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A research on *Babesia* and *Theileria* species in sheep and goats of Kırıkkale province through molecular methods

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Abstract: The aim of the present study was to determine presence and prevalence of *Babesia* and *Theileria* species in the sheep and goats of stock farming in the province of Kırıkkale, which is in Central Anatolia Region of Turkey through microscopic examination and RLB method. Venous blood samples of 3 mL were collected from *vena jugularis* of 300 sheep and 100 goats into tubes containing EDTA. Blood smears were prepared from samples; and stained by Giemsa and examined for *Theileria* and *Babesia* under a light microscope. Genomic DNAs were extracted from blood samples with EDTA through suitable methods; a region with a length of 360 to 430 bp on variable region V4 of the 18 ssu rRNA gene of *Theileria* and *Babesia* species were proliferated; PCR products obtained were hybridized on a membrane where species-specific probes. Piroplasms were detected in 54 (18%) sheep and in none of the goats during microscopic examination of blood smears. With the RLB technique; *T. ovis* 70%, *Theileria* sp. 2.3%, *B. ovis* 5.3%, *T. ovis* + *B. ovis* 2.7% were detected in sheep. In goats; *T. ovis* was detected at a rate of 1%. Consequently, this study is the first for detection of *Theileria* and *Babesia* species in sheep and goats in Kırıkkale. Presence of *T. ovis* and *B. ovis* in the sheep, and *T. ovis* in goats was detected in Kırıkkale.

Key words: Babesia, goat, RLB, sheep, Theileria

1. Introduction

Babesiosis and Theileriosis which are protozoan diseases transmitted by ticks are responsible from significant economic losses in livestock all over the world [1]. Theileriosis is transmitted to their hosts transstadially by Ixodid ticks, while babesiosis is transmitted both transovarially and transstadially [2]. The natural conditions of Turkey cause many tick-borne infections to occur in humans and animals in different regions. A total of 19 tick-borne infections have been reported from seven geographical regions of Turkey. Some tick borne infections, especially babesiosis and theileriosis, have been reported to be common in many parts of Turkey [3]. Up to date, *T. lestoquardi*, *T. ovis*, *T. separata*, *T. uilenbergi* and *T. luwenshuni, T. recondita, T. annulata* [4–6] of *Theileria* species, and *B. ovis*, *B. motasi*, *B. crassa, B. taylori* and *B. foliata* species of *Babesia* were detected in the sheep and goats; it was detected that *T. lestoquardi (=T.hirci)*, *T. uilenbergi* and *T. luwenshuni* cause clinical infections with higher mortality and morbidity [7, 8], *T. ovis*, and *T. separata* cause subclinic infections [9], *B. ovis* has a higher pathogenicity, *B. motasi* presents a lower pathogenicity than *B. ovis* [7], and two subspecies with different pathogenicity may exist (10), *B. crassa* is the nonpathogenic form [11].

Furthermore, three genotypes of *Theileria* including *Theileria* sp. OT1, *Theileria* sp. OT3 and *Theileria* sp. MK were detected in healthy sheep and goats in some countries including Turkey [12–18].

Usually, the diagnosis of babesiosis and theileriosis in sheep and goats is based on clinical signs and microscopic examination of blood smears. While these methods are useful in acute cases, they are insufficient for porter animals [19]. In addition, false positive and false negative results were observed in serological methods for the diagnosis of carrier animals [20,21]. For these reasons, it has been necessary to use molecular diagnostic methods to determine babesiosis and theileriosis in epidemiological studies [22]. The Reverse Line Blotting (RLB) technique is a technique that allows simultaneous identification of *Theileria* and *Babesia* species. This method has been used successfully in the identification of *Theileria* and *Babesia* species [12,16,21].

Several studies were conducted in different provinces of Turkey in order to determine theileriosis and babesiosis in the sheep and goats [1,6,13,14,16,19,22]. The aim of the

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present study was to determine presence and prevalence of *Babesia* and *Theileria* species in the sheep and goats of stock farming in the province of Kırıkkale, which is in Central Anatolia Region of Turkey through microscopic examination and RLB method.

2. Materials and methods

2.1. Collection of blood samples

Within the scope of the study, sheep and goat farms were visited in the province of Kırıkkale, and blood samples were collected from 300 sheep and 100 goats after informing the animal owners between June 2014 and October 2014. Stratified random sampling method was used during blood sample collection, and 10% sampling was performed from each farm, and number of samples collected from each farm were calculated individually. Selection of animals raised in the aforesaid province was regarded. The animal owner was interviewed in order to get information including age, race, sex, previous infection, history of acaricide use; and information gathered were recorded.

The blood to be used for DNA extraction was collected from the *vena jugularis* of each animal, transmitted into sterile, vacuum tubes containing EDTA of 3 mL, and taken to Parasitology laboratory of Veterinary Faculty within Kırıkkale University within a cold chain; the samples were stored at –20 °C until DNA extraction. Thin blood smear was prepared from blood samples for microscopic examination.

Among 300 sheep of which blood samples were collected, 198 (66%) were female, 102 (34%) were male; 73 female and 27 male goats were included into the study. The ratios of age at and below 1 year, and over 1 year were 24.3% and 75.7%, respectively in sheep; such ratios were 28% and 72%, respectively in goats.

2.2. Laboratory analysis

2.2.1. Blood smear staining

Blood smears prepared were fixed by methyl alcohol for 5 min, and stained by 5% Giemsa stain solution at room temperature for 40 min. The stained smears were examined for presence of *Babesia* sp. and *Theileria* sp. piroplasms by 100× magnification of the light microscope (Leica DM 750). Two hundred areas with homogenous distribution of erythrocytes, which were selected randomly, were reviewed on each smear prepared from animals; the animal was accepted positive even one microorganism was detected [23].

2.2.2. DNA extraction

Genomic DNA was extracted from blood samples by using a commercial DNA isolation kit (Macherey-Nagel, Blood Genomic DNA extraction kit, Germany) according to the manufacturer's instructions. DNA samples were stored at –20 °C until used in PCR.

2.2.3. Polymerase chain reaction

In the present study, general primers (RLB-F2 5' GACACAGGGAGGTAGTGACAAG 3' and RLB-R2 5'-biotin-CTAAGAATTTCACCTCTGACAGT 3') (Bioneer, (Daejeon, South Korea) which amplify a part with a varying length of 360 and 430 bp from variable V4 region of 18S ssu rRNA gene of *Babesia* and *Theileria* parasite species as reported by Georges et al. [24]. The reverse primer to be used for RLB procedure was marked with primer biotin. *Babesia* sp. and *Theileria* sp. isolates in our laboratory were used as positive controls, and sterile ultrapure water was used as negative control. Polymerase chain reaction was performed in total reaction volume of 25 µL containing 2.5 µL 10X PCR buffer (500 mM KCl, 100 mM Tris-HCl (pH:8.8), 1% Triton X-100), 2 µL 50mM $MgCl_2$, 0.5 µL 10 mM dNTP mix, 0.5 µL Taq DNA polymerase (5U/µL, 500U) (Vivantis, Malaysia), 0.35 µL from each primer (100 pmol/µL) and 5 µL template DNA. A touchdown PCR program was used in order to minimize nonspecific amplification. The protocol included 2 min at 94 °C, two cycles of 20 s at 94 °C, 30 s at 67 °C, and 30 s at 72 °C. The annealing temperature was lowered every two cycle with 2 °C to a touchdown temperature of 57 °C. Then, an additional 40 cycles each consisting of 20 s at 94 °C, 30 s at 57 °C, and 30 s at 72 °C were performed. The final extension was for 7 min at 72°C [25].

Five microliters of PCR product were visualized by UV transillumination in a 1% agarose gel after electrophoresis and staining ethidium bromide. Remaining PCR products were stored at +4 °C until use in RLB.

2.2.4. Reverse line hybridization

All probes were synthesized by Bioneer (Daejeon, South Korea) and contained N-terminal N-trifluacetamidohexyl-cyanoethyl, N, N-diisopropyl phosphoramidite (TFA)-C6 aminolinker at the 5' ends to bind to the negatively charged Biodyne C membrane (Pall Biosupport Group, Ann Arbor, MI) as reported by Nagore et al. [12], Gubbels et al., [21] and Schnittger et al. [26]. The sequences of probes used is presented in Table 1.

RLB procedure was performed as reported by Gubbels et al. [21]. Biodyne C membrane was activated for 10 min at room temperature by incubation in 10 mL of 16 % EDAC (1-ethyl-3-(3-dimethylamino-propyl) carbodiimide) (BioShop, Canada) and placed in a miniblotter after washed in sterile distilled water. Probes were diluted to give 50–1200 pmol/150µL in 500 mM NaHCO3 (pH 8.4). Blue ink diluted with 2 × SSPE (AppliChem, Germany) and 0.5% sodium dodecyl sulfate (SDS) with a rate of 1% filled in miniblotter the first and last channel slots. One hundred fifty microliters of each probes was filled in the miniblotter channel slots. After membrane was incubated at room temperature for 10 min the liquid in the channels

Table 1. Sequence of probes used in reverse line blotting test.

Probes	Nucleotide sequence (5'…….3')	Reference
Catchall	TAATGGTTAATAGGA(AG)C(AG)GTTG	21
***Theileria* sp.**	TGATGGGAATTTAAACC(CT)CTTCCA	12
T. ovis	TTTTGCTCCTTTACGAGTCTTTGC	12
T. lestoquardi	ATTGCTTGTGTCCCTCCG	26
T. uilenbergi	TGCATTTTCCGAGTGTTACT	26
T. luwenshuni	TCGGATGATACTTGTATTATC	26
***Theileria* sp. OT1**	ATCTTCTTTTTGATGAGTTGGTGT	12
***Theileria* sp. OT3**	ATTTTCTCTTTTTATATGAGTTTT	12
***Theileria* sp. MK**	CATTGTTTCTTCTCATGTC	12
***Babesia* sp.**	CCT(GT)GGTAATGGTTAATAGGAA	26
B. ovis	GCGCGCGGCCTTTGCGTTTACT	12
B. motasi	ATTGGAGTATTGCGCTTGCTTTTT	12
B. crassa	TTATGGCCCGTTGGCTTAT	26

was removed by aspiration. Membrane removed from miniblotter was inactivated in 100 mM NaOH for 10 min. Then it was washed in 2 × SSPE/0.1 % SDS for 5 min at 60 °C.

Twenty microliters of PCR product was diluted in 2 × SSPE/0.1 % SDS with a total 150 µL volume, and it was denatured for 10 min at 99 °C in a thermal cycler. This PCR products were quickly cooled on ice to avoid readhesion. The membrane was washed with 2 × SSPE/0.15% SDS for 5 min at room temperature and placed in a Miniblotter (Immunetics MN45, USA). Denatured PCR product were filled into the slots and hybridized for 1 h at 42 °C in incubator. Then PCR product were aspirated and membrane was washed twice at 52 °C for 10 min in 2 × SSPE/0.5% SDS. The membrane was incubated in 10 mL of 1:4000 diluted peroxidase-labelled streptavidin-POD Konjugat (Amersham, UK) in 2 × SSPE/0.5 % SDS for 30 min at 42 °C. Following incubation, membrane washed twice at 42 °C for 10 min in 2 × SSPE/0.5%SDS and twice at room temperature for 5 min in 2 × SSPE. Membrane was incubated in 10 mL of ECL solution (Amersham, UK) for 1 min, and then it was taken on a hard surface and covered with acetate. The membrane was incubated under an ECL hyperfilm for 1–10 min depending on the strength of signals in a dark room. ECL hyperfilm was processed with developer and fixer solutions. Black spots formed on the films at the intersections of the probe and the PCR rows considered positive.

2.2.5. Statistical analysis

All data were reviewed by a frequency table before significance tests. Chi-square test was used to investigate the difference for presence of parasite detected through microscopic examination and RLB method according to district, age and sex. All statistical analyses were reviewed by an error ratio of 5%. All analyses were performed through Stata 12/MP4 (License No: 50120500264) statistical package program.

3. Results

Piroplasms were detected in 54 (18%) sheep; however, all goats were found negative for piroplasms. Among samples exposed to RLB method, 80.3% of sheep samples and 1% of goat samples were positive for parasite. All samples detected as positive in microscopic examination were also positive in RLB. In microscopic examination; 12.3% *Theileria* sp., 3.3% *Babesia* sp. single and 2.3% *Theileria* spp + *Babesia* sp. mix infection were detected in sheep. No parasites were detected in goats by microscopic examination. In the RLB method; among sheep *T. ovis* was detected 70%, *B. ovis* in 5.3%, *Theileria* sp. in 2.3%, and *T. ovis* + *B. ovis* mixed to be in 2.7%. With the same method, among goats *T. ovis* was detected 1%. The parasites and their ratio detected by microscopic examination and RLB results were presented in Table 2. The goat of which *T. ovis* was detected is a female hair goat over 1 year of age. Since a single sample was detected positive in goats, statistical significance tests were implemented on sheep only.

In terms of the presence of blood parasites, the highest positivity was detected in Karakecili by microscopic examination, and Celebi in the RLB method. There was a statistically significant relationship between the districts of the province in terms of parasites detected both by microscopic examination and by RLB method ($p < 0.05$). According to the microscopic examination the

district of Keskin which had the highest negativity rate was statistically different from other districts. The district of Karakecili was statistically significant for *Theileria* sp. than other districts. According to the RLB method; The district of Balıseyh which had the highest negativity rate was statistically different from other districts. The district of Yahsihan was similar to the district of Balıseyh for *B. ovis* which was statistically significant when compared with other districts. The district of Balıseyh was statistically significant for *T. ovis* + *B. ovis* than other districts (Table 3).

In both microscopic examination and RLB method, the rate of positivity in female sheep was higher than in males. However, there was no statistically significant relationship between sexes in terms of parasites detected by both method ($p > 0.05$). In microscopic examination, 14.6% *Theileria* sp., 3% *Babesia* sp., and 2.5% *Theileria* sp.

Table 2. Parasites and their rates detected by microscopic examination and RLB methods in sheep and goats.

		Microscopic examination (ME)				**Reverse line blotting (RLB)**				
		Theileria sp.	*Babesia* sp.	*Theileria* sp. +*Babesia* sp.	Negative	*T. ovis*	*B. ovis*	*T.ovis*+ *B.ovis*	*Theileria* sp.	Negative
Sheep	n	37	10	7	246	210	16	8	7	59
	%	12.3	3.3	2.3	82	70	5.3	2.7	2.3	19.7
Goat	n	0	0	0	100	1	0	0	0	99
	%	0	0	0	100	1	0	0	0	99

Table 3. Comparison of microscopic examination and RLB results by district in sheep.

			Microscopic examination (ME)				**RLB**					**Total**
			***Theileria* sp.**	***Babesia* sp.**	***Theileria* sp.+ *Babesia* sp.**	**Negative**	***T.ovis***	***B.ovis***	***T.ovis*+ *B.ovis***	***Theileria* sp.**	**Negative**	
District	**Sulakyurt**	n	7	2	1	26	29	2	2	1	2	36
		%	19.4	5.6	2.8	72.2	80.6	5.6	5.6	2.8	5.6	100.0
	Celebi	n	2	2	0	17	19	2	0	0	0	21
		%	9.5	9.5	0.0	81.0	90.5	9.5	0.0	0.0	0.0	100.0
	Keskin	n	1	0	1	46	32	2	1	1	12	48
		%	2.1	0.0	2.1	95.8	66.7	4.2	2.1	2.1	25.0	100.0
	Karakecili	n	8	0	1	14	20	0	1	0	2	23
		%	34.8	0.0	4.3	60.9	87.0	0.0	4.3	0.0	8.7	100.0
	Yahsihan	n	4	1	1	15	14	4	1	1	1	21
		%	19.0	4.8	4.8	71.4	66.7	19.0	4.8	4.8	4.8	100.0
	Bahsili	n	0	0	0	18	17	0	0	0	1	18
		%	0.0	0.0	0.0	100.0	94.4	0.0	0.0	0.0	5.6	100.0
	Center	n	3	0	0	33	23	1	0	3	9	36
		%	8.3	0.0	0.0	91.7	63.9	2.8	0.0	8.3	25.0	100.0
	Delice	n	4	1	0	42	41	1	0	1	4	47
		%	8.5	2.1	0.0	89.4	87.2	2.1	0.0	2.1	8.5	100.0
	Balıseyh	n	8	4	3	35	15	4	3	0	28	50
		%	16.0	8.0	6.0	70.0	30.0	8.0	6.0	0.0	56.0	100.0
Total		n	37	10	7	246	210	16	8	7	59	300
		%	12.3	3.3	2.3	82.0	70.0	5.3	2.7	2.3	19.7	100.0
			Chi-square = 42.462, p = 0.011				Chi-square = 97.452, $p < 0.001$					

+ *Babesia* sp. were detected in female sheep, while these rates were 7.8%, 3.9% and 2% in males, respectively. In the RLB method; 72.2% *T. ovis*, 4.5% *B. ovis*, 2.5% *T. ovis* + *B. ovis* and 2% *Theileria* sp. were detected in female sheep. In male sheep, these rates were determined as 65.7%, 6.9%, 2.9% and 2.9%, respectively (Table 4).

While the rate of positivity in microscopic examination is higher in sheep >1 year old than in sheep ≤ 1 year old, it is the opposite in RLB method. However, there was no statistically significant relationship between age groups in terms of parasites detected by both method ($p > 0.05$). In microscopic examination; *Theileria* sp. was 13.7%, *Babesia* sp. 3.1%, and *Babesia* sp. + *Theileria* sp. 3.1% in sheep >1 year old, while these rates were 8.2%, 4.1% and 0%, in sheep ≤1 year old, respectively. In the RLB method; in sheep older than 1 year, *T. ovis* was 67.8%, *B. ovis* 4.8%, *T. ovis* + *B. ovis* 3.5% and *Theileria* sp. 2.2%. In the same method, in sheep 1 year and younger, *T. ovis* was 76.7%, *B. ovis* 6.8%, *Theileria* sp. 2.7%, while no mixed parasite (*T. ovis*+*B. ovis*) was found (Table 5).

4. Discussion

Several studies were conducted in different provinces of Turkey in order to determine *Theileria* and *Babesia* in the sheep and goats. Such studies were performed through microscopic examination, serological and molecular methods. *T. ovis*, *Theileria* sp. MK, *Theileria* sp. OT3, *Theileria* sp., *T. uilenbergi*, *T. lestoquardi* ve *T. annulata*, *B. ovis*, *B. crassa*, *B. motasi* and *B. separata* were detected in the sheep in Turkey by these studies [1,6,16,19,27–29]. In the sample obtained from sheep and goat samples in province of Kırıkkale and its districts, *T. ovis, Theileria* sp., *B. ovis* and *T. ovis*+*B. ovis* which were also detected in different regions of Turkey were detected.

The studies performed through microscopic examination in different regions of Turkey revealed piroplasm ratios of *Theileria* sp., *Babesia* sp. and *Theileria* sp.+*Babesia* sp. as 3.91%–37.55% [16,30–32], 0% –27.35% [16,33], and 3.12% [34]. These ratios in goats were detected 0–9.09, 0–12.2, 3.03, respectively [1,16,31,34]. In the present study, *Theileria* sp. was 12.3%, *Babesia*

Table 4. Comparison of microscopic examination and RLB results by sex in sheep.

			Microscopic examination (ME)				RLB					
			***Theileria* sp.**	***Babesia* sp.**	***Theileria* sp.+ *Babesia* sp.**	**Negative**	***T.ovis***	***B.ovis***	***T.ovis+ B.ovis***	***Theileria* sp.**	**Negative**	**Total**
Sex	**Female**	n	29	6	5	158	143	9	5	4	37	198
		%	14.6	3.0	2.5	79.8	72.2	4.5	2.5	2.0	18.7	100.0
	Male	n	8	4	2	88	67	7	3	3	22	102
		%	7.8	3.9	2.0	86.3	65.7	6.9	2.9	2.9	21.6	100.0
Total		n	37	10	7	246	210	16	8	7	59	300
		%	12.3	3.3	2.3	82.0	70	5.3	2.7	2.3	19.7	100.0
			Chi-square = 3.123, p = 0.373				Chi-square = 1.661, p = 0.798					

Table 5. Comparison of microscopic examination and RLB results by age in sheep.

			Microscopic examination (ME)				RLB					
			***Theileria* sp.**	***Babesia* sp.**	***Theileria* sp.+ *Babesia* sp.**	**Negative**	***T.ovis***	***B.ovis***	***T.ovis+ B.ovis***	***Theileria* sp.**	**Negative**	**Total**
Age	**≤1**	n	6	3	0	64	56	5	0	2	10	73
		%	8.2	4.1	0.0	87.7	76.7	6.8	0.0	2.7	13.7	100.0
	>1	n	31	7	7	182	154	11	8	5	49	227
		%	13.7	3.1	3.1	80.2	67.8	4.8	3.5	2.2	21.6	100.0
Total		n	37	10	7	246	210	16	8	7	59	300
		%	12.3	3.3	2.3	82.0	70.0	5.3	2.7	2.3	19.7	100.0
			Chi-square = 4.128, p = 0.248				Chi-square = 5.425, p = 0.246					

sp. was 3.3%, and *Theileria* sp.+*Babesia* sp. was 2.3% in sheep; however, no piroplasm was detected in goats by microscopic examination. The results obtained by microscopic examination in both sheep and goats included into the present study were similar to results of other studies conducted in Turkey.

The first study to determine theileriosis in sheep and goats in Turkey was carried out by Aktas et al. [35]. Presence and prevalence of *Theileria* and *Babesia* species were analysed through PCR, Nested-PCR and RLB techniques in different provinces of Turkey. Such studies revealed that the most common species in sheep and goats in Turkey was *T. ovis* [16,27,32,35]. Molecular studies conducted in Turkey detected *T. ovis* by 27.34% to 58.79% and *B. ovis* by 0% to 10.66% in sheep [1,16,19,36,37]. Such ratios were detected as 70% and 5.3% in the present study. The ratios of *B. ovis* and *T. ovis* in goats were 0% to 3% and 3.88% to 17%, respectively [1,16,19,36]. These ratios were 0% and 1% in the present study. The present study has the property to be the only study that has presented the highest positivity for *T. ovis* in Turkey. Such outcome revealed that the most common species in small ruminants of Turkey is *T. ovis*.

In the molecular analysis for *T. ovis*+*B. ovis* up to date, the ratio between 0.35% and 21% was detected in sheep, and 0% and 1.3% was detected in goats in Turkey [6,19,38]. In the present study, mixed infection of *T. ovis*+*B. ovis* was detected by 2.7% in sheep; however, no mixed infection was detected in goats.

Higher positivity was detected in female sheep by microscopic examination and RLB. However, there was not any significant difference between females and males for positivity. Higher rate of positivity in female animals may be connected to the fact that number of sample collected from females was higher than males. The only positive sample detected in goats belonged to a female animal.

While the rate of positivity in microscopic examination is higher in sheep >1 year old than in sheep ≤ 1 year old, it is the opposite in RLB method. However, there was no statistically significant relationship between age groups in terms of parasites detected by both method. Shortly after the sheep are born, they start grazing on the pasture, so vector ticks are encountered at all ages. Therefore, parasite was found in both ≤1 and over 1-year-old sheep.

Statistically significant differences were detected between the districts through both methods. The cause may be the difference in going out to the pastureland and meeting with vector ticks depending on the regions.

In the present study, it has been investigated the presence and distribution of *Babesia* and *Theileria* species in sheep and goats by using both microscopic examination and RLB. Piroplasms were detected in 54 (18%) sheep, while all goats were found negative for piroplasms by microscopic examination. Among samples exposed to RLB method, 80.3% of sheep samples and 1% of goat samples were positive for parasite. These results show that RLB a significantly more sensitive than microscopic examination. RLB is more sensitive than microscopic examination for determining of blood parasites in sheep and goat as those in previous studies [1,16,29].

In the RLB test, 7 (%2.3) sheep samples signaling with the *Theileria* sp. probe did not signal with the *Theileria* species specific probes. This may be due to the low sensitivity of the probes used, or to the removal of the probes from the membrane during the washing and reuse of the membrane. Yıldırım et al. (39), similar to this situation, found that in the RLB method, 16 samples gave positivity at the *Babesia* sp. level, but did not react at the species level. This situation was thought to be due to probes falling from the membrane during washing and reuse of the membrane or the low sensitivity of the probes used. However, in samples detected as *Theileria* sp. in RLB, a species or strain other than the species-specific probes used may be present, or it may be due to a mutation occurring in the existing species.

In present study; in blood samples collected from sheep, 18% positivity was detected in microscopic examination and 80.3% in RLB method, while these rates were 0% and 1% in blood samples collected from goats, respectively. The reason why blood protozoa are less common in goats in the region; these animals feed on pens rather than graze and therefore encounter fewer vector ticks.

Consequently, in this study *Theileria* sp., *T. ovis* and *B. ovis* were determined in sheep and only *T. ovis* species in goats in Kırıkkale. It was concluded that *T. ovis* is more prevalent in sheep of the province; therefore, awareness of animal breeders should be increased for a careful and efficient tick counteracting. Outcomes of the present study revealed the necessity of further investigation on economic loss caused by *T. ovis*.

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