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ORIGINAL PAPER

Prevalence of *Theileria*/*Babesia* Species in Ruminants in Burdur Province of Turkey

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Abstract

Purpose Theileriosis and babesiosis, two tick-borne haemoparasitic diseases (TBHDs) of ruminants, are caused by protozoan parasites of the genus *Theileria* and *Babesia*, respectively. Among them, some species are considered to be highly pathogenic causing serious economic losses to livestock holders especially in tropic and subtropic regions. Local and/or general control measures are needed to be applied to reduce economic impact of TBHDs. Prevalence studies are essential for the implementation and/or design of effective prevention and control measures based on true epidemiological data. Therefore, this study aimed to investigate the presence, prevalence and possible cross infections of *Theileria*/*Babesia* species between sheep, goat and cattle herds in Burdur province in Turkey.

Methods A total of 964 blood samples were collected from sheep ($n=330$), goat ($n=300$) and cattle ($n=334$) from five different districts of Burdur province. The samples were investigated for ovine and bovine *Theileria*/*Babesia* species using reverse line blot (RLB) hybridization assay.

Results In small ruminants, *T. ovis* was the most abundant *Theileria* species detected in sheep with a rate of 79.69%. Among *Babesia* species, *B. ovis* and *B. crassa* were detected only in blood of goats (0.66%) and sheep (1.12%) as single and mixed infections, respectively. In cattle, *T. annulata*, *B. bovis*, *Babesia* spp. were detected in rates of 0.59%, 3.29%, 3.59%, respectively.

Conclusion Obtained results clearly indicated that no cross infections with *Theileria*/*Babesia* species occurred in small ruminant and cattle herds that use the same grazing area.

Keywords *Babesia* · Reverse line blot (RLB) · Ruminant · *Theileria*

Introduction

Theileriosis and babesiosis caused by *Theileria* and *Babesia* species of the apicomplexa phylum, are transmitted by ixodid and argasid ticks and considered to be two of the most prevalent and economically important tick-borne haemoparasitic diseases (TBHDs) in mammalians [1–4]. *Theileria* and *Babesia* species are endemic in tropical and subtropical climatic regions along with the distribution of the vector tick species. Economic losses directly attributed to diseases caused by *Theileria* and *Babesia* species include mortality, production losses with the costs of veterinary diagnostic/treatment and tick control [5]. These losses are associated with a long-term carrier status that is being developed in survivor animals from the acute diseases [1, 2, 5, 6].

Several *Theileria* and *Babesia* species have been detected in cattle and small ruminants up to now. *Theileria annulata*, *T. parva*, *T. mutans*, *T. sergenti/buffeli/orientalis*, *T. taurotragi*, *T. velifera*, *T. sinensis*, *B. bigemina*, *B. bovis*, *B. divergens*, *B. major*, *B. ovata*, *B. occultans*, *B. jakimovi* and *B. beliceri* were observed in cattle [7–9]. In sheep and goats, the presence of *T. ovis*, *T. lestoquardi* (=*T. hirci*), *T. recondita*, *T. separata*, *T. luwenshuni* (=T. sp. China 1), *T. uilenbergi* (=T. sp. China 2), *T.* sp. OT1, *T.* sp. OT3, *T.* sp. MK, *B. ovis*, *B. motasi*, *B. crassa*, *B. foliata*, *B. taylori*, *B.* sp. Xinjiang and *B.* sp. BQ1 were shown [10–14].

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Among *Theileria* and *Babesia* species observed in cattle and small ruminants; *T. annulata*, *T. parva*, *T. lestoquardi*, *T. luwenshuni*, *T. uilenbergi*, *B. bovis*, *B. bigemina*, *B. divergens*, *B. ovis* and *B. motasi* are considered to be pathogenic [1, 3, 15–17]. Although *Theileria* and *Babesia* species are considered to be host-specific parasites [1–4], some studies have reported that these parasites can be detected in other hosts apart from their known hosts [16, 18–21].

In endemic regions, conventional methods such as microscopical examination of Giemsa-stained blood smears are widely used to detect *Theileria* and *Babesia* species in animals with clinical outcome. However, following recovery from primary infection with *Theileria* and *Babesia* species, animals become persistent carriers of the parasites for an extended period of time [22, 23], which is characterised by the presence of very low numbers of parasites circulating within the blood. This situation makes it difficult and unreliable to obtain a positive diagnosis by examination of stained blood smears [24]. Moreover, it is extremely difficult to conclusively discriminate pathogenic species from non-pathogenic ones by morphology, especially when they simultaneously occur within the same host [6, 11, 25, 26]. Molecular based diagnosis techniques such as reverse line blot (RLB) hybridisation assay have been frequently used to detect and discriminate *Theileria* and *Babesia* species in ruminants especially over the last decade [11, 27–30]. Molecular methods provide much more sensitive and specific results in terms of detecting carrier animals compared to microscopic diagnosis [6, 11, 26, 31].

In the present study, the prevalence of *Theileria* and *Babesia* species in sheep, goat and cattle was determined using RLB hybridisation technique in Burdur province of Turkey. Additionally, possible cross infections between small ruminants and cattle that are using the same grazing area were also investigated.

Materials and Methods

Sample Collection and DNA Extraction

The present study was carried out in five districts of Burdur province (Fig. 1). Collection of 964 total blood samples were made from herds of cattle, sheep and goat grazing in the same or neighbouring pastures. The number of samples and sampled herds according to animal species and districts are given in Table 1. According to this, an average of 5–6 animals were sampled per herd. Blood samples were collected in EDTA blood collection tubes from *vena jugularis* or *v. coccygea* of randomly selected animals in herds. DNA was extracted via Promega Wizard genomic DNA extraction kit (Madison, WI, USA) following the manufactural protocol. Isolates of *T. lestoquardi* (Lahr) and *B. crassa* from Iran, isolates of *T. uilenbergi* (Longde), *T. luwenshuni* (Lintan) and *B. motasi* (Lintan) from China and isolates of *T. ovis* (Kayseri), *T.* sp. MK (Kayseri), *B. ovis* (Kayseri) and *T. annulata* (Aydin) from Turkey were used as positive control DNA samples.

RLB Hybridization Assay for Detection of Theileria and Babesia Species

RLB hybridisation assay was used to detect the prevalence of *Theileria* and *Babesia* species in collected samples. Initially: 460–540 bp fragment of the V4 variable region of 18S small subunit ribosomal RNA gene of all *Theileria* and *Babesia* species was amplified by PCR, using genus specific primers of RLB-F (5'-GAC ACA GGG AGG TAG TGA CAA G -3') and RLB- R (5' biotin-CTA AGA ATT TCA CCT CTG ACA GT -3') [6]. Final volume of each PCR reaction mix were 50 µL and consisted of; 1 × PCR buffer, 1.5 mM $MgCl_2$, 200 mM of each dNTP, 2.5 U of hotstart Taq polymerase (HOT FİREpol-Solis Biodyne), 25 pmol of each primer RLB-F and RLB-R (5′ biotin-labelled) and 2 µL of template DNA. Reaction conditions were as follows: an initial denaturation step at 94 °C for 10 min was followed by a touchdown programme including two cycles at each temperature, i.e. 20 s at 94 °C, 30 s at an annealing temperature of 67 °C, 30 s at 72 °C, with the annealing temperature being decreased from 67 to 57 °C in steps of 2 °C. Then, 40 cycles of 20 s at 94 °C, 30 s at 57 °C and 30 s at 72 °C were performed and followed by 72 °C for 10 min as a final extension.

Oligonucleotide probes used to detect all known *Theileria*/*Babesia* species both at species and genus level are listed in Table 2 and exemplar pictures of the RLB showing the reaction of the various positive controls with the probes can be seen in Supplementary Figs. 1 and 2. Probes were immobilised on a Biodyne-C nitrocellulose membrane (Pall Biosupport), via N-terminal N-trifluoracctamidohexyl-cyanocthyl, N, N-diisopropyl phosphoramidite [TFA]-C6 amino linker as described previously [11]. Then, 20 µl of biotine-labelled PCR products were diluted in 2 × SSPE/0.1% SDS solution to a total volume of 150 µl and screened by RLB hybridisation assay as previously described [11]. Each membrane was reused up to 12 times following post-hybridization and documentation of the membrane [11, 27, 29].

Statistical Analysis

Statistical analysis was performed using Pearson chi-square test with help of MiniTab16 Statistics program. The Chi-square test was used to compare the positivity rates of the detected species and different districts. Observed differences were considered to be statistically significant when $P < 0.05$.

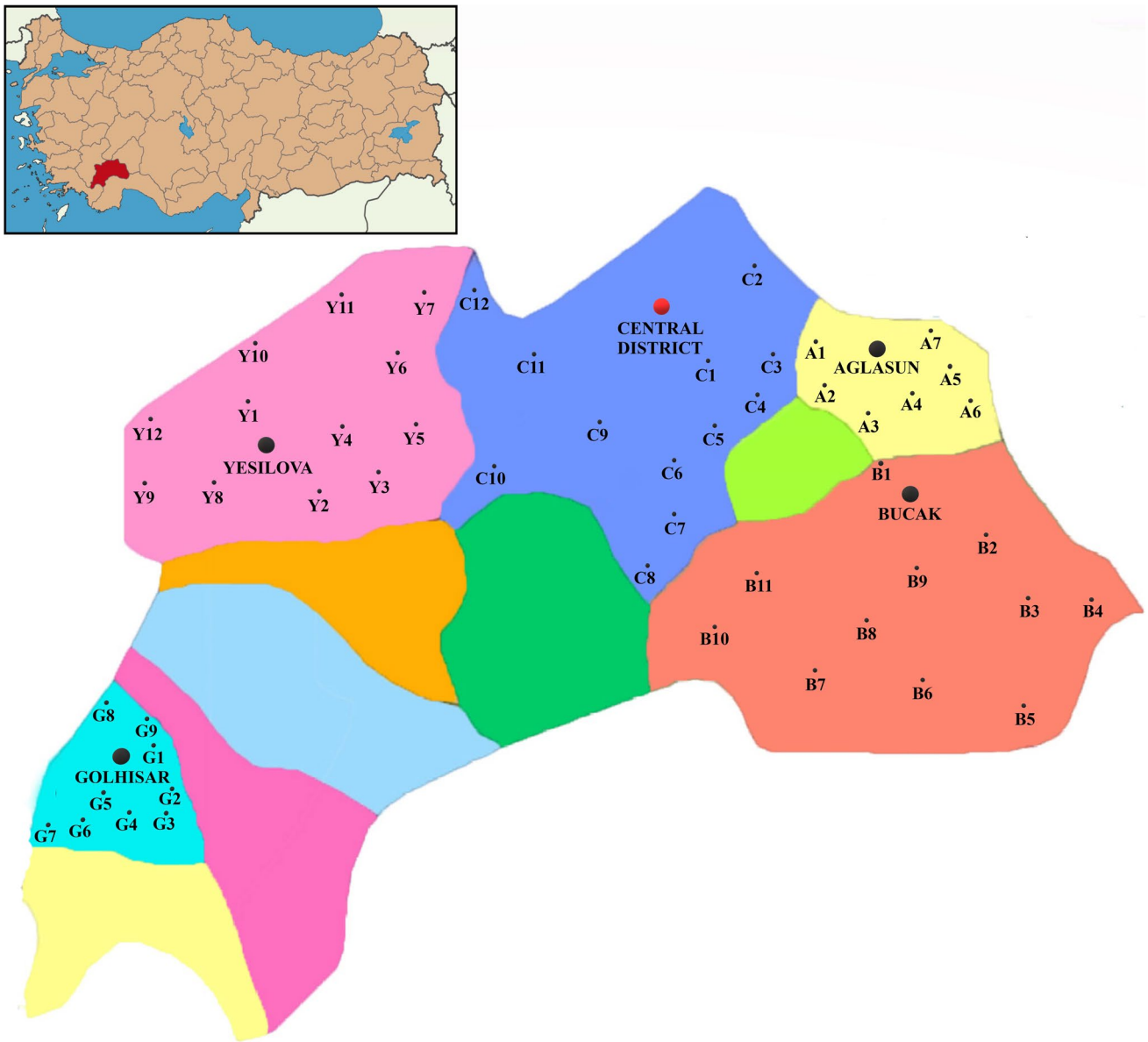

Fig. 1 Villages of Burdur province where the sampled herds located. Locations (villages) of the sampled herds are as follows; A1: Yesilbaskoy, A2: Kiprit, A3: Canakli, A4: Hisar, A5: Yumrutas, A6: Asagiyumrutas, A7: Camlidere, B1: Seydikoy, B2: Beskonak, B3: Cobanpinari, B4: Kizilli, B5: Kizilseki, B6: Karaot, B7: Bogazkoy, B8: Gundogdu, B9: Kuyubasi, B10: Urkutlu, B11: Yuregil, C1: Yakakoy, C2: Gokcebag, C3: Halicilar, C4: Cine, C5: Beskavak, C6: Aksu, C7: Kapakli, C8: Bozlar, C9: Karacaoren, C10: Callica, C11: Yassigume, C12: Basmakci, G1: Yamadi, G2: Uylupinar, G3: Hisarardi, G4: Kargali, G5: Karapinar, G6: Asmali, G7: Yesildere, G8: Camkoy, G9: Sorkun, Y1: Kayadibi, Y2: Caltepe, Y3: Bedirli, Y4: Bayirbasi, Y5: Baskuyu, Y6: Harmanli, Y7: Yarisli, Y8: Niyazlar, Y9: Horozkoy, Y10: Doganbaba, Y11: Yukarikirli, Y12: Guney

Table 1 Number of collected samples and herds according to districts and animal species

	Aglasun	Bucak	Golhisar	Center	Yesilova	Total
Sheep	70[s] (10)[h]	50 (9)	50 (10)	85 (12)	75 (12)	330 (53)
Goat	85 (10)	50 (11)	50 (11)	55 (10)	60 (9)	300 (51)
Cattle	100 (14)	51 (10)	71 (12)	52 (10)	60 (10)	334 (56)
Total	255 (34)	151 (30)	171 (33)	192 (32)	195 (31)	964 (160)

[s]Number of samples

[h]Number of herds

Table 2 Oligonucleotide probes, sequences, specificity and references

Probs	Sequences	Specifity	References
T/B catch all	TAATGGTTAATAGGARCRGTTG	All *Theileria/Babesia* spp.	[6]
Theileria all	TGATGGGAATTTAAACCYCTTCCA	All *Theileria* spp.	[11]
T. annulata	CCTCTGGGGTCTGTGCA	*T. annulata*	[42]
T. buffeli	GGCTTATTTCGGWTTGATTTT *W: A or T	*T. buffeli/orientalis*	[6]
T. ovis	TTGCTTTTGCTCCTTTACGAGTCTTTGC	*T. ovis*	[11]
T. lesto II	ATTGCTTGTGTCCCTCCG	*T. lestoquardi*	[11]
T. luwenshuni	TCGGATGATACTTGTATTATC	*T. luwenshuni*	[11]
T. uilenbergi	TGCATTTTCCGAGTGTTACT	*T. uilenbergi*	[11]
T. sp. OT1	ATCTTCTTTTTGATGAGTTGGTGT	*T.* sp. OT1	[32]
T. sp. OT3	ATTTTCTCTTTTTATATGAGTTTT	*T.* sp. OT3	[32]
T. sp. MK	CATTGTTTCTTCTCATGTC	*T.* sp. MK	[36]
T. separata	GGTCGTGGTTTTCCTCGT	*T. separata*	[11]
Babesia all	CCTKGGTAATGGTTAATAGGAA	All *Babesia* spp.	[11]
B. divergens	GTTAATATTGACTAATGTCGAG	*B. divergens*	[6]
B. bovis	CAGGTTTCGCCTGTATAATTGAG	*B. bovis*	[42]
B. bigemina	CGTTTTTTCCCTTTTGTTGG	*B. bigemina*	[6]
B. major	TCCGACTTTGGTTGGTGT	*B. major*	[42]
B. ovis	TGCGCGCGGCCTTTGCGTTACT	*B. ovis*	[11, 32]
Bm3	TTTCAAGCAGACTTTTGTCTTG	*B. motasi, B.* sp. China, *B. crassa* spp.	[11]
Bm2-2	GAATGATGCCGACTTAAACCCT	*B. motasi, B.* sp. China	[11]
Bm1	GCTTGCTTTTTTGTTACTTTTG	*B. motasi*	[11]
BcG	GTTGGCTTATCTTTTTACTTT	*B. crassa* spp.	[11]
BcT	TCTGATCGAGTTGGCTTA	*B. crassa* Turkey	[11]
BcI	TTATGGCCCGTTGGCTTAT	*B. crassa* Iran	[11]
B. motasi	ATTGGAGTATTGCGCTTGCTTTTT	*B. motasi*	[32]

Table 3 RLB positivity rates of animals according to districts

Districts	Sheep	Goat	Cattle
Aglasun	61.42% (43/70)^{cd}	8.23% (7/85)^{b}	2% (2/100)^{b}
Bucak	76% (38/50)^{bc}	6% (3/50)^{b}	0% (0/51)^{c}
Golhisar	96% (48/50)^{a}	58% (29/50)^{a}	0% (0/71)^{c}
Center	76.47% (65/85)^{b}	0% (0/55)^{c}	0% (0/52)^{c}
Yesilova	96% (72/75)^{a}	16.66% (10/60)^{b}	36,66% (22/60)^{a}
P	<0.05	<0.05	<0.05
Total ($P<0.05$)	80.6% (266/330)^{x}	16.33% (49/300)^{y}	7.18% (24/334)^{z}

^{a,b,c,d}Values in the same column with different superscripts are significantly different

^{x,y,z}Values in the same row with different superscripts are significantly different

Results

The number of blood samples infected at least with one *Theileria* or *Babesia* species according to districts are given in Table 3. The overall positivity rate in sheep, goat and cattle samples were 80.6%, 16.33%, 7.18%, respectively. The positivity rate (266/330) of sheep was significantly higher ($P<0.05$) compared to cattle and goat.

The overall single infections in sheep, goat and bovine samples were 46.66%, 16.33% and 6.88%, respectively. Overall mixed infection rates were 33.93%, 0% and 0.29% in sheep, goat and cattle, respectively. The positivity rates of single (154/330) and mixed (112/330) infections in sheep were significantly higher ($P<0.05$) compared to cattle and goat (Table 4).

Differences in positivity rates of each host between districts were statistically significant ($P<0.05$). The highest prevalence of overall infections in sheep was detected in Golhisar (96%) and Yesilova (96%), followed by Center (76.47%), Bucak (76%) and Aglasun (61.42%). Infection rates in goat samples were 58%, 16.66%, 8.23% and 6% in Golhisar, Yesilova, Aglasun, Bucak, respectively and the differences between districts were statistically significant ($P<0.05$). All goat samples from Center were negative. The highest positivity rate in cattle samples was detected in Yesilova (36.66%), followed by Aglasun (2%) while none of cattle samples were positive in Bucak, Golhisar and Center ($P<0.05$) (Table 3).

Table 4 The prevalence of overall *Theileria* and *Babesia* species and single and mixed infection status according to animals

	Overall *Theileria* spp.	Overall *Babesia* spp.	Single inf	Mixed inf
Sheep	79.69% (263/330)[a]	34.24% (113/330)[a]	46.66% (154/330)[a]	33.93% (112/330)[a]
Goat	0.33% (1/300)[b]	15% (45/300)[b]	16.33% (49/300)[b]	0% (0/300)[b]
Cattle	0.59% (2/334)[b]	6.88% (23/334)[c]	6.88% (23/334)[c]	0.29% (1/334)[c]
P	< 0.05	< 0.05	< 0.05	< 0.05

[a,b,c]Values in the same column with different superscripts are significantly different

Regardless of single or mixed infection status, the overall prevalences of *Theileria* spp. and *Babesia* spp. were significantly higher ($P < 0.05$) in sheep than those in goat and cattle samples (Table 4).

The prevalence between single or mixed infections in overall sheep samples was statistically significant ($P < 0.05$), and the most prevalent was *T. ovis* single infection (46.36%), followed by *T. ovis* + *Babesia* spp. mixed infection (32.12%) as seen in Table 5. Single *T. ovis* infection was also significantly higher between districts ($P < 0.05$) with rates of 94.7%, 65.9% and 52% in Yesilova, Center and Bucak, respectively. According to districts, the prevalence of mixed infections with *T. ovis* + *Babesia* spp. in Golhisar, Aglasun, Bucak and Center was also significantly different ($P < 0.05$) with rates of 92%, 55.71%, 24% and 10.58%, respectively. Also regardless of being single or mixed infection, *T. ovis* and *Babesia* spp. were the most prevalent in sheep samples with a rate of 79.69% and 32.42%, respectively. The overall prevalence of *T. ovis* was 41.9% (264/630) in small ruminants. While *B. crassa* was detected in four sheep samples (1.12%) as mixed infections, *B. ovis* was not detected in any of the sheep samples. The prevalence of unidentified *Babesia* positive sheep samples (i.e. positive at *Babesia* spp. or Bm3, but not bind any species-specific probe) was 33.03% (109/330).

Bm3 (comprising *B. motasi*, *B.* sp. China and *B. crassa*) (11%) and *Babesia* spp. (3.33%) were found to be most prevalent ($P < 0.05$) in goats compared to other detected parasites (Table 6). The prevalence of Bm3 was significantly different ($P < 0.05$) among districts and was most commonly detected in Golhisar with a rate of 50%. Unclassified *Theileria*/*Babesia* were detected in three goat samples collected from Golhisar and Yesilova. The prevalence of *B. ovis* was 0.66% in goat and was detected in only two samples from Aglasun and Golhisar. The prevalence of unidentified *Babesia* or *Theileria* positive goat samples (i.e. positive at *Babesia* spp., Bm3 or T/B catch all, but not binding to any species-specific probe) was 15.33% (46/300).

A significantly higher ($P < 0.05$) number of positive samples was detected in cattle from Yesilova (36.66%) compared to Aglasun district (2%), while cattle samples collected from Bucak, Golhisar and Center districts were negative. The most prevalent *Babesia* species in Yesilova were *B. bovis* (18.33%) and *Babesia* spp. (18.33%) regardless of single or mixed infection status. Two cattle from Aglasun and Yesilova were infected with *T. annulata* as single infection and

Table 5 RLB detections of *Theileria*/*Babesia* species in sheep samples according to districts

Districts	Detected species and infection rates							
	T. ovis	*Babesia* spp.	*T. ovis* + *Babesia* spp.	*T. ovis* + Bm3	BcG	*T. ovis* + Bm3 + BcG	*T. ovis* + Bm3 + BcI	Total
Aglasun	0% (0/70)[c]	1.42% (1/70)	55.71% (39/70)[b]	0% (0/70)	2.85% (2/70)	0% (0/70)	1.42% (1/70)	61.42% (43/70)
Bucak	52% (26/50)[b]	0% (0/50)	24% (12/50)[c]	0% (0/50)	0% (0/50)	0% (0/50)	0% (0/50)	76% (38/50)
Golhisar	0% (0/50)[c]	0% (0/50)	92% (46/50)[a]	4% (2/50)	0% (0/50)	0% (0/50)	0% (0/50)	96% (48/50)
Center	65.9% (56/85)[b]	0% 0/85	10.58% (9/85)[d]	0% (0/85)	0% (0/85)	0% (0/85)	0% (0/85)	76.47% (65/85)
Yesilova	94.7% (71/75)[a]	0% (0/75)	0% (0/75)[e]	0% (0/75)	0% (0/75)	1.33% (1/75)	0% (0/75)	96% (72/75)
P	< 0.05		< 0.05					
Total ($P < 0.05$)	46.36% (153/330)[x]	0.3% (1/330)[z]	32.12% (106/330)[y]	0.6% (2/330)[z]	0.6% (2/330)[z]	0.3% (1/330)[z]	0.3% (1/330)[z]	80.6% (266/330)

[a,b,c,d,e]Values in the same column with different superscripts are significantly different

[x,y,z]Values in the same row with different superscripts are significantly different

Table 6 RLB detections of *Theileria/Babesia* species in goat samples according to districts

Districts	Detected species and infection rates					
	T. ovis	*B. ovis*	*Babesia* spp.	Bm3	T/B catch all	Total
Aglasun	0% (0/85)	1.17% (1/85)	5.88% (5/85)	1.17% (1/85)[b]	0% (0/85)	8.23% (7/85)
Bucak	0% (0/50)	0% (0/50)	0% (0/50)	6% (3/50)[b]	0% (0/50)	6% (3/50)
Golhisar	0% (0/50)	2% (1/50)	4% (2/50)	50% (25/50)[a]	2% (1/50)	58% (29/50)
Center	0% (0/55)	0% (0/55)	0% (0/55)	0% (0/55)[c]	0% (0/55)	0% (0/50)
Yesilova	1.66% (1/60)	0% (0/60)	5% (3/60)	6.66% (4/60)[b]	3.33% (2/60)	16.66% (10/60)
P				< 0.05		
Total ($P < 0.05$)	0.33% (1/300)[z]	0.66% (2/300)[z]	3.33% (10/300)[y]	11% (33/300)[x]	1% (3/300)[z]	16.33% (49/300)

[a,b,c]Values in the same column with different superscripts are significantly different

[x,y,z]Values in the same row with different superscripts are significantly different

Table 7 RLB detections of *Theileria/Babesia* species in bovine samples according to districts

Districts	Detected species and infection rates				
	T. annulata	*B. bovis*	*Babesia* spp.	*T. annulata* + *Babesia* spp.	Total
Aglasun	1% (1/100)	0% (0/100)	1% (1/100)	0% (0/100)	2% (2/100)[b]
Bucak	0% (0/51)	0% (0/51)	0% (0/51)	0% (0/51)	0% (0/51)[c]
Golhisar	0% (0/71)	0% (0/71)	0% (0/71)	0% (0/71)	0% (0/71)[c]
Center	0% (0/52)	0% (0/52)	0% (0/52)	0% (0/52)	0% (0/52)[c]
Yesilova	0% (0/60)	18.33% (11/60)	16.66% (10/60)	1.6% (1/60)	36.66% (22/60)[a]
P					< 0.05
Total ($P < 0.05$)	0.29% (1/334)[y]	3.29% (11/334)[x]	3.29% (11/334)[x]	0.29% (1/334)[y]	7.18% (24/334)

[a,b,c]Values in the same column with different superscripts are significantly different

[x,y,z]Values in the same row with different superscripts are significantly different

T. annulata + *Babesia* spp. as mixed infection, respectively (Table 7). The overall prevalence of *T. annulata* was 0.59%. The prevalence of unidentified *Babesia* positive bovine samples (i.e. positive at *Babesia* spp., but not binding to any species-specific probe) was 3.59% (12/334).

According to single or multiple infection status, of the 330 sheep sampled from five districts; 154 (46.66%), 110 (33.33%) and two (0.6%) sheep were found to be infected with single, two and three species, respectively. Of 300 goats, 49 (16.33%) were infected with single species, while no mixed infections were detected. Twenty-three of 334 (6.88%) cattle were infected with one species and only one (0.29%) was infected with two species. No cross-infection was observed between small ruminants and cattle.

Discussion

Theileriosis and babesiosis are considered among the most important diseases responsible for major health problems and economic losses for small ruminant and cattle production, especially in countries located in tropic and subtropic climates [1, 3, 5, 32]. There are several *Theileria* and *Babesia* species including highly pathogenic species detected in sheep, goat and cattle causing theileriosis and babesiosis [1, 3, 16]. Conventional methods such as microscopic examination can be used to diagnose acute clinical forms of these diseases, however, these are inadequate to detect persistent carrier animals and differentiate species in mixed infections [16, 23, 33]. In contrast, RLB hybridisation assay, also used in the present study, is a molecular based method, and allows more specific, sensitive and simultaneous detection of small ruminant and bovine *Theileria* and *Babesia* species and possible cross-infections [6, 11, 33].

Theileria ovis, considered as non-pathogenic [12, 32], was the only *Theileria* species detected in small ruminants in Burdur province with an overall prevalence of 41.9%. A significant difference ($P < 0.0001$) was observed among sheep and goats for the presence of *T. ovis* and the prevalence rates were 79.69% and 0.3% in sheep and goats, respectively. Similarly, in studies conducted in the Eastern Black Sea Region [33] and in Nigde province [34] of Turkey, significant differences were observed between sheep and goats for prevalence of *Theileria* infections. In Sivas [17], and Kayseri [28, 35] significant differences were also observed in the prevalence of *T. ovis* among sheep and goats. Factors including seasonal activity of vector tick species and their infection rates together with, infestation rates of animals, different grazing behaviours of sheep and goat and parasite epidemiology were stated to be related with the observed differences among sheep and goats [28, 35].

In the present study, samples were collected from sheep and goat herds grazing in the same or neighbouring areas at similar days. This increased the possibility of being exposed to same tick species; however, the possible differences in infection and/or infestation rates among tick and hosts should not be ignored. Another factor effecting the infection rate is the difference in the immunological response among sheep and goats that needs to be further investigated. *Theileria* species considered as pathogenic were not detected in sheep and goats in this study. This indicated the presence of asymptomatic but carrier animals with very low parasitaemia of non-pathogenic *Theileria* species among sampled sheep.

Although *T. ovis* was the only *Theileria* species detected in small ruminants in the present study, the presence of pathogenic species *T. uilenbergi* and *T. luwenshuni* have been revealed in Burdur region in a previous study [29]. According to that; *T. ovis* (62.77%) and *T.* sp. OT1 (2.91%) were found by RLB and *T. ovis* (70.8%), *T. uilenbergi* (8.75%), *T. luwenshuni* (2.18%) and *T.* sp. MK (0.72%) were detected by species-specific PCR in sheep samples from Burdur province [29]. Therefore, local and/or general control measures such as controlled herd movements and anti-tick applications are still needed for effective prevention of epidemics.

The presence of ovine babesiosis in small ruminants caused by *B. ovis* (0.4–5.43%) [28, 29, 35, 36], *B. motasi* (0.1%) [29], *B. crassa* (4%) [29] and *Babesia* spp. (5.4%) [29] have been reported with similar prevalence rates in Turkey, also *B. crassa* and *Babesia* spp. were found in rates of 3.64% and 5.84% respectively in Burdur province [29]. In this study, *B. ovis* was only detected in two goat samples with a prevalence of 0.66% and no *B. ovis* was detected in sheep. Besides, *B. crassa* was detected as a part of mixed infection in sheep with a rate of 1.12% (4/330) but not in goat. The most prevalent *Babesia* infections in small ruminants were *Babesia* spp. (18.57%) and Bm3 group (5.87%) comprising *B. motasi*, *B.* sp. China and *B. crassa*. The significantly high prevalence of unclassified *Babesia* spp., detected in the present study, needs further investigation using species-specific PCR, sequence and phylogenetic analysis and other techniques to reveal the small ruminant babesiosis status in this region.

This study revealed the presence of *T. annulata* in cattle populations in Burdur province with a low prevalence of 0.59%, while *T. buffeli/orientalis* were not detected in any samples. The presence of *T. annulata*, causative agent of tropical theileriosis, has been reported in bovine samples in Turkey with a range of 1.28–18.1% [37–39]. The data obtained in previous studies indicated that the prevalence of *T. annulata* is closely related with the endemic status of the sampling region. Regions with higher prevalence show signs of being endemic or having an endemic stability for tropical theileriosis, while some regions having lower prevalence seems to be sporadic for *T. annulata*. The lower prevalence of *T. annulata* detected in the present study clearly indicated that tropical theileriosis is not a major problem for farmers in Burdur region. These results resembled a sporadic situation rather than an endemic stability in regions where samples were collected for tropical theileriosis. This is supported by Yukarı and Umur [40], where the prevalence of vector tick species (*Hyalomma marginatum*) of tropical theileriosis was shown to be 0.8% in Burdur. However, it should be noted that uncontrolled cattle movement and neglected tick control programmes may potentially cause outbreaks especially in regions where host and vector ticks species overlap.

So far, the most important causative agents of bovine babesiosis *B. bovis*, *B. bigemina*, *B. divergens* and less pathogenic species *B. major* and *B. occultans* were detected in Turkey [15, 41]. In the present study, only *B. bovis* and *Babesia* spp. were detected in cattle with prevalence rates of 3.29% and 3.59% respectively. It should be noted that all *B. bovis* and all except one *Babesia* spp. positive samples were found in Yesilova, only one bovine sample from Aglasun was infected with *Babesia* spp. and all bovine samples from other districts were negative for *Babesia* species. Hence, prevalence of bovine babesiosis is significantly higher in Yesilova district with a rate of 36.66% (22/60). Animals that survive from acute bovine babesiosis develop persistent infection and are carriers of *Babesia* species, which causes more transmissions and persistence of parasites in endemic regions, where usually high prevalence of *Babesia* parasites could be detected but number of clinical bovine babesiosis is low because of endemic stability [41].

Another subject that needs to be discussed is possible cross-infections. In a study conducted in Vietnam [20]; *B. bigemina* was detected in a blood sample of a goat and this finding was also confirmed by sequence analysis. As a result of the AMA-1 (Apical Membrane Antigen) gene sequence analysis, it was interpreted that *B. bigemina* is in the same

group with other isolates detected in cattle in Vietnam, but the genetic diversity in *B. bigemina* populations can be better determined with the identification of more genes and the development of markers [20]. In another similar study conducted in Egypt [16]; *B. bovis* and *B. bigemina* were detected in two sheep via species-specific PCR method. As a result of the sequence analysis; the genes encoding RAP-1 (Rhoptri Associated Protein) of *B. bovis* and AMA-1 protein of *B. bigemina* were preserved at high rates of 99.3–100% and 95.3–100%, respectively, in these isolates [16]. Elsify *et al*. [16] suggests that, although the pathobiological meaning behind the parasite's host change is unknown, it may be a survival strategy used by blood parasites. Furthermore, the unidentified isolates in the present study may have different genetic characteristics from the identified species, and may become more adaptive for the different hosts other than their own ones. Piroplasms can be transmitted to different host animal species other than their own ones due to the blood-sucking habits of vector ticks, hence it could be expected to detect DNA of piroplasms in blood samples of different hosts. Supporting Elsify *et al*. [16], we can consider that the piroplasm species may be adapting to a new similar or familiar hosts, however, phylogenetic analysis is particularly needed for evaluation and discussion.

In the present study, no cross-infection was observed between cattle, sheep and goat sharing the same grazing area. However, a cross infection among animal species may not be expected in herds where infection rates are at such low levels. On the other hand, samples positive at only genus level needs to be further identified. These isolates were attempted to be cloned for DNA sequence analysis for multiple times without success, so they are still unclassified (denoted) species of *Theileria* or *Babesia*.

More detailed studies covering investigation of tick species and their vector potential for this region are needed to improve our knowledge about prevalence, pathogenicity, host preference of these parasites and epidemiology and impacts of diseases to develop more effective control strategies.

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Declarations

Conflict of Interest The authors declare there are no conflicts of interest.

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