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Seroprevalence of theileriosis and babesiosis of cattle*)

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Summary

This study was carried out on cattle to detect the seroprevalence of theileriosis and babesiosis around the Antakya province. A total of 214 randomly selected cattle were examined from selected locations for Theileria annulata, Babesia bigemina, B. bovis and B. divergens. Blood samples were collected from the cattle by jugular vene puncture to obtain sera for IFAT. Thin blood smears were prepared from the punctured ear veins of each animal. The blood smears were stained with 5% Giemsa's stain and examined microscopically at 100 × magnification. None of the Babesia species was detected but T. annulata observed in 5 (2.33%) blood smears. The sera were tested for the presence of antibodies to the T. annulata, Babesia bigemina, B. bovis and B. divergens by IFAT. Antibodies were detected against T. annulata in 24 and B. bigemina in 2 sera of the tested 214 cattle. Antibodies for B. bovis and B. divergens were not detected in any sera. It has been concluded that detailed molecular biological, serological and epidemiological studies needed to clarify the genetic and antigenic diversity of the blood parasites in Turkey.

Keywords: Babesia spp., IFAT, seroprevalence, Theileria annulata

Theileria annulata and *Babesia spp*. are tick-borne protozoan parasites that infects cattle in tropical and sub tropical regions, which can cause huge losses to livestock. Research into these diseases are vital due to the great economic importance of cattle in developing countries where these parasites are prevalent. The protozoan parasite *Theileria annulata* is the causative agent of tropical theileriosis and is endemic in the area around the Mediterranean and the Middle East and reaches the Southern parts of Asia (5, 9, 10, 13). The parasite is transmitted from cattle to cattle by ticks of the genus *Hyalomma* (1, 13, 14, 22, 24).

In Turkey, *T. annulata* is considered to be a major threat to the cattle industry since the disease causes mortality and economic losses, particularly in crossbred cattle (6, 9, 18, 24). It has been reported that the disease occurs throughout the country. In serological studies carried out in different regions of Turkey, the seroprevalence of tropical theileriosis has been determined to vary from 0 to 92% in cattle (4, 6, 8, 11). Molecular biological tecniques have been applied for more specific and sensitive detection of *T. annulata* and *Babesia spp*. (6, 7, 9, 10, 14, 17).

Babesiosis, which is caused by intraerythrocytic parasites of the protozoan genus *Babesia*, is one of the more common diseases of farm animals worldwide and is gaining increasing attention as an emerging tick-borne zoonosis in humans (12, 16, 26, 27). The prevalences of *Babesia spp*. have been reported to be between 0.60% and 54.96% from different regions of Turkey (2, 4, 8, 17). Babesiosis is considered to be another major treat to the cattle industry since it causes mortality and economical losses in cattle farms as well (2, 17).

The present study was carried out to determine the frequency of infections on the *Theileria annulata* and *Babesia spp*. in cattle in Antakya province.

Material and methods

Area of study and animals. The study was conducted in Antakya province located in the south of Turkey where infections of blood parasites are thought to be endemic. Cattle of the Holstain breed crossbreeds are dominant in the province. A total of 214 cattle were included in the study. Blood samples were collected from randomly selected healthy cattle, in 11 different locations of seven different area namely Serinyol-Bakras (39), Anayazi-Zulufluhan (35), Harbiye-Gumusgoze (33), Antakya (21), Samandag (14), Altinozu (42) and Degirmendere-Tahtakopru (30).

Sample collection. Since, tick born diseases have a seasonal increase from May to October with the peak period in July to August, samples were collected in July in the middle of the disease season. Two thin blood smears were prepared with peripheral blood taken from ear vein of each animal. These samples were air dried and stored in a slide box until stained with 5% Giemsa's stain. Thin blood smear slides were examined by light microscopy (× 100).

For serum samples, 10 ml blood was collected in plain test tubes by jugular venepuncture. After collection, blood samples were stored in ice box for 4-6 hr. The sera were seperated from the blood within 24 hr by centrifugation at 3000 × g for 10 min. and divided into aliquots. The samples were labeled and stored at –20°C until used.

Serological tests. The IFAT, using both the schizont and piroplasm stage of the *T. annulata* and *Babesia* species piroplasm stage as antigens, was used to examine serum samples for the precence of appropriate specific parasite antibodies. *T. annulata*

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Tab. 1. The distribution of the positive results according to the locations

Location	n	*T. annulata*		*B. bigemina*		*B. bovis*		*B. divergens*	
		M	S	M	S	M	S	M	S
Serinyol-Bakras	39 (18.22)	2 (5.12)	7 (17.94)	–	–	–	–	–	–
Anayazi-Zulufluhan	35 (16.35)	3 (8.57)	5 (14.28)	–	–	–	–	–	–
Harbiye-Gumusgoze	33 (15.42)	–	4 (12.12)	–	1 (3.03)	–	–	–	–
Samandag	14 (6.54)	–	1 (7.14)	–	–	–	–	–	–
Antakya	21 (9.81)	–	4 (19.04)	–	–	–	–	–	–
Altinozu	42 (19.62)	–	2 (4.76)	–	1 (2.38)	–	–	–	–
Tahtakopru-Degirmendere	30 (14.01)	–	1 (3.33)	–	–	–	–	–	–
Total	214 (100)	5 (2.33)	24 (11.21)	–	2 (0.93)	–	–	–	–

Explanations: M – Microscopy (Thin blood smears); S – Serology (IFAT); Dash (–): indicates that there is no positive result; In Brackets: (%)

antigens and control serums (positive-negative) were prepared in the Parasitology Department of Faculty of Veterinary Medicine at Ankara University used to detect antibodies to *T. annulata*, *B. bigemina*, *B. bovis* and *B. divergens*. Anti-bovine IgG, FITC Conjugate was obtained from SIGMA (Cat. No. F-7509).

Slides were examined in dark room following the IFAT procedure using a flourescein microscope (Zeiss) with Neoflaur objective (40 ×).

Results and discussion

T. annulata was observed in five samples from two locations while *Babesia spp*. were not detected in any blood smears. Antibodies against *T. annulata* were found in 24 sera and in 2 sera against *B. bigemina*. No antibodies were detected against *Babesia bovis* and *B. divergens*. The distribution of the positive results according to the locations is summarised in tab. 1.

Positive microscopy and serology results for *T. annulata* were obtained from all age group but higher infection rate observed in the animal group of over five years age using IFAT. *B. bigemina* was detected from animals older than three years of age but there was no signals for *B. bovis* and *B. divergens* with IFAT (tab. 2).

All the positive results determined by microscopy were from animals kept indoor. On the other hand, animals kept outdoor conditions had higher infection rate than the animals kept indoors according to the IFAT results. The positive result distribution between keeping conditions were summarised in tab. 3.

Sampling period were choosen July because cases of blood parasites occur from April to September, with the highest number occuring in June-July, in paralel with the increase of tick populations (9, 13, 18, 21). The prevalences in the earlier studies were determined very high in different regions (2, 8, 9, 11, 24). The highest prevalences were found in Southeast Anatolia 91.40% and Central Anatolia 92.65% (11, 24, 28). However, in some studies the prevalences have been reported that about 10% in different regions. Cakmak and Oz (4) found *T. annulata* prevalence 10.68% in Adana which is the neighbour city to Antakya. These results were close to our finding for *T. annulata* (11.21%).

Tab. 2. The distribution of positive results according to the animals age

Age Group	n	*T. annulata*		*B. bigemina*		*B. bovis*		*B. divergens*	
		M	S	M	S	M	S	M	S
1 < Year	32 (14.95)	1 (3.12)	3 (9.37)	–	–	–	–	–	–
1-2 Year	88 (41.12)	2 (2.27)	9 (10.22)	–	–	–	–	–	–
3-5 Year	50 (23.36)	1 (2.00)	5 (10.00)	–	1 (2.00)	–	–	–	–
5 > Year	44 (20.56)	1 (2.27)	7 (15.90)	–	1 (2.27)	–	–	–	–
Total	214 (100)	5 (2.33)	24 (11.21)	–	2 (0.93)	–	–	–	–

Explanations: as in tab. 1

Tab. 3. The distribution of positive results according to the management

Management System	n	*T. annulata*		*B. bigemina*		*B. bovis*		*B. divergens*	
		M	S	M	S	M	S	M	S
Indoor	156 (72.89)	5 (3.20)	16 (10.25)	–	2 (1.28)	–	–	–	–
Outdoor	58 (27.10)	–	8 (13.79)	–	–	–	–	–	–
Total	214 (100)	5 (3.20)	24 (11.21)	–	2 (0.93)	–	–	–	–

Explanations: as in tab. 1

The first serological cattle theileriosis and babesiosis screening was conducted in Ankara using IFAT and reported that 4.80% *B. bigemina* and 9.78% *B. bovis* seropositive (3). Although latter serological studies showed that the prevalences fluctuate among the studied areas (3, 4, 8, 9, 11, 17, 23, 24). The fluctuations could be due to instability of the areas for the blood parasites and/or antigenic diversity of the parasites in the different regions. *B. bigemina* was found in two indoor cattle but no *B. bovis* and *B. divergens* observed in Antakya using IFAT. The serological studies conducted in Turkey were used mostly same antigens for detections. The

antigens may not be capable to detect the parasite species collected from different regions.

Sayin et al. (25) reported that the incidence of appearance of piroplasm was higher than that of seroconversion for the *T. annulata* in cattle. They have been concluded that either the IFAT is not sensitive enough to detect the Theileria antibodies or more than one *Theileria* species or strains of *T. annulata* causing the diseases in the animals in central Anatolia. Furthermore, it has been identified for the first time using reverse line blotting that there is, *T. buffeli/orientalis* group in the Turkey (6). The blood parasites were thought to be endemic in the Antakya province according to the local veterinary surgeons. Theileria species were observed only in five blood smears. However, IFAT screening resulted with 24 (11.21%) *T. annulata* and 2 (0.93%) *B. bigemina* seropositive out of 214 animals. The serological and microscopical tests resulted in the absence of *B. bovis* and *B. divergens*. In contrary to local veterinarian observations, the prevalences of *T. annulata* and *B. bigemina* indicated that these infections are not endemic in the province. It migth suggested that the animals are subjected to infections of *T. annulata* with different antigenic and/or genetic characters. Other blood parasite species such as *T. buffeli/orientalis* group and/or *Anaplasma spp*. may be confused and misdiagnosed as Theileiosis or Babesiosis. These parasites species were not investigated in this study.

In the province, some of the cattle are reared using a traditional management system characterized by partly extensive grazing on natural pasture and/or mostly kept in stable and gardens of houses. During the winter months, they are mostly fed indoors. The other parts of the animals always sheltered in stable next to the houses. The shelters where the cattle are housed for indoor feeding are not usually hygienic. Acaricides are applied irregularly to the animals for tick control. The management system of the animal in the province in which cattle were not moved to pasture but mostly kept indoors may reducing effect on the diseases prevalence. In addition, it has been observed that most of the indoor animal had tick infestation. This could be due to irregular acariside applications and transportation of ticks by grass which collected from field and stored next to animal shelter to use as feed or bedding for the animals. Supporting these obsevations, the seropositivity distribution was not meaningful between the animals kept indoor or outdoor but all the latent infections were observed in the animals kept indoor.

Molecular biological studies offers more sensitive and spesific identifications applied for epidemiological field studies where lots of factors may interfear the results. The results of the studies showed that molecular biological tests are more sensitive than other tests (6, 10, 17, 28). However, Dumanli et al. (9), found no significant differences between IFAT and molecular biological detection of *T. annulata*. The differences between results could be due to the test applied for the studies. On the other hand, in molecular biological tests, the genetic diversity of organisms according to geographic distribution might have influences on the results as well. The genetic and antigenic diversity of *T. annulata* has been already reported by a number of author (7, 15, 19, 20). Therefore, organisms from different regions or countries may produce bias with false positive or negative results in the studies unless they have been tested for genetic and/or antigenic diversity in the study region. Turkey has seven big geographic and climatically different regions which may effects on blood parasites antigenic and genetic characteristics. There are two new study starting regarding to *T. annulata* and *Babesia spp*. antigenic and molecular biological diversity in Turkey.

In conclusion, detailed molecular biological, serological and epidemiological studies needed to clarify the genetic and antigenic diversity of the blood parasites in Turkey.

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