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ORIGINAL PAPER

Molecular identification of *Theileria* and *Babesia* in sheep and goats in the Black Sea Region in Turkey

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Abstract This study was carried out to investigate presence and distribution of *Theileria* and *Babesia* species via microscopic examination and reverse line blotting (RLB) techniques in sheep and goats in the Black Sea region of Turkey. For this purpose, 1,128 blood samples (869 sheep and 259 goats) were collected by active surveillance from sheep and goats in different provinces of various cities in the region in the years 2010 and 2011. Smears were prepared from the blood samples, stained with Giemsa, and examined under the light microscope for *Theileria* and *Babesia* piroplasms. The genomic DNAs were extracted from blood samples. The length of 360–430-bp fragment in the variable V4 region of 18S SSU rRNA gene of *Theileria* and *Babesia* species was amplified using the gDNAs. The polymerase chain reaction products were hybridized to the membrane-connected species-specific probes. A total of 38 animals (3.37 %) including 34 sheep (3.91 %) and 4 goats (1.54 %) were found to be positive for *Theileria* spp. piroplasms in microscopic examination of smears while *Babesia* spp. piroplasm could not detected. Infection rates were 34.64 % in sheep, 10.04 % in goats, and totally 28.99 % for *Theileria ovis* while 0.58 % in sheep and totally 0.44 % for *Babesia ovis*. However, *Theileria* sp. OT3 was detected in 2.65 % of sheep and 2.04 % of all animals; besides *Theileria* sp., MK had 0.58 % prevalence in sheep and 0.77 % in goats, with a total 0.62 % with RLB. Although *T. ovis* and *Theileria* sp. MK were determined in both sheep and goats, *B. ovis* and *Theileria* sp. OT3 were observed only in the sheep. These results provide the first detailed molecular data for sheep and goat theileriosis and babesiosis in the region.

Nucleotide sequence data reported in this paper are available in GenBank, EMBL 21, and DDBJ databases under accession numbers from JQ867384 to JQ867387.

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Introduction

Tick-borne hemoprotozoan parasites are widespread in tropical and subtropical climates. Piroplasmosis caused by *Theileria* and *Babesia* species leads to clinical and subclinical infections in domestic and wild animals with high mortality and morbidity (Friedhoff 1997; Jongejan and Uilenberg 2004). *Theileria lestoguardi*, *Theileria uilenbergi*, and *Theileria luwenshuni* are highly pathogenic for small ruminants and cause lenphoproliferative diseases with high mortality and morbidity while *Theileria ovis* and *Theileria separata* have low pathogenicity (Friedhoff 1997; Hoosmand-Rad and Hawa 1973; Luo and Yin 1997; Schnittger et al. 2004; Yin et al. 2007, 2008). *Theileria* sp. OT1, *Theileria* sp. OT3, and *Theileria* sp. MK are newly described *Theileria* species; also there is no information about their vector and pathogenicity (Ahmed et al. 2006; Altay et al. 2007b; Nagore et al. 2004; Yin et al. 2008).

Ovine babesiosis is the most important hemoparasitic tick-borne disease of small ruminants caused by *Babesia ovis*, *Babesia motasi*, *B. crassa* (Alani and Herbert 1988; Friedhoff 1997; Levine 1985; Uilengberg et al. 1980; Hashemi-Fesharki and Uilenburg 1981) *Babesia taylori* (Sarwar 1935), *Babesia foliata* (Ray and Raghavachari

1941), and recently described *Babesia* sp. Xinjiang and *Babesia* sp. BQ1 (Lintan) (Guan et al. 2009). *B. ovis* and *Babesia* sp. Xinjiang are highly pathogenic especially in sheep and cause severe infections which are characterized by fever, anemia, icterus, and hemoglobinuria (Friedhoff 1997; Guan et al. 2009).

Diagnosis of theileriosis and babesiosis in acute cases are based on clinical signs and microscopic examination of Giemsa-stained blood smears (Guo et al. 2002; Yin et al. 2003). Animals with theileriosis and babesiosis become porter after infection, and the value of these animals in the population is important in the epidemiology of disease (Brown 1990). It has been proposed that microscopic methods are insufficient; also, false-positive and false-negative results were observed in serological methods for diagnosis of porter animals (Burridge et al. 1974; Gubbels et al. 1999). For these reasons, it has been needed using molecular diagnostic methods for the epidemiological studies to determine *Theileria* and *Babesia* infections (Aktas et al. 2005b; Heidarpour Bami et al. 2009).

In the recent years, it has began to be used some molecular techniques like polymerase chain reaction (PCR)-based reverse line blotting method (RLB) which allows diagnozing several agents simultaneously and discovery of new species and genotypes used to assess parasitic DNA for diagnosis of subclinical *Theileria* and *Babesia* infections. Because more than one species could not be detected simultaneously, new techniques which allow diagnosis multiply agents in blood at the same time as needed. RLB method was developed in 1995 for differentiate four *Borrelia* species in ticks (Rijpkema et al. 1995). This method successfully used for diagnosis *Theileria* and *Babesia* species and is increasing use in the parasitological researches (Schnittger et al. 2004; Nagore et al. 2004; Altay et al. 2008a, b; Gubbels et al. 1999).

Piroplasmosis has been reported in some parts of Turkey (Aktas et al. 2005b, 2007; Altay et al. 2007b, 2012; Inci et al. 2010), but there is no detailed molecular survey on ovine theileriosis and babesiosis in Black Sea Region of Turkey. In this study, we investigated presence and frequency of *Theileria* and *Babesia* species infecting small ruminants in the region using microscopy and RLB.

Materials and methods

Study area and collection of field samples

The Black Sea region covers 18 % of Turkey's land with an area of 143,537 km^2 and the third largest region in terms of area. The region which has the highest rainfall is the first place between the regions with respect to the existence of the forest with the rate of 27 %. The region is dominated by two different climates. Inland areas of the region have a terrestrial climate, and the stretch of coast has cool summers and mild winters.

This study was planned to represent the whole of Black Sea region. The number of sheep and goats in the provinces was obtained from the database of Turkey Statistical Institute. Sheep and goat populations were seen 1,063,666 and 139,939, respectively, in the region (Anonymous 2011). Bolu, Kastamonu, Corum, Samsun, Tokat, Giresun, and Bayburt provinces were selected as study areas according to the evaluation criteria of geographical distribution of the provinces, animal population, climatic conditions, and vegetation. Therefore, at least 121 sheep and 32 goats were included from each province in the study (Fig. 1 and Table 1).

Blood samples were collected into tubes containing EDTA from 1128 clinically healthy small ruminants (869 sheep and 259 goat) randomly selected from 43 towns in Bolu (Center, Gerede, Kıbrısçık, Mengen, Seben, and Mudurnu), Kastamonu (Center, Araç, Bozkurt, Çatalzeytin, Daday, İnebolu, and Taşköprü), Çorum (Center, Alaca, Dodurga, Kargı, Osmancık, Sungurlu, and Uğurludağ), Samsun (Center, Bafra, Havza, Ladik, Tekkeköy, and Terme), Tokat (Center, Almus, Erbaa, Niksar, Reşadiye, Turhal, and Zile), Gireun (Center, Alucra, Çamoluk, Dereli, Keşap, Piraziz, and Şebinkarahisar), and Bayburt (Center, Aydıntepe, and Demirözü) provinces in the Black Sea Region of Turkey in 2010 and 2011, spring and summer, when ticks were active (Fig. 1 and Table 1).

DNA extraction

Blood samples were defrosted and homogenized at room temperature for 10–15 s. DNA extractions were performed by a commercial DNA isolation kit or the manual method. In manual method, 125 μl of blood was added to 250 μl of lysis solution (0.32 M sucrose, 0.01 M Tris, 0.005 M $MgCl_2$, 1 % Triton X-100, pH 7.5). The mixture was centrifuged at 11,600×*g* for 1 min. The pellet was washed three times by centrifugation with 250 μl lysis buffer. The supernatants were discarded, and the final pellets were resuspended in 100 μl of PCR buffer (50 mM KCl, 10 mM Tris–HCl (pH 8), 0.1% TritonX-100, pH 8.3). Proteinase K (50 μg/ml) was added to the pellet suspension, and the mixture was incubated at 56 °C for 1 h. Finally, the samples were heated at 100 °C for 10 min (Aktas et al. 2005a). Genomic DNAs were maintained at −20 °C until use.

Polymerase chain reaction and agarose gel electrophoresis

For the amplification of *Theileria* and *Babesia* species, one set of primers was used to amplify an approximately 360–430 bp fragment of the hypervariable V4 region of the 18S

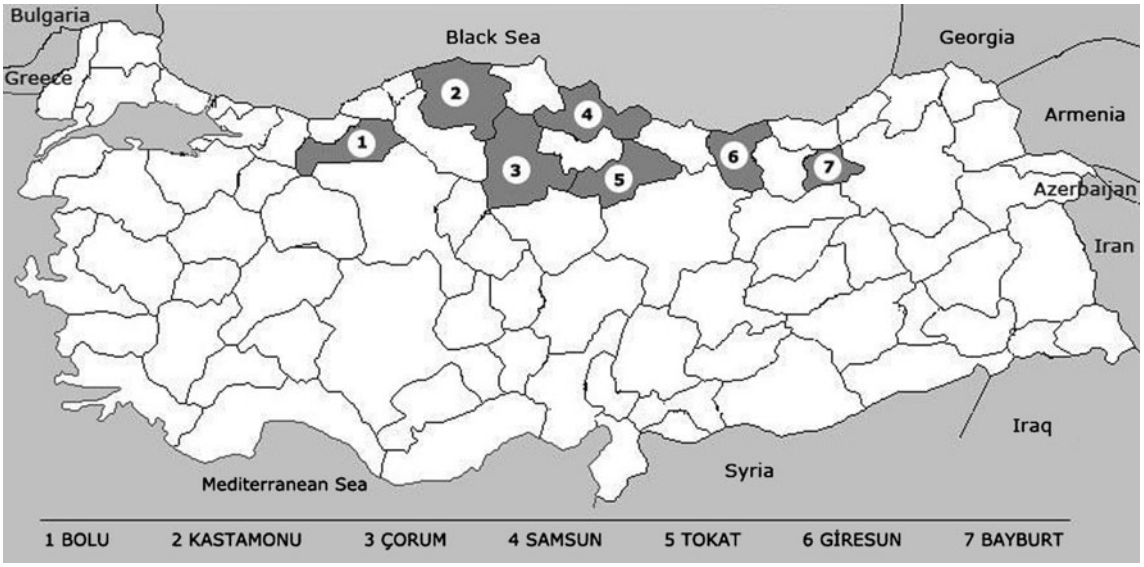

Fig. 1 Map of the provinces, showing the locations investigated in the study

rRNA gene. The forward [RLB-F2 (5'-GACACAGGGAG GTAGTGACAAG-3')] and the reverse [RLB-R2 (biotin-5'-CTAAGAATTTCACCTCTGACAGT-3')] primers were described by Georges et al. (2001). Touchdown PCR is a modification of conventional PCR. It involves the use of an annealing temperature that is higher than the target optimum in early PCR cycles. The annealing temperature was decreased every second cycle with 2 °C to a "touchdown" temperature of 57 °C. This allows for the enrichment of the correct product over any non-specific product.

The PCR was performed in a touchdown thermocycler in a total reaction volume of 25 μl containing PCR buffer [750 mM Tris–HCl (pH 8.8), 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20], 5 mM $MgCl_2$, 125 μM deoxynucleotide triphosphates, 1.25 U Taq DNA polymerase, primers (20 pmol/μl), and template DNA. Five microliters of PCR product were visualized by UV transillumination in a 1.5 % agarose gel after electrophoresis and staining with ethidium bromide.

Reverse line blotting (RLB)

Binding of species-specific oligonucleotides (probes) on the biodyne C membrane

Probes of *Theileria*/*Babesia* catchall, *Theileria* spp., *T. ovis, Theileria lestoquardi, T. uilenbergi, T. luwenshuni, Theileria* sp. OT1, *Theileria* sp. OT3, *Theileria* sp. MK, *Babesia* spp., *B. ovis, B. motasi,* and *B. crassa* were used with a range of 200–400 pmol/150 μl concentration and contain N-terminal *N*-(trifluoracetamidohexyl-cyanoethyl,*N*,*N*-diisopropyl phosphoramidite [TFA])-C6 amino linker in the study (Gubbels et al. 1999; Nagore et al. 2004; Schnittger et al. 2004; Altay et al. 2007b).

Biodyne C membrane was activated in 10 ml of 16 % EDAC 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide for 10 min at room temperature and placed in a miniblotter after washed demineralized water. Residual liquid on the membrane was aspirated. One hundred fifty microliters of each probe which diluted to a 50 to 1,200 pmol/150 ml concentration in 500 mM $NaHCO_3$ (pH 8.4) was filled the miniblotter except for the first and last channel slots. India ink diluted with 2×SSPE and 0.5 % sodium dodecyl sulfate (SDS) with a rate of 1% filled the first and last slots. Then, they were incubated for 10 min at room temperature. Liquids in slots were aspirated after incubation. The membrane was inactivated in 100 mM NaOH for 10 min after removing from miniblotter at room temperature. Then it was washed in 2×SSPE/0.1 % SDS for 5 min at 60 °C.

Reverse line blotting

The membrane was washed for 5 min at room temperature with 2× SSPE/0.15 SDS %, and it was placed in the miniblotter with the slots vertical on the previously connected probes. Residual liquid on the membrane was aspirated. Twenty microliters of PCR product was diluted in 2X SSPE/0.1 % SDS with a total 150 μl volume, and it was denatured for 10 min at 99 °C. Denaturated PCR products were cooled on ice immediately for uncombined DNA strands again. Denatured PCR samples were filled into the slots and hybridized on a flat surface for 1 h at 42 °C. PCR products on the membrane were aspirated. The membrane was washed twice in 2× SSPE / 0.5 % SDS for 10 min at 52 °C. The membrane was incubated in 10 ml of 1:4,000-diluted peroxidase-labeled streptavidin in 2× SSPE/0.5 % SDS for 30 min at 42 °C. Then membrane was washed twice in 2× SSPE/0.5 % SDS for 10 min at 42°C and twice in 2×

Table 1 Distribution of *Theileria* and *Babesia* species via RLB and comparison with microscopy and PCR results according to the provinces of the Black Sea Region in sheep and goats

Information of samples			Microscopy result		PCR result	RLB result										
						Total infection				Single infection				Mix infection		
Province	Host	Number of samples	*Theileria* spp.	*Babesia* spp.	Positive	*T. ovis*	*Theileria* sp. OT3	*Theileria* sp. MK	*B. ovis*	*T. ovis*	*Theileria* sp. OT3	*Theileria* sp. MK	*B. ovis*	*T. ovis*+ *B. ovis*	*T. ovis*+*Theileria* sp. OT3	*T. ovis*+*Theileria* sp. MK
Bolu	Sheep	123	9	–	46	66	6	1	–	63	4	–	–	–	2	1
	Goat	40	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	∑	163	9	–	46	66	6	1	–	63	4	–	–	–	2	1
Kastamonu	Sheep	125	3	–	22	35	3	–	2	33	2	–	1	1	1	–
	Goat	38	–	–	1	1	–	–	–	1	–	–	–	–	–	–
	∑	163	3	–	23	36	3	–	2	34	2	–	1	1	1	–
Çorum	Sheep	125	10	–	54	101	3	–	–	99	1	–	–	–	2	–
	Goat	40	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	∑	165	10	–	54	101	3	–	–	99	1	–	–	–	–	–
Samsun	Sheep	123	7	–	33	51	1	3	1	48	–	2	–	1	1	1
	Goat	35	2	–	3	6	–	1	–	5	–	–	–	–	–	1
	∑	158	9	–	36	57	1	4	1	53	–	2	–	1	1	2
Tokat	Sheep	123	2	–	20	30	7	1	2	27	6	–	1	1	1	1
	Goat	39	1	–	6	8	–	1	–	7	–	–	–	–	–	1
	∑	162	3	–	26	38	7	2	2	34	6	–	1	1	1	2
Giresun	Sheep	129	2	–	6	14	–	–	–	14	–	–	–	–	–	–
	Goat	32	1	–	5	10	–	–	–	10	–	–	–	–	–	–
	∑	161	3	–	11	24	–	–	–	24	–	–	–	–	–	–
Bayburt	Sheep	121	1	–	4	4	3	–	–	4	3	–	–	–	–	–
	Goat	35	–	–	1	1	–	–	–	1	–	–	–	–	–	–
	∑	156	1	–	5	5	3	–	–	5	3	–	–	–	–	–
Total	Sheep	869	34	–	185	301	23	5	5	288	16	2	2	3	7	3
	Goat	259	4	–	16	26	–	2	–	24	–	–	–	–	–	2
	∑	1,128	38	–	201	327	23	7	5	312	16	2	2	3	7	5

SSPE for 5 min at room temperature. The membrane was incubated in 10 ml of ECL detection fluid 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 30 s to 30 min depending on the strength of signals in a dark room. Finally, ECL hyperfilm was processed with developer and fixer solutions. Black spots occurring in rows where PCR products and probes were crossed was evaluated as positive to the related species.

Stripping of PCR-products from membrane

The membrane was washed twice in 1 % SDS for 30 min at 85 °C to strip PCR products from the membrane. Finally, the membrane was rinsed in 20 mM EDTA for 15 min at room temperature and stored in 20 mM EDTA at 4 °C for reuse.

Statistical analysis

A Pearson chi-square ($\chi 2$) test was used to evaluate the comparison of microscopy, PCR, and RLB results, assessment of positive rates in the provinces, and evaluation on the basis of the prevalence of *Theileria* and *Babesia* species in the host and cities. Values of $P<0.05$ were accepted statistically significant. The 15.00 SPSS package program was used for the tests.

Results

Distribution and frequency of *Theileria* and *Babesia* species detected by PCR and RLB are presented in Tables 1 and 2 and results demonstrated in Figs. 2 and 3. Of the 1,128 blood samples examined, PCR revealed 201 (17.82 %) positive for piroplasms, whereas 362 (32.09 %) of DNA-amplified products hybridized with the probes for catchall, genera, and species-specific probes. These results demonstrated that there was a significantly higher rate of detection of *Theileria* and *Babesia* infections ($P<0.01$) between microscopy PCR and RLB. Single and mixed infections were detected in animals. Dominant species is *T. ovis. Theileria* sp. OT3 was found as the second dominant species. *Theileria* sp. MK and *B. ovis* were also detected in animals.

Table 2 Provincial distribution of microscopy, PCR, and RLB results

Province		Test					
		Microscopy		PCR		RLB	
	n	+	%	+	%	+	%
Bolu	163	9	5.52	46	28.22	73	44.79
Kastamonu	163	3	1.84	23	14.11	41	25.15
Çorum	165	10	6.06	54	32.73	104	63.03
Samsun	158	9	5.70	36	22.78	63	39.87
Tokat	162	3	1.85	26	16.05	49	30.25
Giresun	161	3	1.86	11	6.83	24	14.91
Bayburt	156	1	0.64	5	3.21	8	5.13
Total	1,128	38	3.37	201	17.82	362	32.09

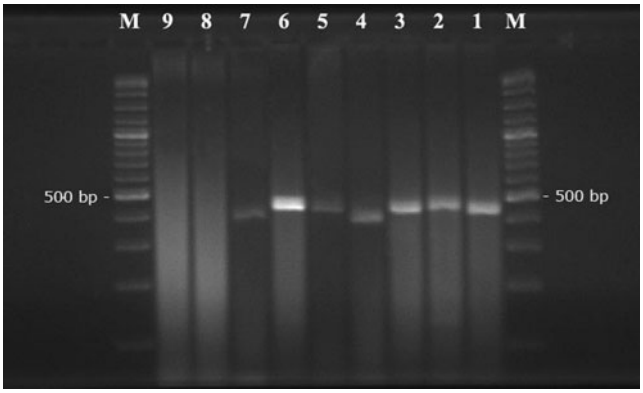

Fig. 2 Ethidium bromide stained agarose gel electrophoresis of amplification products from *Babesia* and *Theileria* species using *Babesia*- and *Theileria*-specific primers RLB-F2 and RLB-R2. *M*: marker (100 bp), *lane 1 T. ovis*, *lane 2 Theileria* sp. OT3, *lane 3 Theileria* sp. MK, *lane 4 B. ovis*, *lane 5 T. ovis+B. ovis* (mix sample), *lane 6 T. ovis* positive sample, *lane 7 B. ovis* positive sample, *lane 8* PCR negative control (distilled water), *lane 9* extraction negative control (genomic DNA of uninfected sheep)

Microscopic examination results

Theileria spp. piroplasms were detected in 34 sheep, 4 goats (1.54 %), and a total of 38 small ruminants (3.37 %); however, no *Babesia* spp. piroplasm was detected in blood smears by microscopic examination. Maximum positive rate was determined in Çorum (6.06 %) and the minimum in Bayburt (0.64 %). According to the results of microscopy, the

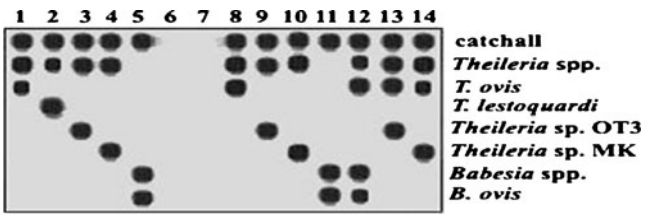

Fig. 3 Reverse line blot assay of the PCR products generated by amplification of genomic DNA from sheep and goat samples infected with *Theileria* and *Babesia* species, and from negative and positive samples as template. Oligonucleotide probes are shown in *rows*, and samples are applied in *columns*. Samples bearing identified single and mixed infections and negative and positive controls are showed as follows: *lane 1*: *T. ovis* (positive control); *2*: *T. lestoquardi* (positive control); *3*: *Theileria* sp. OT3 (positive control); *4*: *Theileria* sp. MK (positive control); *5*: *B. ovis* (positive control); *6*: negative control (genomic DNA of uninfected sheep); *7*: negative PCR control (distilled water); *8*: *T. ovis* (field sample); *9*: *Theileria* sp. OT3 (field sample); *10*: *Theileria* sp. MK (field sample); *11*: *B. ovis* (field sample); *12*: *T. ovis+B. ovis* (mix field sample); *13*: *T. ovis+Theileria* sp. OT3 (mix field sample); *14*: *T. ovis+Theileria* sp. MK (mix field sample)

incidence of *Theileria* spp. in sheep and goats does not have a statistically significant (*P* value 0.064), whereas there was statistically significant (*P* value 0.016) between provinces.

PCR results

The results of PCR with RLB-R2-biotin, RLB-F2 revealed that 201 of 1,128 samples were positive for *Theileria* spp. or *Babesia* spp. One hundred eighty-five of 869 sheep and 16 of 259 goats were found positive by PCR. The highest rate of PCR positivity was observed in animals from Çorum (32.73 %), and the lowest positivity rate was observed in the animals from Bayburt (3.21 %). Positivity rates in sheep and goats and the differences between provinces were statistically significant.

RLB results

T. ovis, *B. ovis*, *Theileria* sp. OT3, and *Theileria* sp. MK species/genotypes were detected in the region via RLB. Maximum infection rate was detected in Çorum (63.03 %) while minimum rate was in Bayburt (5.13 %).

As a result, molecular prevalence of *T. ovis* in sheep was 34.64 % (301/869); in goats, 10.04 % (26/259) with a total 28.99 % (327/1,128). Molecular prevalence of *B. ovis* in sheep was 0.58 % (5/869) and in goats was 0 % (0/259), with a total 0.44 % (5/1128), molecular prevalence of *Theileria* sp. OT3 in sheep was 2.65 % (23/869) and in goats was 0 % (0/259), with a total 2.04 % (23/1,128). Molecular prevalence of *Theileria* sp. MK in sheep was 0.58 % (5/869) and in goats was 0.77 % (2/259) with a total 0.62 % (7/1,128) (Table 1). According to the results of RLB, provincial distribution and frequency of *Theileria* and *Babesia* infections in sheep and goats were statistically significant ($P<0.01$).

Discussion

Diagnosis of piroplasm infections in vertebrate hosts has been mainly carried out by microscopic examination of blood smear. However, piroplasms have similar morphological features. PCR-based molecular techniques allow detection and discrimination of the parasites at low parasitemia rates and mixed infected animals. Additionally, RLB is able to determine more than one parasite in the same samples.

RLB was successfully used for diagnosis of *Theileria* and *Babesia* species, with increasing use in the parasitological researches (Geoges et al. 2001; Nagore et al. 2004; Altay et al. 2007b, 2012; Inci et al. 2010).

New *Theileria* and *Babesia* species or genotypes were discovered in sheep and goats with molecular techniques in recent years. *Theileria* sp. OT1 and *Theileria* sp. OT3 genotypes were determined in small ruminants with RLB technique in Spain (Nagore et al. 2004). *Theileria* sp. MK was discovered in sheep and goats in East Anatolia of Turkey (Altay et al. 2007b). In China, new *Babesia* genotypes which are morphologically and genetically different were detected in sheep and goats and named as *Babesia* sp. Xinjiang and *Babesia* sp. Lintan (Liu et al. 2007; Guan et al. 2009, 2010; Niu et al. 2009).

In this study, microscopic prevalence of theileriosis and babesiosis in sheep and goats was determined as 3.37 % and 0 %, respectively. It was shown that microscopic prevalence of these agents was low when compared with other regions (Inci et al. 1998, 2002; Sevinc and Dik 1996).

Ovine and caprine piroplasmosis has been investigated by RLB in some parts of Turkey (Altay et al. 2007b, 2012; Inci et al. 2010). This method was used for the detection of sheep and goat *Theileria* and *Babesia* species in Kayseri province for the first time in Turkey, and *B. ovis* and *T. ovis* were detected with a rate of 2.7 % and 34.2 % respectively (Içа et al. 2005).

B. ovis (5.43 %), *T. ovis* (34.56 %), *Theileria* sp. MK (1.30 %), and *Theileria* sp. OT3 (0.43 %) were determined via RLB in sheep and goats in Eastern Anatolia, and *Theileria* sp. MK genotype was first defined (Altay et al. 2007b). The results demonstrated that *T. ovis* (50.55 %) is the most common species, and there is no *T. lestoquardi* in sheep and goats in this region (Aktas et al. 2005a; Altay et al. 2007a).

In the Yeşilhisar province of Kayseri, *B. ovis* (3.7 %) and *T. ovis* (37.6 %) were detected with RLB in sheep and goats (Sarayli et al. 2006).

Randomly selected 421 sheep and 152 goats from Kayseri, Sivas, and Yozgat provinces of central Anatolia region were examined with RLB, and *B. ovis* and *T. ovis* were determined with a rate of 2.6 % and 33.9 %, respectively (Inci et al. 2010). A total of 201 apparently healthy animals from Artvin, Giresun, Gumushane, and Tokat of East Black Sea Region of Turkey were investigated for the blood protozoans. *Theileria* piroplasms were identified in nine (4.47 %) samples by microscopic examination, and *T. ovis* (18.90%), *Theileria* sp., MK (0.99%), and *Theileria* sp. OT3 (0.43 %) were detected by RLB.

Theileria sp. MK genotype was determined with a rate of 0.62 % similarly, and *Theileria* sp. OT3 (2.04 %) was detected higher than to East Anatolia. *T. ovis* was found the most prevalent species (28.99 %) in Black Sea Region, like the other regions (37.6–33.3 %) of Turkey. In addition, *B. ovis* was found with very low levels when compared with the other regions.

In this study, *B. ovis*, *T. ovis*, *Theileria* sp. MK, and *Theileria* sp. OT3 were detected in small ruminants in the region. The other *Theileria* (*T. lestoquardi*, *Theileria* sp. OT1, *T. luwenshuni*, and *T. uilenbergi*) *B. motasi*, and *B. crassa* were not detected. The most prevalent parasite was *T. ovis* with 28.99 %. These results organized the preliminary detailed molecular information for sheep and goat theileriosis

and babesiosis in the region and agree with similar the previous studies (Altay et al. 2007a, b, 2012; Inci et al. 2010).

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