilum* was found in tick vectors in both humid and terrestrial climates, despite *I. ricinus* being the predominant species in humid regions. This is also similar to other findings from the region [31]. This could support the theory that *A. phagocytophilum*'s ecology and epidemiology, particularly its transmission to animal host species, may be significantly influenced by other tick species.

In conclusion, *A. ovis* and *A. phagocytophilum* were investigated in a large geographic area of the Black Sea Region of Türkiye. *Anaplasma ovis* was detected with high prevalence (MLE 34.1) in six tick species *(R. bursa, R. turanicus, R. sanguineus* s.l., *D. marginatus, Hae. parva and Hae. punctata*) and in all investigated provinces. As the first record in Türkiye, *A. phagocytophilum*-like 1 and *A. phagocytophilum*-like 2 strains were also found in tick species *(A. phagocytophilum*-like 1 in *Hae. concinna, Hae. sulcate*, *Hy. excavatum* and *R. bursa*; *A. phagocytophilum*-like 2 in *R. turanicus* and *R. sanguineus* s.l.,) but not in its main European vector, *I. ricinus*. Additionally, this bacterium was detected only in four provinces (Bolu, Kastamonu, Çorum and Giresun) and with a lowest MLE of infection rate (3.61%) when compared to *A. ovis*. These findings showed that *A. ovis* and *A. phagocytophilum* are common in the area and pose a serious risk to the health of people and animals. We hope these data will help to sensitize for the implementation of anaplasmosis control methods in the region.

Declarations

Availability of Data and Materials: When needed, the corresponding author (M.F. Aydın) can provide the data and materials used in this study.

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