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RESEARCH ARTICLE

Molecular Investigation of Ovine and Caprine Anaplasmosis in South-eastern Anatolia Region of Turkey

Ceylan Ceylan[1*] and Özlem Derinbay Ekici[1]

[1]Selcuk University, Faculty of Veterinary Medicine, Department of Veterinary Parasitology, Konya, Turkey
*Corresponding author: ceylanilhan@selcuk.edu.tr

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A B S T R A C T

Ovine and caprine anaplasmosis is an endemic tick-borne disease in Europe, the Middle East and some African and Asian countries, including Turkey. This study was conducted to determine the prevalence of *Anaplasma ovis* and *Anaplasma phagocytophilum* in sheep and goats in the Southeastern Anatolia region of Turkey. For each animal group, 384 individuals were included in the study. Microscopy and Rt-PCR were used to determine the prevalence of anaplasmosis in sheep and goats. Microscopic examination revealed 21.88% (n:84) and 39.58% (n:152) *Anaplasma* sp. positivity in sheep and goats, respectively. The study revealed that the molecular prevalence values were higher than the microscopic prevalence values for each microorganism. The molecular prevalence of *A. ovis* was 89.32% (n:343) in sheep and 78.91% (n:303) in goats and the difference was statistically significant ($P<0.05$). On the other hand, *A. phagocytophilum* was detected at 42.97% (n:165) in sheep and 15.89% (n:61) in goats. This is the first study in Turkey in which the Rt-PCR method was used for the detection of *Anaplasma* species in small ruminants. This study demonstrated that Rt-PCR provides more accurate and specific results than microscopic examination. The present study is expected to contribute to determining the prevalence of *Anaplasma* species, one of the tick-borne pathogens, in Turkey and provide data on future control strategies for small ruminant anaplasmosis.

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INTRODUCTION

Turkey is a country located in the subtropical climate zone and its economy is based on industrial and agricultural products (Ceylan *et al.*, 2021a). There are 6,174,348 sheep and 2,669,638 goats in the Southeastern Anatolia Region of Turkey. This region has a significant share of Turkey's small ruminant population and significantly contributes to the country's meat and milk production (Tuik, 2018; Gunlu and Mat, 2021). Many factors adversely affect sheep and goat farming in Turkey, one of which is *Anaplasma* infection transmitted by ticks. The primary etiological agent of small ruminant anaplasmosis is *Anaplasma ovis*, an intracellular rickettsia. Small ruminant anaplasmosis is characterized by hemolytic anemia. *Anaplasma ovis* causes subclinical infections with a low fever. However, the infection may be severe in the case of coinfections (Stoltsz, 2004; Yasini *et al*., 2012). The main vector tick species of *A. ovis* are *Rhipicephalus (Rh.) bursa*, *Rh. turanicus*, *Dermacentor (D.) andersoni, D. marginatus*, *D. silvarum*, and *Haemaphysalis sulcata*. These agents can also be transferred mechanically by arthropods such as blood-sucking flies, fleas, basids, and Hypoboscide flies or by veterinary equipments such as syringes and scissors (Hornok *et al*., 2011; Cabesaz-Cruz *et al*., 2019).

Another species causing anaplasmosis in sheep and goats is *A. phagocytophilum*, a zoonotic rickettsial agent infecting neutrophils of many mammals (Stuen *et al*., 2003). It is a small (approximately 0.3 μm), gram-negative, pleomorphic microorganism settling in the mononuclear phagocytic system cells, erythrocytes, and platelets (Dantas-Torres and Otranto, 2017). Sequence analysis of rRNA gene revealed that the agents of granulocytic anaplasmosis detected in ruminants, horses, and humans are different variants of the same species. The infection caused by *A. phagocytophilum* is called granulocytic anaplasmosis in humans, tick fever in animals, and pasture fever in cattle. The disease causes high fever, neutropenia, low milk yield, and abortion in non-immune pregnant animals (Atas *et al*., 2016).

Although the prevalence of *A. ovis* and *A. phagocytophilum* has been investigated in small-scale epidemiological studies in Turkey, no study has been found covering the Southeastern Anatolia Region, which has an important place in small ruminant breeding. This study was planned to determine the prevalence of *Anaplasma* agents in sheep and goats in the Southeastern Anatolia Region.

MATERIALS AND METHODS

Study area and sample collection: The samples were collected from sheep (n:384) and goats (n:384) in 9 cities of the Southeastern Anatolia region of Turkey in September 2019. Blood sample (5-8 ml) collection was carried out in different herds (ranging from 1 to 6) in each city and blood samples were collected in EDTA-anticoagulated tubes using a vacutainer and sterile needles. Collected blood samples were preserved and carried to the laboratory in a mini cooler box, and centrifuged (5000 rpm for 5 min) to separate plasma and blood cells. All blood samples were stored at -20°C until used in laboratory tests. In the study, a power analysis was performed to determine the number of samples to be collected from each province. Fig. 1 shows a map of sample collection sites and the number of samples.

Preparation of thin blood smears and microscopic examination: Thin blood smears were prepared from the EDTA-anticoagulated blood, dried in open-air, fixed with absolute methanol for 3-5 min, stained with 10% Giemsa solution for 45 min, washed under tap water, dried and stored until microscopic examination. The smears were examined for *Anaplasma* spp. under a light microscope (100X), and 50 microscopic fields were scanned to detect the pathogens.

DNA extraction: Genomic DNA extraction from the blood samples were performed using EZNA Blood DNA Mini Kit (OMEGA Bio-Tek, Georgia, USA), following the manufacturer's instructions. The extracted DNA samples were stored at -20°C until the molecular analysis.

Rt-PCR amplification: In the study, during the diagnosis of *A. ovis* by Rt-PCR, 5 µl of Master mix (Fast start Essential DNA probes master, Roche), 0.5 µl of anti-sense primer 5' AGGTTTGGATCTGCCTCTCTGTGA-3' (Microsynth), 0.5 µl of sense primer 5′-AGGTACCGGGTATCGTTGCA-3′ (Microsynth), 0.5 µl of TaqMan probe FAM 5'ACATTTACAGGCACACCTCTGGCATGC-3′ BHQ1 (Microsynth) (Chi *et al*., 2013), 1 µl of ddH2O (double distilled water), 2.5 µl of template DNA was used and loaded into the Rt-PCR device (LightCycler 96, Roche) with a total volume of 10 µl in each well. Previously confirmed DNA samples (Ceylan *et al*., 2021b) were used as positive controls. Double distilled water was used as negative control. During the diagnosis of *A. phagocytophilum* by Rt-PCR, 0.75 µl of ApMSP2 F 5' ATGGAAGGTAGTGTTGGTTATGGTATT-3', 0.75 µl of ApMSP2 R 5'-TTGGTCTTGAAGC GCTCGTA-3', 5 µl of Master mix (Sso Advanced Universal SYBR green Supermix, 1 ml, Biorad) (Courtney *et al*., 2004; Almazán *et al*., 2020), 1 µl of ddH2O (double distilled water), 2.5 µl of template DNA was used and loaded into the Rt-PCR device (LightCycler 96, Roche) with a total volume of 10 µl in each well.

Statistical analysis: The significance level was indicated to be $\alpha = 0.05$. The IBM SPSS statistics 22 (IBM Corp. lisensors 1989, 2012. IBM SPSS Statistics 22 for Windows, NY: IBM Corp.) statistical package program was employed to analyze the data. Chi-square values (χ^2) and P values were calculated to compare the methods and to determine the level of statistical significance between sheep and goats.

Ethics statement: The owners of the sheep were informed concerning the study, and their approval was obtained before sampling. All practices were carried out following the ethical guidelines for the use of animal samples permitted by the Faculty of Veterinary Medicine, Selcuk University (Approval: 2019/10, Date: 31.01.2019).

RESULTS

Microscopic examination results: Blood smears were microscopically examined for *A. ovis* and *A. phagocytophilum*. *A. phagocytophilum* was not found in any of the smears. *Anaplasma* spp. was detected in 21.88% of sheep samples. On the other hand, *Anaplasma* spp. was detected in 39.58% of goat samples. Microscopy results are indicated in Fig. 2.

Rt-PCR results: The molecular prevalence of *A. ovis*, *A. phagocytophilum* and total infection was found 89.32%, 42.97% and 94.01% respectively, in sheep. Detailed information regarding Rt-PCR results of sheep are indicated in Table 1.

The molecular prevalence of *A. ovis*, *A. phagocytophilum* and total infection was found 78.91%, 15.89% and 81.25% respectively, in goats. Detailed information regarding Rt-PCR results of goats are indicated in Table 2.

Statistical analysis results: A chi-square (χ^2) test was performed to compare the results of methods. A statistically significant difference was determined between Rt-PCR and microscopy in the diagnosis of *A. ovis*, *A. phagocytophilum*, and coinfections in sheep ($P<0.001$). In the study, the molecular prevalences of *A. ovis* (89.32%), *A. phagocytophilum* (42.97%), and coinfections (94.01%) were higher as compared to prevalences diagnosed through microscopy. The data obtained as a result of statistical analysis are given in Table 3.

A statistically significant difference was determined between Rt-PCR and microscopy in diagnosing *A. ovis*, *A. phagocytophilum*, and coinfections in goats ($P<0.001$). In the study, the molecular prevalences of *A. ovis* (78.91%), *A. phagocytophilum* (15.89%), and coinfections (81.25%) were higher as compared to prevalences determined through microscopy. The data obtained as a result of statistical analysis are given in Table 4.

Microscopic examinations revealed a higher *Anaplasma* spp. prevalence in goats (39.58%) when compared with sheep (21.88%) with a statistically significant difference ($P<0.001$). Since *A. phagocytophilum* was not detected in microscopy, statistical data of *A. phagocytophilum* were not evaluated. The data obtained as a result of statistical analysis are given in Table 5.

A statistically significant difference was determined between molecular prevalence of *A. ovis* and coinfections in sheep and goats ($P<0.001$). The molecular prevalences

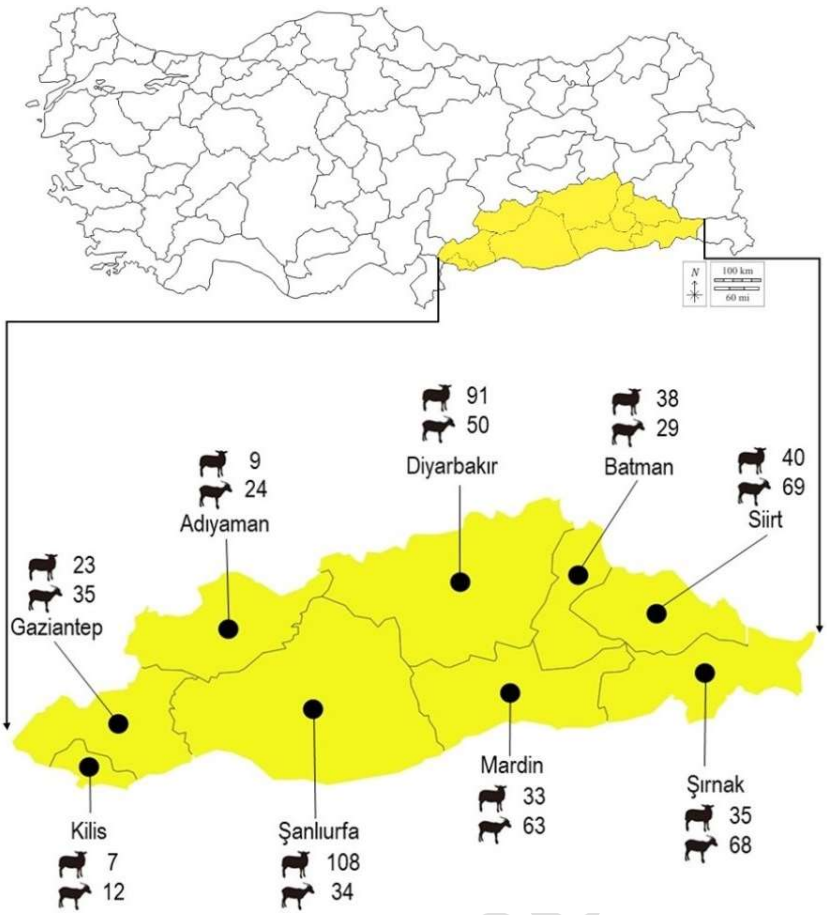

Fig. 1: Map of Turkey and Southeastern Anatolia region showing sample collection sites and the number of samples collected by the province.

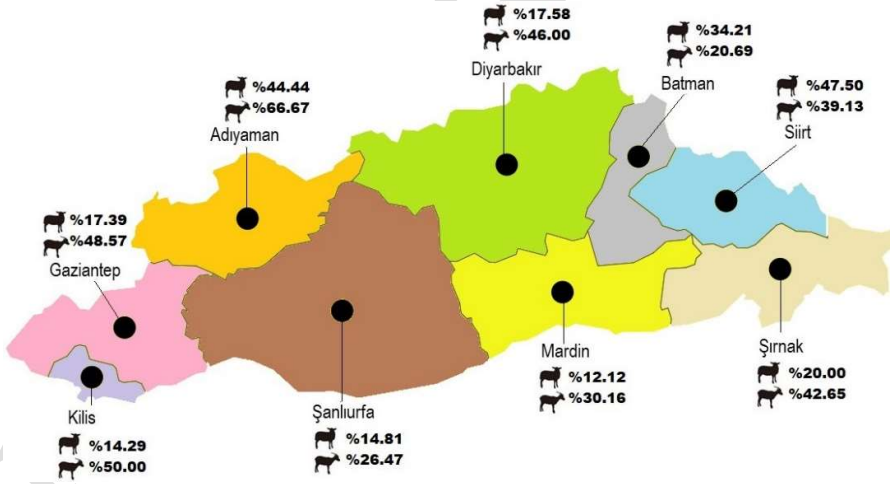

Fig. 2: The microscopic prevalence of *Anaplasma* spp. in sheep and goats in the Southeastern Anatolia Region.

of *A. ovis* (P<0.001) and coinfections (P<0.001) were found to be higher in sheep than in goats. As a result of statistical analysis, no significant difference was found in the data of *A. phagocytophilum* in sheep and goats (P>0.05). The reason for this is that the rates of single infection are low in *A. phagocytophilum*. However, when the total infection rates are considered, the rate of *A. phagocytophilum* was higher in sheep than in goats in Rt-PCR, like other results obtained. The statistical analysis results are given in Table 6.

DISCUSSION

Diagnosis of haemoparasites has been made by observing disease-specific clinical findings and microscopic examination of blood smears for many years. Microscopically, the prevalence of *A. ovis* in sheep varies between 0.2-10% in studies conducted in different parts of Turkey (Hoffman *et al*., 1971; Guralp *et al*., 1975; Ozer *et al*., 1993; Ekici, 2016). In the study, the microscopic prevalence of *A. ovis* was determined as 21.88% and 39.58% in sheep

Table 1: Molecular prevalences of *Anaplasma* spp. in sheep in Southeastern Anatolia Region of Turkey

Order	City	n	*Ao* (%)	*Ap* (%)	*Ao* +*Ap* (%)	Total infection rate		
						Ao (%)	*Ap* (%)	*Ao*/*Ap* total
1	Kilis	7	-	3	-	-	3 (42.86)	3 (42.86)
2	Gaziantep	23	2	9	3	5 (21.74)	12 (52.17)	14 (60.87)
3	Şanlıurfa	108	23	1	83	106 (98.15)	84 (77.78)	107 (9.07)
4	Adıyaman	9	1	1	4	5 (55.56)	5 (55.56)	6 (66.67)
5	Diyarbakır	91	82	-	9	91 (100)	9 (9.89)	91 (100)
6	Mardin	33	11	1	21	32 (96.97)	22 (66.67)	33 (100)
7	Batman	38	30	-	8	38 (100)	8 (21.05)	38 (100)
8	Siirt	40	19	3	13	32 (80.00)	16 (40)	35 (87.50)
9	Şırnak	35	28	-	6	34 (97.14)	6 (17.14)	34 (97.14)
Total		384	196 (51.04)	18 (4.69)	147 (38.28)	343 (89.32)	165 (42.97)	361 (94.01)

*n: Number of sampled animals Ao: *Anaplasma ovis* Ap: *Anaplasma phagocytophilum*

Table 2: Molecular prevalences of *Anaplasma* species in goats in Southeastern Anatolia Region of Turkey

Order	City	n*	*Ao*(%)	*Ap*(%)	*Ao*+*Ap* (%)	Total infection rate		
						Ao (%)	*Ap* (%)	*Ao*/*Ap* total
1	Kilis	12	3 (25.0)	3 (25.0)	3 (25.0)	6 (50.00)	6 (50.00)	9 (75.0)
2	Gaziantep	35	13 (37.14)	3 (8.57)	1 (2.86)	14 (40.00)	4 (11.43)	17 (48.57)
3	Şanlıurfa	34	28 (82.35)	-	3 (8.82)	31 (91.18)	3 (8.82)	31 (91.18)
4	Adıyaman	24	11 (45.83)	-	1 (4,17)	12 (50.00)	1 (4.17)	12 (50.00)
5	Diyarbakır	50	42 (84.0)	-	-	42 (84.00)	-	42 (84.00)
6	Mardin	63	35 (55.55)	1 (1.59)	19 (30.16)	54 (85.71)	20 (31.75)	55 (87.3)
7	Batman	29	13 (44.82)	1 (3.45)	7 (24.14)	20 (68.97)	8 (27.59)	21 (72.41)
8	Siirt	69	47 (68.12)	1 (1.45)	11 (15.94)	58 (84.06)	12 (17.39)	59 (85.5)
9	Şırnak	68	59 (86.77)	-	7 (10.29)	66 (97.06)	7 (10.29)	66 (97.06)
Total		384	251 (65.36)	9 (2.34)	52 (13.54)	303 (78.91)	61 (15.89)	312 (81.25)

n*: Number of examined animals Ao: *Anaplasma ovis* Ap: *Anaplasma phagocytophilum*

Table 3: Statistical comparison of Rt-PCR and microscopy results in sheep.

	Ao	Ap	Miks	Ao+Miks	Ap+miks	Total
Rt-PCR	196/384[a]	18/384[a]	147/384[a]	343/384[a]	165/384[a]	361/384[a]
Microscopy	84/384[b]	0/384[b]	0/384[b]	0/384[b]	0/384[b]	84/384[b]

[a, b]: Different letters in the same column are statistically significant(P<0.05)

Table 4: Statistical comparison of Rt-PCR and microscopy results in goats.

	Ao	Ap	Miks	Ao+Miks	Ap+miks	Total
Rt-PCR	251/384[a]	9/384[a]	52/384[a]	303/384[a]	61/384[a]	312/384[a]
Microscopy	152/384[b]	0/384[b]	0/384[b]	0/384[b]	0/384[b]	152/384[b]

[a, b]: Different letters in the same column are statistically significant(P<0.05)

Table 5: Statistical comparison of microscopy results in sheep and goats.

	Ao	Ap	Miks	Ao+Miks	Ap+miks	Total
Sheep-Microscopy	84/300[a]	0/384	0/384	0/384	0/384	84/300[a]
Goat- Microscopy	152/232[b]	0/384	0/384	0/384	0/384	152/232[b]

*[a, b]: Different letters in the same column are statistically significant (P<0.05)

Table 6: Statistical comparison of Rt-PCR results in sheep and goats.

	Ao	Ap	Miks	Ao+Miks	Ap+miks	Total
Sheep-Rt-PCR	188/196[a]	18/366	147/237[a]	41/343[a]	165/219[a]	23/361[a]
Goat-Rt-PCR	133/251[b]	9/375	52/332[b]	81/303[b]	61/323[b]	72/312[b]

[a, b]: Different letters in the same column are statistically significant(P<0.05)

and goats, respectively. In the study, the infection rates were found to be higher than the rates in other studies conducted with microscopic examination in Turkey (Hoffman *et al*., 1971; Guralp *et al*., 1975; Ozer *et al*., 1993; Ekici, 2016).

Advances in molecular biology have greatly improved the sensitivity and specificity of the diagnostic techniques. After the creation of a specific PCR protocol for the detection of *A. ovis* DNA (de la Fuente *et al*., 2007), new data on this pathogen have been obtained. In studies using molecular methods throughout Turkey, the prevalence of *A. ovis* has been determined as 18-67.06% (Renneker *et al*., 2013b; Altay *et al*., 2014; Oter *et al*., 2016; Bilgic *et al*., 2017; Zhou *et al*., 2017; Aktas and Ozubek, 2018; Sevinc *et al*., 2018; Benedicto *et al*., 2020; Ceylan *et al*., 2021b). In the present study, *A. ovis* was detected in 89.32% of sheep samples and 78.91% of goat samples. The results obtained in our study are similar to the findings obtained in previous studies.

There are some studies on *A. ovis* and *A. phagocytophilum* in sheep and goats in Turkey, but there is no study representing the prevalence covering the whole Southeastern Anatolia Region. Renneker *et al*., (2013b) determined the molecular prevalence of *A. ovis* as 10.6% in sheep in Van and Sanliurfa provinces. In this study, the molecular prevalence of *A. ovis* was found higher (98.15%) in Sanliurfa. Aktas and Ozubek (2018) determined the molecular prevalence of *A. ovis* in Gaziantep (17.64% in sheep, 18.42% in goats) and Adıyaman (19.04% in sheep, 11.36% in goats) provinces. The molecular prevalence of *A. ovis* was 21.74% in sheep and 40% in goats in Gaziantep; 55.56% in sheep, and 50% in goats in Adıyaman in the present study. The prevalences determined in this study were found to be higher than the prevalences determined by Aktas and Ozubek (2018). In a study by Benedicto *et al*. (2020) in which they investigated tick-borne diseases in sheep and goats from 10 cities of

Turkey using microscopy and PCR, they found *A. ovis* in Diyarbakır with a rate of 22.6% in microscopy and 46.6% in PCR. In our study, the prevalence of *A. ovis* was determined as 17.58% by microscopy and 100% by Rt-PCR in sheep in Diyarbakır. In a study conducted by Ceylan *et al*. (2021b), the molecular prevalence of *A. ovis* was detected as 43.37% in Batman and 35% in Mardin. In our study, *A. ovis* was found in sheep with a high rate of 100% in Batman and 96.97% in Mardin. The rates we determined were found to be higher than the other studies conducted in the Southeastern Anatolia Region. This situation might be attributed to different tick species involved in the transmission of the disease, different seasonal conditions during sample collection and the location of the sample collection area where close to the border regions (Syria, Iraq) where the disease is seen at a high rate. There are also many molecular researches around the world regarding *A. ovis* has been reported in many continents such as Asia, Europe, Africa, and America (Ahmadi-Hamedani *et al*., 2009; Torina *et al*., 2010; Renneker *et al*., 2013a). This and other molecular studies (de la Fuente *et al*., 2005; Hornok *et al*., 2007; Liu *et al*., 2012) show that *A. ovis* is endemic in many geographic regions (Renneker *et al*., 2013b).

Chi *et al*., (2013) revealed that Rt-PCR is more sensitive and useful than conventional PCR. In this study, primers and probes designed by Chi *et al*., (2013) were used for the detection of *A. ovis* DNA by Rt-PCR. The main reason for using microscopic examination and Rt-PCR in our study is that microscopic examination can be performed even under field conditions and rapid results are obtained, while Rt-PCR gives more specific results. In the present study, there was a high difference between microscopic (21.88%/39.58%) and molecular prevalence (89.32%/78.91%) of *A. ovis*, since the Rt-PCR is a more specific and sensitive method than the microscopy. This difference can also be attributed to blood collection from apparently healthy animals and unsuitable blood sample collection period. Moreover, in acute cases where the parasitemia level is above 0.1-0.2%, the agent can be detected easily by microscopic examination, while the low parasitemia in subclinical or latent infections makes it difficult to detect the agents (Renneker *et al*., 2013a).

There are few studies regarding microscopic prevalence of *A. phagocytophilum* in small ruminants. As a result of these studies, microscopic prevalence of *A. phagocytophilum* has been reported as 0-9.86% (Gokce *et al*., 2008; Sevinc *et al*., 2018) in Turkey. The results obtained in our study show similarities with the previous findings. *A phagocytophilum* could not be detected by microscopy in any of the smears in this study. It is thought to be the presence of *A. phagocytophilum* in granulocytes for 3-4 weeks following infection after entering the body, they are seen in blood smears between 3-15 days after infection. For this reason, the microscopic diagnosis can only be made in the first 3-8 days of the infection, which is called the febrile period. The definitive microscopic diagnosis of *A. phagocytophilum* is very difficult, since the chance of catching infected animals in the febrile period is low in regional studies, and the majority of infected animals are in the chronic period, and single forms with unclear morphological details are observed in the smears prepared in this period (Gokce *et al*., 2008). The molecular prevalence of *A. phagocytophilum* has been reported as 0-57.19% (Gokce *et al*., 2008; Altay *et al*., 2014; Oter *et al*., 2016; Bilgic *et al*., 2017; Zhou *et al*., 2017; Sevinc *et al*., 2018; Ceylan *et al*., 2021b) in Turkey. *A. phagocytophilum* was detected in 42.97% of sheep and 15.89% of goats by Rt-PCR in the present study. Although there are some studies on anaplasmosis in the Southeastern Anatolia Region of Turkey, there is no comprehensive epidemiological study in the region. In a study conducted by Atas *et al*., (2016), the molecular prevalence of *A. phagocytohilium* was determined as 5.97% and 1.35% in sheep and goats, respectively in Sanliurfa. In our study, *A. phagocytophilum* was found with a higher rate of 77.78% in sheep and 8.82% in goats by Rt-PCR in Sanliurfa. Benedicto *et al*., (2020) could not microscopically detect *A. phagocytophilum* but determined the molecular prevalence as 66.7% in Diyarbakır. In our study, while *A. phagocytophilum* was not detected in microscopy in sheep, the molecular prevalence of the agent was determined as 9.89% in Diyarbakir. The large difference in molecular prevalences can be attributed to the fact that the blood samples were collected from tick-infested animals by Benedicto *et al*., (2020). In a comprehensive study conducted by Ceylan *et al*., (2021b), the molecular prevalence of *A. phagocytophilum* was detected as 70% in Mardin and 42.11% in Batman. The molecular prevalence of *A. phagocytophilum* was detected 66.67% in Mardin and 21.05% in Batman in sheep in the present study. The prevalences we obtained were found to be lower than the rates obtained in the study of Ceylan *et al*., (2021b). It is thought to be due to the differences in the method and the fact that the sheep blood samples used in the study were collected in the warmer months (May-August), which is more suitable for the ticks. Although the results are consistent with many studies, there are differences with some studies (Grøva *et al*., 2011; Yang *et al*., 2016; Song *et al*., 2020; Benedicto *et al*., 2020; Bauer *et al*., 2021; Ceylan *et al*., 2021b). This difference can be attributed to the geographical and climatic diversity between the regions where the studies were carried out, the presence of vector tick species, and the presence of reservoir hosts that play a role in the continuity of the infection, which directly affects the incidence of anaplasmosis (Ahmadi-Hamedani *et al*., 2009; Altay *et al*., 2014). On the other hand, the animals were examined for ticks and no tick infestation was detected. This may cause a change in the status of the infection, as the weather conditions during the collection period are also not suitable for the vector ticks (September 2019). In addition, inadequate tick control practices and the lack of awareness about protection from diseases can be counted among these factors.

Researchers from different countries reported that sheep are more exposed to tick-transmitted agents than goats, and they attributed this to the natural resistance of goats to these pathogens and the presence of extensive hair cover in sheep, which causes tick infestation to be missed (Gebrekidan *et al*., 2014; Rjeibi *et al*., 2014; Lee *et al*., 2018). In the present study, a statistically significant difference was found between the molecular prevalences of *A. ovis* and *A. phagocytophilum* in sheep and goats. For both species and both diagnostic methods, the infection rate was higher in sheep than in goats. The statistical data obtained in the study were found to be similar to the results

of studies conducted worldwide. In our study, the rate of *A. ovis* and *A. phagocytophilum* coinfection in sheep (38.28%) and goats (13.54%) was found to be relatively high. Coinfections can adversely affect the prognosis of tick-borne diseases compared to single-species-infected animals, especially in stressful conditions (Zhou *et al*., 2017; Sevinc *et al*., 2018). Further studies are needed to discover new interactions, determine the clinical effects of the disease, and select the best therapeutic practice to be applied in sheep and goats with mixed infections with *Anaplasma* species and other haemoparasites.

In conclusion, this study revealed that ovine and caprine *Anaplasma* species have a widespread distribution in the Southeastern Anatolia Region of Turkey. The study provides information about the microscopic and molecular prevalence of *A. ovis* and *A. phagocytophilum*, which are of zoonotic importance, in sheep and goat populations in the region, where small ruminant farming is intense, in Turkey. It is expected that the current study will contribute to the data concerning the epidemiology of ovine and caprine anaplasmosis in Turkey and the envisaged control programs of the diseases in the future. This situation needs to be supported by more comprehensive molecular epidemiological studies covering more geographic areas.

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Authors contribution: CC and ÖDE conceived and designed the research. CC executed the research under the supervision of ÖDE. CC and ÖDE analyzed the data. CC wrote the manuscript. CC and ÖDE reviewed the manuscript.

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