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Seroepidemiological survey of bovine tick-borne infections in the Black Sea Region of Turkey

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Abstract: Babesiosis, theileriosis, and anaplasmosis are the tick-borne diseases of cattle in most of the tropical areas and Turkey. A total of 270 cattle were randomly selected from 27 villages and 77 farms in the Black Sea Region of Turkey for the determination of infections by *Babesia bigemina* and *Anaplasma marginale* by using the indirect fluorescent antibody (IFA) test and the competitive enzyme-linked immunosorbent assay (cELISA). Of those, 242 and 256 sera were also analyzed for antibodies against *Theileria annulata* and *Babesia bovis* infections using the IFA test, respectively, during the period of June 2006 to June 2007. Of the 256 tested sera, 99 (38.6%) were positive for *B. bovis*; from the total 270 sera, 40 (14.8%) were positive for *B. bigemina*; of the 242 sera tested, 31 (12.8%) were positive for *T. annulata* by IFA; and from the total 270 sera, 102 (37.8%) were positive for *A. marginale* by cELISA. A total of 1125 ixodid ticks were collected and 10 species were identified. Antibodies produced by infections with *Babesia* spp., *Anaplasma* spp., and *Theileria* spp. were found highly prevalent. Infections caused by the agents and the tick species may cause severe economic damage to cattle production from the Black Sea Region of Turkey.

Key words: Anaplasmosis, babesiosis, theileriosis, ticks, Turkey

1. Introduction

Tick-borne diseases are a major cause of widespread morbidity and mortality in cattle in tropical and subtropical areas and in northern Australia (1,2). Tick-borne hemoparasitic diseases, which include babesiosis, theileriosis, and anaplasmosis, have been previously reported in Egypt and the Philippines (3,4). In cattle, tick-borne diseases such as babesiosis, theileriosis, and anaplasmosis occur throughout the world, especially in tropical and subtropical areas (1,5,6). Babesiosis and theileriosis are of worldwide importance and are characterized by icterus, hemoglobinuria, and death, and as a result, they have a high economic impact in several parts of the world, especially tropical and subtropical countries (1,3,6).

To contribute to previous studies in Turkey, the aim of the present study was to investigate the presence of the tick-borne diseases babesiosis, theileriosis, and anaplasmosis; determine the prevalence of ixodid ticks of cattle in different areas of the Black Sea Region of Turkey; and obtain detailed information on the regional occurrence of these diseases by employing microscopic examination in conjunction with the indirect fluorescent antibody (IFA) test and the competitive enzyme-linked immunosorbent assay (cELISA).

2. Materials and methods

2.1. Study area and field sampling

This study was conducted on a total of 270 cattle that were randomly selected from a total of 27 villages and 77 farms in the Samsun, Sinop, Kastamonu, Amasya, Tokat, Ordu, and Giresun provinces of the Black Sea Region during the period of June 2006 to June 2007. The minimal sampling conferred 95% confidence degree and 5% error probability. A total of 270 sera were collected from 8 Holstein, 6 Simmental, 109 Jersey crossbred, 46 Montafon crossbred, 84 Holstein crossbred, and 17 Simmental crossbred aged 2 years old and were analyzed using the IFA and cELISA tests. Peripheral thin blood smears and blood samples were prepared from the tip of the tail and the jugular vein of the animals. Of those, 270 were tested for *B. bigemina* and *A. marginale*, and 242 and 256 were tested for *T. annulata* and

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B. bovis, respectively. Serum samples were collected more frequently in spring, summer, and autumn. Collected ticks from cattle were placed in 70% ethanol in vials.

2.2. Microscopical examination

Peripheral thin blood smears were stained with 5% Giemsa stain and each slide was examined for the presence of parasites. Each blood smear was examined under at least 200 microscopic fields before being considered negative. Identification of the ticks was performed under stereomicroscope with the aid of previously published taxonomic keys (7).

2.3. Serological analyses

Babesia bigemina and *T. annulata* antigens were provided by the Department of Parasitology in the Faculty of Veterinary Medicine, Ankara University. Preparation of the antigens for the IFA test was performed according to Office International Des Epizooties (OIE) recommendations (8). Twelve-well IFA substrate slides (BV-12-030722) for *B. bovis* antigens were acquired from Fuller Laboratories (Fullerton, CA, USA).

The indirect fluorescent antibody tests for *B. bigemina*, *T. annulata*, and *B. bovis* were conducted as described by the OIE (8) and according to the manufacturer's instructions for use. Sera at working dilutions of 1/80, 1/160, and 1/320 were placed in the antigen slide wells and incubated in a humid chamber at 37 °C for 1 h. The slides were subsequently incubated in a humid chamber at 37 °C for 1 h with antibovine fluorescein isothiocyanate conjugate (FITC) with a minimum dilution of 1/300. Slides were examined in a darkroom with a Nikon Eclipse 80*i* microscope with an epifluorescence attachment at 492 nm and a 40× fluorescence oil objective. The presence of fluorescence in the ≥1/80 serum dilution was considered as a positive titer for the reaction.

As described in the manufacturer's instructions, a cELISA (VMRD Inc., Pullman, WA, USA) was employed to detect *A. marginale* antibodies with a cut-off equal to or higher than 30% inhibition. The resulting color was read with a Bio-Tek Model ELX800 Universal ELISA Microplate Reader at 405 nm.

The positive and negative control sera for the tests were obtained from Fuller Laboratories and the Department of Parasitology in the Faculty of Veterinary Medicine at Ankara University.

2.4. Statistical analysis

The prevalences of *B. bovis*, *B. bigemina*, *T. annulata*, and *A. marginale* antibodies in cattle breeds based on IFA and cELISA reactions and the results of microscopic examination of blood smears were statistically analyzed employing the chi-square test as provided by SPSS 20.0 (IBM Corp, Armonk, NY, USA). Data were considered statistically significant if $P < 0.05$.

3. Results

Of the 256 tested sera, 99 (38.6%) were positive for *B. bovis*, and of the 270 sera tested, 40 (14.8%) were positive for *B. bigemina* with IFA and 102 (37.8%) for *A. marginale* with cELISA. Of the 242 tested sera, 31 (12.8%) were positive for *T. annulata* by IFA. Across the 7 provinces, the prevalence of seropositive animals for the tick-borne parasites ranged from 8.6% to 55% for *B. bovis*, 9.8% to 21.4% for *B. bigemina*, 4.3% to 35% for *T. annulata*, and 24.6% to 70% for *A. marginale* (Table 1). *Babesia bovis* and *B. bigemina* infections were seen in all the provinces, while *A. marginale* and *T. annulata* levels were significantly higher in Sinop and Amasya provinces ($P < 0.05$). Mixed infections were detected in 33 (12.2%) serum samples tested. According to the serological test results, antibodies of the mixed infections were determined to be 21 (63.6%) *B. bovis* + *A. marginale*, 3 (9%) *B. bovis* + *T. annulata*, 4 (12.1%) *T. annulata* + *A. marginale*, 2 (6%) *B. bovis* + *B. bigemina*, and 3 (9%) *B. bovis* + *T. annulata* + *A. marginale*.

There were no acute clinical cases of babesiosis, anaplasmosis, or theileriosis in the cattle of this study during its duration. Microscopic examination of the total of 270 blood smears revealed that 40 (14.8%), 16 (5.9%), and 3 (1.1%) animals were positive for *Babesia* spp, *Anaplasma* spp., and *Theileria* spp., respectively.

Seroprevalence of *B. bovis* were observed at 47.8%, 39.2%, 38.6%, 33.3%, and 31.2% in Montafon crossbreds, Holstein crossbreds, Jersey crossbreds, Simmentals, and Simmental crossbreds, respectively. However, antibodies to *B. bovis* were not detected in the 8 Holsteins.

A total of 1125 ticks were collected from the animals, of which 0.9% were nymphs, 44.2% males, and 54.7% females (Table 2). In the present study, of the 270 cattle examined, 248 (91.8%) animals were infested with one or more tick species. Ten tick species were recorded as follows: *Hyalomma marginatum*, *H. excavatum*, *H. detritum*, *Ixodes ricinus*, *Rhipicephalus bursa*, *R. turanicus*, *Rhipicephalus* (*Boophilus*) *annulatus*, *Haemaphysalis punctata*, *Hae. Parva*, and *Dermacentor marginatus*. The genera *Rhipicephalus* and *Hyalomma* were found more prevalent at 44.0% and 41.9%, respectively, and *Haemaphysalis* was the least prevalent (1.0%) tick genus (Table 2). *H. marginatum* was the most common species identified at the rate of 32.9%, which was collected from animals at localities ranging from 230 to 1230 m in altitude. *Rhipicephalus annulatus* was recorded in Kastamonu, Sinop, Amasya, and Samsun provinces during the study. In terms of seasonal activity of the ticks, the activity peak of *Rhipicephalus* species occurred in spring, summer, and early autumn; of *Hyalomma* species in early summer, autumn, and winter; of *Dermacentor* species in winter and spring; of *Haemaphysalis* species in autumn and early winter; and of *Rhipicephalus* (*Boophilus*) and *Ixodes* species in all seasons.

Table 1. Prevalence of the tick-borne hemoparasites in cattle according to provinces in the Black Sea Region.

Provinces	Seropositivity (%)				Prevalence (%)		
	IFA			cELISA	Microscopic assay		
	Babesia bovis	*Babesia bigemina*	*Theileria annulata*	*Anaplasma marginale*	*Babesia* spp.	*Theileria* spp.	*Anaplasma* spp.
	11.92*	4.02	19.18*	24.38*	20.36*	8.32	19.09*
Amasya	14/40[a] (35.0)	6/40(15.0)	9/40 (22.5)	21/40(52.5)	4/40(10.0)	2/40(5.0)	8/40(20.0)
Giresun	2/23(8.6)	4/23(17.4)	1/23(4.3)	13/23(56.5)	0/23(0.0)	0/23(0.0)	0/23(0.0)
Kastamonu	18/42(42.9)	9/42(21.4)	5/42(11.9)	16/42(38.1)	11/42(26.2)	0/42(0.0)	1/42(2.3)
Ordu	18/42(42.9)	8/42(19.0)	3/14 (21.4)	11/42(26.2)	9/42(21.4)	1/42(2.3)	2/42(4.7)
Samsun	19/47(40.4)	6/61(9.8)	4/61(6.6)	15/61(24.6)	14/61(22.9)	0/61(0.0)	2/61(3.2)
Sinop	11/20(55.0)	2/20(10.0)	7/20(35.0)	14/20(70.0)	1/20(5.0)	0/20(0.0)	2/20(10.0)
Tokat	17/42(40.5)	5/42(11.9)	2/42(4.8)	12/42(28.6)	1/42(2.3)	0/42(0.0)	1/42(2.3)
Total	99/256(38.6)	40/270(14.8)	31/242(12.8)	102/270(37.8)	40/270(14.8)	3/270(1.1)	16/270(5.9)

[a] : Examined animals; * : $P < 0.05$.

Table 2. Distribution of tick species collected from cattle in the Black Sea Region.

Tick species	Amasya		Giresun			Kastamonu		Ordu		Samsun			Sinop			Tokat		Number of ticks	Tick species (%)
	M[a]	F[b]	M	F	N[c]	M	F	M	F	M	F	N	M	F	N	M	F		
H. marginatum	22	24	-	-	-	100	60	-	-	3	2	-	41	41	-	39	28	370	32.9
H. excavatum	16	13	-	-	-	-	-	-	-	5	7	-	11	9	-	8	2	71	6.3
H. detritum	-	-	-	-	-	5	3	-	-	1	1	-	5	2	-	-	-	17	1.5
Hyalomma spp.	-	9	-	-	-	-	-	-	-	-	3	-	-	-	-	-	2	14	1.2
I. ricinus	-	-	44	139	6	-	-	3	15	5	15		-	-	-	-	-	227	20
R. bursa	12	11	-	-	-	-	-	2	5	24	45	-	13	12	-	-	-	214	19
R. turanicus	31	36	-	-	-	-	-	-	-	15	22	-	63	62	-	4	3	236	21
R. (*Boophilus*) *annulatus*	1	2	-	-	-	2	1	-	-	4	11	3	3	16	2	-	-	45	4.0
Hae. punctata	-	-	-	-	-	-	-	-	-	1	2	-	1	-	-	2	2	6	0.5
Hae. parva	-	-	-	-	-	1	3	-	-	1	-	-	-	-	-	-	-	5	0.4
D. marginatus	2	4	-	-		1	1	-	-	4	4	-	3	5	-	-	2	24	2.0
Total	84	97	44	139	6	109	68	5	20	63	112	3	140	147	2	53	39	1125	

[a] Male, [b] Female, [c] Nymph.

4. Discussion

Bovine babesiosis and anaplasmosis are widespread in most Latin American countries (9). For the southern Italian region prevalences of *B. bigemina* (23.1%) and *A. marginale* (11.5%) were reported by Cringoly et al. (5). In the Philippines, prevalences of *Anaplasma* spp., *B. bigemina*, and *B. bovis* were 54.7%, 15.4%, and 10.0%, respectively (4). In this study, only slight differences were recorded in prevalences of *B. bovis*, *B. bigemina*, and *T. annulata* determined by antibody detection in cattle from the Black Sea Region, which were 38.6%, 14.8%, and 12.8%, respectively. However, *A. marginale* prevalence (37.8%) was found higher in the present study.

In Turkey, identification of tick-borne infection prevalence has been performed with various molecular and serological methods (10–12). Bovine babesiosis (32.21%, 75%) and anaplasmosis (7.88%) are widely distributed in the Black Sea Region of Turkey (13,14). *Anaplasma* species (9.0%) were detected at similar proportions by reverse line blood (RLB) hybridization in the Eastern Black Sea Region (10). Additional information was gleaned from the present study in which a serosurvey revealed that 38.6% of cattle were positive for *B. bovis*, 37.8% for *A. marginale*, 14.8% for *B. bigemina*, and 12.8% for *T. annulata*.

For the Black Sea Region, prevalences of *B. bovis* (44%), *B. bigemina* (62%), and *T. annulata* (63%) infection were reported by Dinçer et al. (14). Furthermore, these infections by tick-borne parasites were reported in other areas of Turkey (12,15,16). In the present study, the prevalence of *B. bovis* (38.6%) was higher than that recorded in the Aegean Region (22.4%), Central Anatolia (20.3%), the Marmara Region (10.6%), and East and Southeast Anatolia (35.1%) (17). The differences between the regions are probably based on principal vector distributions, such as that of *R. annulatus* and *R. bursa* (6,18,19).

The prevalence of *B. bovis* (38.6%) was higher than that of *B. bigemina* (14.8%) for the Black Sea Region in the present study. Similarly, seroprevalence of *B. bovis* and *B. bigemina* were reported as 25.5% and 12% in cattle, respectively, in the same region (15). These results indicated that *Babesia* infections are widespread in the Black Sea Region and the current study shows that there is endemic instability for babesiosis in the Black Sea Region. According to a previous study, endemic stability is more likely to develop for *B. bigemina* than for *B. bovis* when both parasites are present in the same tick species (20). In addition, an endemically stable situation occurs when 81%–100% of the herd is infected with a particular *Babesia* species (21).

The least prevalent blood parasite antibody recorded in the present study was *T. annulata* (12.8%), whereas previous studies showed that *T. annulata* was the most prevalent blood parasite in cattle in Turkey, as follows: Central Anatolia at 67.5% (22), Marmara Region at 33.3% (17), Aegean Region at 31% (23), East Anatolia at 34.9% (12), and Southeast Anatolia at 91.4% (17).

The high prevalence of *T. annulata* is likely to be related to the distributions of their principal vectors, including *H. anatolicum*, *H. detritum*, and *H. asiaticum* (24). In the present study, *H. marginatum* was the most widely distributed tick species in the sampled region, which was in contrast to an earlier report that the percentage infection prevalences and the intensities were similar in *H. anatolicum* and *H. excavatum* but much lower in *H. marginatum* (25).

It was also reported that the prevalence of *A. marginale* in cattle in the Eastern Black Sea Region ranged between 2.8% and 17%, based on RLB and microscopic examinations, respectively (10,13). The high percentage of *A. marginale* (37.8%) detected with serology in the present study is somewhat consistent with results from studies in the same region (10,13). In the present study, *B. bovis*, *A. marginale*, and *T. annulata* infections were also recorded at different rates among the provinces. Prevalence of *A. marginale* infections was higher in Sinop than in the other provinces, and the prevalence of *T. annulata* infections was low, except for Sinop ($P < 0.05$). The findings imply that tick-borne infections such as babesiosis and anaplasmosis are common in cattle in the Black Sea Region. While *A. marginale* was the second most prevalent (37.8%) infectious agent, *T. annulata* was the least prevalent (12.8%) in the current study. Blood smear examination also showed that *Theileria* spp. prevalence was lower (1.1%) than those detected for the other 3 species in this study. These data are similar to those of the previous studies on *Babesia*, *Theileria*, and *Anaplasma* infections (13,14,22).

In the present study, 10 tick species were recorded that have also been reported previously from other areas of Turkey (18,26). The most prevalent tick species determined in the present study was *H. marginatum*. That result is in agreement with the findings of Aktas et al. (27). Previous studies have also shown the genus *Rhipicephalus* to be the most frequently occurring tick genus in cattle in the Black Sea, Aegean, and other regions of Turkey (19,28). In the present study, distribution of the genus *Rhipicephalus* was recorded as 44.0% of cattle. In this study, babesiosis was the most prevalent disease in the Black Sea Region since it is probably transmitted by ticks of the genus *Rhipicephalus*, like *R. annulatus* and *R. bursa*. Also in the current study, *I. ricinus* was more prevalent than *R. annulatus* in the 7 provinces of the Black Sea Region. It has great medical and veterinary importance because of being a vector of disease agents such *Rickettsia* spp. and *B. divergens* (27,28).

In conclusion, tick-borne diseases such as babesiosis, anaplasmosis, and theileriosis and their main tick vectors, which impact the general health of cattle in the Black Sea Region of Turkey, should be considered and need to be further investigated with molecular and immunological

techniques. Moreover, investigation of the roles of the different vector tick species in the transmission of different species of blood parasites in cattle in each province is recommended.

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