

Prevalence of *Babesia bigemina* and *Theileria annulata* in Cattle in Ereğli, Turkey

Esin GÜVEN¹, Ayşe ÇAKMAK², Ömer ORKUN², Aytaç AKÇAY³, Asiye KOÇAK²

¹ Atatürk Üniversitesi Veteriner Fakültesi Parazitoloji Anabilim Dalı, Erzurum

² Ankara Üniversitesi Veteriner Fakültesi Parazitoloji Anabilim Dalı, Ankara

³ Erciyes Üniversitesi Veteriner Fakültesi Biyometri Anabilim Dalı, Kayseri

Summary: Babesiosis and theileriosis are tick-borne diseases widespread in tropical and sub-tropical regions with high economic impact worldwide. Carrier animals have an important role in the transmission of the infection by ticks and also in the epidemiology of the diseases. The aim of this study was to determine the seroprevalence of hemoprotozoan parasites (*Babesia bigemina* and *Theileria annulata*) in cattle in Ereğli district of Konya, Turkey. The serum samples of 287 cattle were examined using the indirect fluorescence antibody test (IFAT). The antibodies against *B. bigemina* were detected in 34.84% and *T. annulata* in 0.69% sera of the cattle. On the other hand statistical analysis of IFAT results of *T. annulata* couldn't perform as numbers of positive results are inadequate. The results of this study showed that babesiosis was a fairly common disease in comparison to theileriosis in cattle of Ereğli district at the study period.

Key Words: *B. bigemina*, *T. annulata*, cattle, Indirect Fluorescence Antibody test

Ereğli Yöresi Sığırlarında *Babesia bigemina* ve *Theileria annulata*'nın Prevalansı

Özet: Babesiosis ve theileriosis, tropikal ve subtropikal bölgelerde sıklıkla karşılaşılan ve dünya genelinde ekonomik öneme sahip, kene kaynaklı hastalıklardır. Portör hayvanlar hastalığın epidemiolojisinde ve kenelerce nakledilmesinde büyük öneme sahiptir. Bu çalışmanın amacı Konya'nın Ereğli ilçesindeki sığırların bazı hemoprotozoan parazitlerinin (*Babesia bigemina* ve *Theileria annulata*) seroprevalansının belirlenmesidir. Bu amaçla İndirekt Floresan Antikor testi (IFAT) ile 287 sığır serumu incelenmiş, serumların % 34.84'ünde *B. bigemina*'ya, % 0.69'unda ise *T. annulata*'ya karşı antikor olduğu tesbit edilmiştir. Sonuç olarak, çalışma döneminde ve alanında babesiosisin theileriosise oranla daha sık karşılaşılan bir hastalık olduğu anlaşılmıştır.

Anahtar Kelimeler: *B. bigemina*, *T. annulata*, sığır, İndirekt Floresan Antikor testi

Introduction

Among tick-borne hemoprotozoan parasites of vertebrates, *Theileria* and *Babesia* are the species that have a major effect on livestock health (Mehlhorn and Schein, 1984). These constitute major health and management problems in developing countries; therefore, research into these diseases is vital (Kaya et al, 2006).

Bovine babesiosis by *B. bovis*, *B. bigemina*, *B. divergens* and *B. major* causes significant morbidity and mortality in cattle worldwide (Mc Cosker, 1981; Eckert et al., 2005). *B. bigemina* is one of the major causative agents

of bovine babesiosis and is transmitted by *Rhipicephalus (Boophilus) annulatus* in Turkey (Dincer et al., 1991; Inci et al., 2002). Tropical theileriosis is one of the most lethal types of theileriosis in North Africa, Europe and Asia besides in Turkey. Ticks of the genus *Hyalomma* is known to transmit the etiological agent of tropical theileriosis (Uilenberg, 1981; Sayin et al., 1997).

In serological studies carried out in different regions of Turkey, the seroprevalence of *B. bigemina* has been reported to vary between 0.93 and 100% whereas *T. annulata* has been determined between 0 and 92.65% in

cattle (Cakmak and Oz, 1993; Eren et al., 1995; Sevinc et al., 2001; Kaya et al., 2006; Sevgili et al., 2010).

The diagnosis of hemoprotozoan parasites is made by microscopic examination of blood smears, observing clinical symptoms and in addition to theileriosis examination of lymph node smears. These techniques are usually satisfactory for detection of acute infections but not for chronically ill animals with low parasitaemias. After recovery, an abiding carrier status occurs in animals with low numbers of erythrocytes infected with *Theileria* and *Babesia* piroplasms. It is important to identify carrier animals as they are carriers for ticks and also cause natural transmission of the disease (Pipano and Chana, 1969). Many diagnostic methods, including serological and biotechnological techniques, have been investigated to identify haemo-parasite species, monitor their distribution, assess the risk of disease in a given area, and certify the status of animals for trade requirements (Cringoli et al, 2002). Several serological tests are used in the diagnosis of subclinical infections in epidemiological studies. IFAT is one of those and is considered to be one of the most sensitive and effective test (Johnston et al., 1977; Kuttler et al., 1977). The objective of this study was to determine the prevalence of *B. bigemina* and *T. annulata* antibodies in bovine sera by IFAT in Eregli district of Konya, Turkey.

Materials and Methods

Study area and sampling: The study was performed in Eregli district of Konya province located in the Central Anatolia region of Turkey. Blood samples of randomly selected 287 Holstein cows were collected from dairy farms. Of these, 175, 64 and 48 samples belonged to 3, 4 and 5 years old cattle, respectively. During the day they graze on proprietary pastures for 7–9 hour per day and at night, cattle are kept in the cow house. The farmers were applying acaricides to cattle monthly to control ticks. No clinical cases of babesiosis and theileriosis were observed during the study period.

Tick borne diseases have a seasonal increase

from May to October with the peak period in July to August in Turkey related with their vectors activity. Keeping this in mind, blood samples were collected in July, 2008. The sera were separated, divided into aliquots and stored at -20°C until used (Pipano and Chana, 1969).

Serological test: The IFAT, using both the schizont and piroplasm stages of *T. annulata* and *B. bigemina* piroplasms as antigens, was used to examine serum samples for the presence of appropriate specific parasite antibodies. Parasite antigens and control sera (positive, negative), prepared in Department of Parasitology- Faculty of Veterinary Medicine-University of Ankara, were used to detect antibodies to *T. annulata* and *B. bigemina*. Anti-bovine IgG fluorescein isothiocyanate (FITC) conjugate was obtained from SIGMA (Cat. No. F-7250). Slides were examined in dark room following the IFAT procedure using a fluorescein microscope (Zeiss) with Neoflaur objective (40 x).

Statistical analysis: The Chi-Square test was applied to compare the rates of seropositivity between age groups. Statistical significance was defined as $P < 0.05$. The statistical analysis was performed using SPSS 14.01 statistics software.

Results and Discussion

Babesiosis and theileriosis are tick-borne diseases widespread in tropical and sub-tropical regions with high economic impact worldwide (Uilenberg, 1995). The diseases directly and indirectly hamper the growth of the livestock sector. In Turkey ticks of the genera *Haemaphysalis*, *Hyalomma*, *Boophilus* and *Rhipicephalus* are widespread throughout Anatolia and some species of these genera are vectors of cattle *Babesia* and *Theileria sp* (Aydin and Bakirci, 2007). Animals recovered from the infection are carriers and they have an important role in the transmission of the infection by ticks and also in the epidemiology of the diseases (Brown, 1990; d'Oliveira et al., 1995).

In the present study, antibodies against *B. bigemina* were detected in 100 (34.84 %) and *T. annulata* in 2 (0.69 %) of 287 cattle sera by IFA test which is in

agreement with other studies (Cakmak and Oz, 1993; Eren et al., 1995; Sevinc et al., 2001; Kaya et al., 2006; Sevgili et al., 2010). In sera of a 3 years old cattle antibodies against both *B. bigemina* and *T. annulata* were detected. The highest seroprevalence of *B. bigemina* was

in 5 year age group (43.8%), followed by 3 (34.3%) and 4 (29.7%) year age groups with non-significant difference (Table 1). The positivity for *T. annulata* was found only in 2 (1.1%) cattle at the age of 3.

Table 1. Prevalence of antibodies to *B. bigemina* and *T. annulata* in cattle.

| Age | Sample numbers | <i>B. bigemina</i> (%) | <i>T. annulata</i> (%) |
|-----|----------------|------------------------|------------------------|
| 3 | 175 | 60 (34.3%) | 2 (1.1%) |
| 4 | 64 | 19 (29.7%) | - |
| 5 | 48 | 21 (43.8%) | - |

Based on the information gained from a serological study, the immune status of cattle in an area can be classified into an endemically stable (81–100 % positive sera) situation, a situation approaching stability (61–80%), an unstable (21–60%) situation, a minimal disease situation (1–20%), and a disease-free situation (0% positive sera) (Cringoli et al., 2002). On the basis of our study results it is understood that there is an unstable situation for babesiosis and a minimal disease situation for theileriosis. Definitely, the sample size of our study isn't enough to show general situation of the region but can point the situation of the surroundings of the dairy farm.

Compared to *Theileria annulata*, the high (34.84 %) sero-prevalence of *B. bigemina* indicates the widespread distribution of babesiosis in the sampled area. The results highlighted that *B. bigemina* and its potential vectors are extremely common in the examined area and pointed out the likely risks for cattle that are imported from babesiosis-free areas.

Age dependent immunity plays a role in most tick-borne diseases which is independent of colostral antibodies. Age resistance, perhaps in combination in some cases with maternal antibodies, is reflected in the reduced number of clinical outbreaks in young animals (Zwart, 1985). The observed increase of infection in 5 year age group can be explained by the age dependent immunity to Babesia infection that has earlier been explained for babesiosis. However, the infection rate is higher in 3 years age group compared to 4 years and this condition can't be explained with age dependent immunity.

Breed differences are also important in the susceptibility of cattle to tickborne diseases. Cattle of European origin like Holstein are usually highly susceptible. Outbreaks of clinical illness do not occur uniformly in tick-infected areas due to the differences in genetic or age resistance of the cattle, variations in tick populations, numbers of infected ticks and established control methods (de Castro, 1997).

In this study, it was observed that the seroprevalence of *B. bigemina* was 34.84 % and *T. annulata* was 0.69 % by IFAT. The results of this study showed that babesiosis was a fairly common disease in comparison to theileriosis in cattle of Eregli district at the study period. It is important to have more data on prevalence of these infections which have importance on livestock production in Turkey. Urgent measures such as vaccines, chemotherapy, chemoprophylaxis and tick control programs should be taken for the prevention of these diseases.

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Yazışma Adresi:

Yrd. Doç. Dr. Esin GÜVEN
 Atatürk Üniversitesi Veteriner Fakültesi Parazitoloji
 A.B.D. 25240 Yakutiye/Erzurum
 e-mail: esinguyen@atauni.edu.tr