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RESEARCH

Investigation of *Anaplasma* Species with Veterinary and Public Health Significance in Sheep and Goats

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Abstract

Purpose This study was carried out to investigate *Anaplasma* important for veterinary and public health in sheep and goats in Niğde province in Türkiye by using molecular methods.

Methods Blood samples were taken from randomly selected 690 animals (520 sheep and 170 goats), which were between 1 and 10 years old and from different study sites in Niğde by using the vacutainer tubes containing EDTA. After the genomic DNA extractions samples, the *Anaplasma* spp. 16S rRNA genes were amplified by PCR. Species-specific polymerase chain reaction (PCR) assays were performed on positive samples for the presence of *A. bovis*, *A. capra*, *A. ovis, A. platys*-like, and *A. phagocytophilum*. At the same time, the animals were tested for ixodid tick infestation and collected ticks were examined for identification under the stereo-microscope.

Results The results of PCR analysis show that the overall *A. ovis* prevalence was 63.3% (437/690) in small ruminants sampled. A total of 361 sheep (69.4%) and 76 goats (44.7%) were found to be infected with *A. ovis*, whereas no positivity was detected for *A. bovis*, *A. capra*, *A. platys*-like, and *A. phagocytophilum. Anaplasma ovis* positivity was observed at the highest percent in May (%74.6) while the lowest in June (%52.4). In total, 1361 ticks (579♀, 782♂) were collected from sheep and goats in Niğde. Ticks were identified as *Rhipicephalus bursa* (383, 28.1%), *R. turanicus* (607, 44.6%), *Hyalomma marginatum* (7, 0.5%), *Hy. excavatum* (247, 18.1%), *Hy. anatolicum* (23, 1.7%), *Haemophsalis parva* (21, 1.5%), *Hae. punctata* (7, 0.5%), *Hae. sulcata* (40, 2.9%) and *Dermacentor marginatus* (26, 1.9%).

Conclusion The present study reports a high prevalence of *A. ovis* 63.3% (437/690) in sheep and goats in Niğde province.

Keywords Sheep · Goat · *Anaplasma* · Tick

Introduction

Anaplasmosis is caused by organisms of the genus *Anaplasma*, which are gram negative bacterium and transmitted to cattle, sheep, goats, wild ruminants, carnivores and humans by ticks [1, 2]. *Anaplasma ovis* and *A. phagocytophilum* species have been reported to cause clinical and subclinical infections in sheep and goats [2]. *Anaplasma ovis* usually causes mild disease in ruminants. It has been observed that it causes clinical infections in sheep and goats, especially in the presence of various factors (co-infections or stressful situations). *Anaplasma ovis* infections have been reported to be endemic throughout the world, including Europe, China and the United States [3]. *Anaplasma phagocytophilum*, which causes the infection called tick-borne fever in ruminants, causes important infections in ruminants, equines, canines, and humans. Tick-borne fever can cause direct (lamb death) and indirect loss (growth reduction) in sheep, moreover, up to 30% lamb deaths have been reported due to *A. phagocytophilum* [4]. In 2015, a new species named *A. capra* was discovered in goats and ticks in China. In addition, this species has been reported to cause infection in humans [5]. *Anaplasma bovis* causing monocytic anaplasmosis in cattle has been reported in sheep and goats [6]. Similarly, *A. platys* causing cyclic thrombocytopenia in dogs, it has been reported in sheep and goats as *A. platys*-like [6, 7]. The importance of *A. bovis* and *A. platys*-like in sheep and goats is unknown. This study aimed to investigate

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Anaplasma species which have veterinary and public health importance in sheep and goats in Niğde, Türkiye.

Materials and Methods

Study Area and Sample Collection

Blood samples were collected from apparently healthy sheep (n = 520) and goats (n = 170) belonging to 28 flocks of Niğde province, in Central Anatolia of Türkiye (with an altitude of 1240 m, 37° 58′ N longitude-34° 41′ E latitude) (Fig. 1). The region is characterized by a semi-arid climate, with an average annual precipitation of 348.8 mm, a mean temperature of 11.1 °C, and average relative humidity of 55%.

Tick Collection and Identification

Ticks were collected from sheep and goats during clinical examination and blood sampling. Specimens were gently removed using blunt-tipped forceps, placed individually into labeled tubes with 70% ethanol, and transported to the

Fig. 1 Geographic location of the study area. The map displays Türkiye in red, situated at the crossroads of southeastern Europe and western Asia. The Niğde province, where the study was conducted, is highlighted in blue in the inset map. The figure was created using mapchart.net

laboratory. Adult ticks were morphologically identified to species level under a stereomicroscope using standard taxonomic keys [8].

Molecular Detection of *Anaplasma* spp.

Genomic DNA was extracted from the thawed blood with a GF-1 Blood DNA Extraction Kit (Vivantis Technologies Sdn. Bhd. Revongen Corporation Center, Malaysia) according to manufacturer's instructions. The DNA concentration (ng/µL) and purity (A260nm/A280nm) of each sample was determined using a nanodrop spectrophotometer. To determine the presence and frequency of *A. bovis* (*16S rRNA*) [9], *A. capra* (*16S rRNA* and *gltA*) [5, 10], *A. ovis* (*groEL*) [11], *A. platys*-like (*groEL*) [12] and *A. phagocytophilum* (*msp4*) [13] in small ruminants, species specific PCRs were set up using different primer sets. The PCR reactions were performed in PCR Sprint (Sensoquest, Germany) as previously described [14].

Statistical Analysis

The descriptive statistics were analyzed by SAS program. The means and confidence internals were calculated using relevant SAS procedures. The presence of *Anaplasma* species were grouped by the age of animals as well as sampling seasons, i.e., groups of April, May, June and July. Then, these groups were subjected to significance test by chi-square ($\chi 2$) homogeneity tests. In these tests, $p < 0.05$ were considered to be statistically significant.

Results

Molecular Detection and Overall Prevalence of *Anaplasma* spp.

Of the 690 samples examined by PCR, 437 (%63.3; CI 59.1–66.9) were infected with *Anaplasma* spp. As a result of the species specific PCR, only *A. ovis* positivity was detected sheep and goat, %69.4 (CI 65.3–73.3) and %44.7 (CI 37.1–52.5) respectively. No positivity was detected in type specific PCRs of different gene regions specific to *A. phagocytophylium* (Msp4), *A. bovis* (16S rRNA), *A. capra* (16S rRNA and gltA) and *A. platys*-like (groEL).

Seasonal Distribution of *Anaplasma ovis*

The monthly distribution of *A. ovis* positivity is shown in Table 1 and Fig. 2. *Anaplasma ovis* positivity was observed to be the highest in May (74.6%) and the lowest in June (52.4%). It was observed that there was the highest positivity for sheep in May (84.3%) and for goats in July (55%). The difference between the months was not statistically significant ($p > 0.05$).

Distribution of *Anaplasma ovis* Infections by Age

According to Table 2 and Fig. 3, *A. ovis* positivity was determined in each age group. It was observed that the highest positivity was at the age of 5 years and over (73%), and the least positivity was in the 2 ages group (52.5%). The highest positivity in sheep was 5 years old and over (80%), and the highest positivity in goats was determined in the 3 ages group (59.4). The difference between the age groups was not statistically significant ($p > 0.05$).

Species Distribution of Ticks Collected from Small Ruminants

In our previous study using the same material [15], a total of 1361 (579♀, 782♂) ticks were collected from sheep and goats with tick infestation, 383 (28.1%) of them were *R. bursa*, 607 (44.6%) were *R. turanicus*, 7 (0.5%) were *Hy. marginatum*, 247 (18.1%) of *Hy. excavatum*, 23 (1.7%) of *Hy. anatolicum*, 21 (1.5%) *Hae. parva*, 7 (0.5%) *Hae. punctata*, 40 (2.9%) *Hae. sulcata* and 26 (1.9%) *D. marginatus* were identified.

Discussion and Conclusion

The current study detected a high prevalence of *A. ovis* infection among sheep and goats in Niğde province located in Central Türkiye, but tests did not provide any evidence for other *Anaplasma* species, including the zoonotic *A. capra* and *A. phagocytophilum*. Previous studies conducted in Türkiye have reported varying prevalence rates of *A. ovis* (31.4–67%) and *A. phagocytophilum* (2.4–66.7%) in small ruminants [16–20]. Moreover, *A. ovis* has been recorded in different countries with various ecological conditions such as Iraq (66.6%), Sudan (41.6%), Portugal (84.2%) [16], Tunisia (70.1%) [21], Hungary (72.7%) [22], Italy (87%) [23], China (14.3%) [24] and Slovakia (22.6%) [25], showing its wide geographical distribution and adaptability.

One explanation for the detection of *A. ovis* only in this study is based on the ecology of the area and the distribution of tick vectors. The climate of Niğde province is arid to semi-arid, generally being unsuitable for the persistence of *Ixodes ricinus*, the main vector of *A. phagocytophilum* [26]. Although the specific vector of *A. capra* is yet to be identified, there have been reports implicative of some relation with *Haemaphysalis* species, particularly in East Asia [27].

Table 1 Distribution of the species-specific PCR results by months

Months	Host	Number of samples	*Anaplasma* spp.	*A. ovis*	*A. phagocytophylium*	*A. bovis*	*A. capra*	*A. platys*-like
April	Sheep	130	99 (%76.1; 67.9–83.2)	99; (%76.1; 67.9–83.2)	–	–	–	–
	Goat	40	16 (%40; 24.9–56.7)	16 (%40; 24.9–56.7)	–	–	–	–
	∑	170	115 (%67.6; 60–74.6)	115 (%67.6; 60–74.6)	–	–	–	–
May	Sheep	140	118 (%84.3; 77.2–89.9)	118 (%84.3; 77.2–89.9)	–	–	–	–
	Goat	45	20 (%44.4; 29.6–60)	20 (%44.4; 29.6–60)	–	–	–	–
	∑	185	138 (74.6; 67.7–80.7)	138 (74.6; 67.7–80.7)	–	–	–	–
June	Sheep	140	79 (%56.4; 47.8–64.8)	79 (%56.4; 47.8–64.8)	–	–	–	–
	Goat	45	18 (%40; 25.7–55.7)	18 (%40; 25.7–55.7)	–	–	–	–
	∑	185	97 (%52,4; 45–59.8)	97 (%52.4; 45–59.8)	–	–	–	–
July	Sheep	110	65 (%59,1; 49.3–68.4)	65 (%59.1; 49.3–68.4)	–	–	–	–
	Goat	40	22 (%55; 38.5–70.7)	22 (%55; 38.5–70.7)	–	–	–	–
	∑	150	87 (%58; 49.7–66)	87 (%58; 49.7–66)	–	–	–	–
Total	Sheep	520	361 (%69.4; 65.3–73.3)	361 (%69.4; 65.3–73.3)	–	–	–	–
	Goat	170	76 (%44.7; 37.1–52.5)	76 (44.7; 37.1–52.5)	–	–	–	–
	∑	690	437 (%63.3; 59.1–66.9)	437 (%63.3; 59.1–66.9)	–	–	–	–

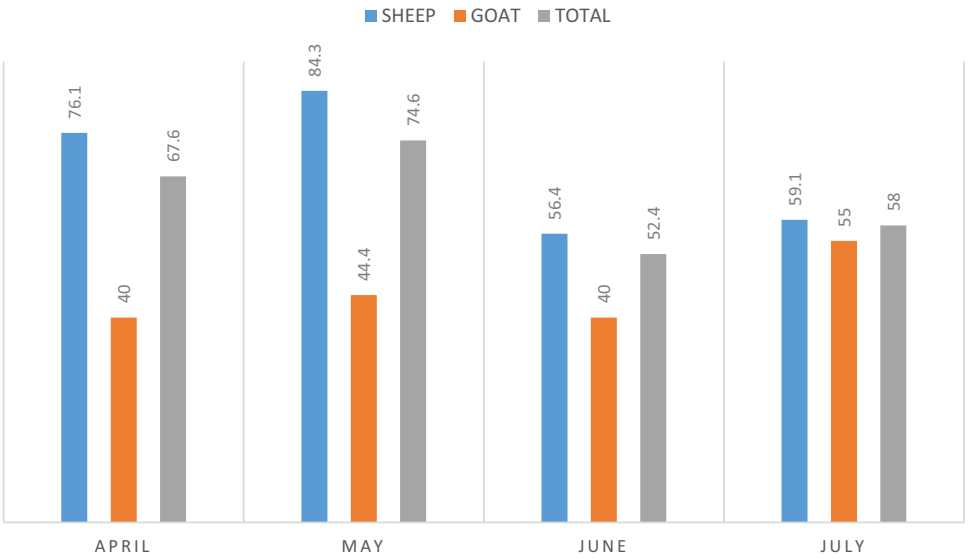

Fig. 2 Distribution of *Anaplasma ovis* positivity according to the months in which the samples were collected

Table 2 Distribution of *Anaplasma ovis* in sheep and goats by age

Age	Host	n	*A. ovis* (%; 95% CI)
1 year	Sheep	82	53 (64.6; 53.3–74.9)
	Goat	26	6 (23.1; 9.0–43.6)
	∑	108	59 (54.6; 44.8–64.2)
2 years	Sheep	110	66 (60; 50.2–69.2)
	Goat	52	19 (36.5; 23.6–51.0)
	∑	162	85 (52.5; 44.5–60.3)
3 years	Sheep	119	85 (71.4; 62.4–79.3)
	Goat	32	19 (59.4; 40.6–76.3)
	∑	151	104 (68.8; 60.8–76.1)
4 years	Sheep	114	81 (71; 61.8–79.1)
	Goat	18	8 (44.4; 21.5–69.2)
	∑	132	89 (67.4; 58.7–75.3)
5 years and older	Sheep	95	76 (80; 70.5–87.5)
	Goat	42	24 (57.1; 41–72.3)
	∑	137	100 (73; 67.5–82.8)
Total	Sheep	520	361 (69.4; 65.3–73.3)
	Goat	170	76 (44.7; 37.1–52.5)
	∑	690	437 (63.3; 59.1–66.9)

The sporadic detection of *Haemaphysalis* spp. in the present study may indicate a limited role for these ticks in the local transmission dynamics of *A. capra*. Additionally, the finding of *R. bursa* and *R. turanicus* species, which are the possible vector of the *A. ovis*, agrees with the high infection rates recorded and reflects the presence of active circulation and transmission in the small ruminant population [28–30].

The age-related distribution of *A. ovis* infection in this study showed varying prevalence across groups, with the highest rate observed in animals aged five years and older. However, the difference among age groups was not statistically significant. While the absence of a significant association suggests that age may not be a major determinant under the current conditions, higher prevalence in older animals has been reported in other studies and is often attributed to prolonged environmental exposure during grazing and trade activities [31, 32]. Conversely, some investigations have reported no age-related pattern, indicating that local management practices, vector density, and host immunity may play more influential roles [30, 33].

No clinical signs were observed in any of the animals sampled, but the high prevalence of infection indicates a generalized subclinical circulation of *A. ovis*. These infections may lead to decreased performance, lack of resistance against secondary infections and may become immunosuppressive, especially in stress and/or co-infection situations [34]. Although *A. ovis* has traditionally been regarded as non-zoonotic, a single report from Cyprus documented an *A. ovis*-like variant detected in a human patient, raising questions about its potential zoonotic capacity [35]. While further studies are required to confirm pathogenicity in humans, this finding highlights the need to reassess the public health implications of *A. ovis* in endemic areas.

This study reports that *A. ovis* is the most prevalent *Anaplasma* species of small ruminants in Niğde province, Central Türkiye, with a high prevalence in total and subclinically circulating. The inability to detect zoonotic strains such as *A. capra* and *A. phagocytophilum*, could be due to local ecological barriers and low vector competence. *Anaplasma ovis* has conventionally been perceived as non-zoonotic; however, a human case was reported which indicates its potential public health concern. These findings emphasize the need for increased awareness of anaplasmosis among farmers, veterinarians, and public health authorities, especially in endemic rural areas. Continued surveillance and molecular

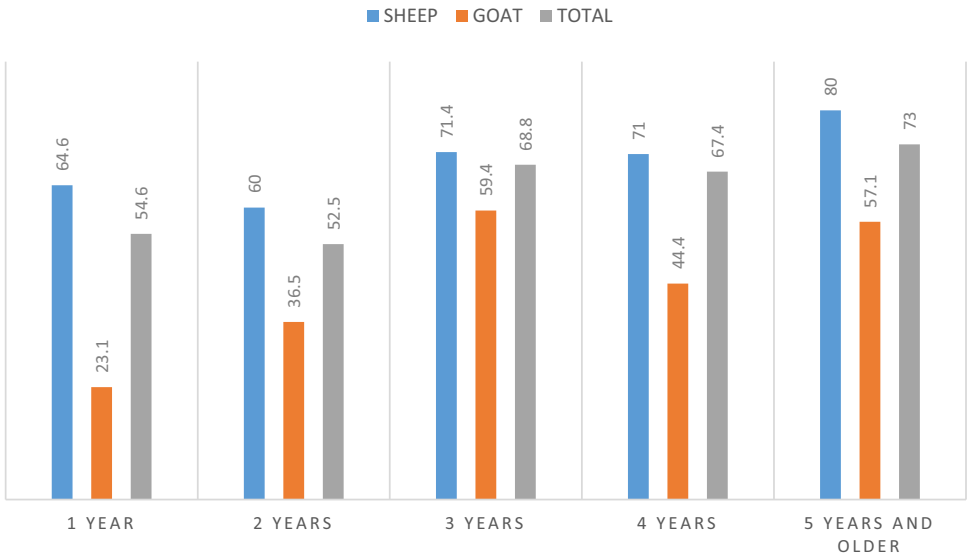

Fig. 3 Distribution of *Anaplasma ovis* positivity according to the ages

characterization of circulating *Anaplasma* species are essential not only for improving animal health management but also for anticipating emerging zoonotic threats.

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Author Contribution M.K., M.A., B.K and S.Ö. wrote the main manuscript text, M.A., S.Ö. prepared Fig. 1 and M.K., B.K. prepared Figs. 2-3. All authors reviewed the manuscript.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of Interest The authors declare no competing interests.

Ethics Committee Approval This study was approved by the Niğde Ömer Halisdemir University Local Ethics Committee for Animal Experiments (date: 06.02.2018, no: 2018/02).

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