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# Prevalence of *Theileria annulata* and *Babesia bigemina* in Cattle in the Vicinity of Sanliurfa

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**Abstract:** This study was carried out on cattle to detect the seroprevalence of *Theileria annulata* and *Babesia bigemina* around the Sanliurfa province. A total of 191 randomly selected cattle were examined from selected locations for *Theileria annulata* and *Babesia bigemina*. Blood samples were collected from the cattle by jugular vene puncture to obtain sera for Indirect Fluorescence Antibody Test (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 100x magnification. In 19 of 191 cattle (9.94%) *T. annulata* and in 17 cattle (8.90%) *Babesia* sp. were observed. The sera were tested for the presence of antibodies to the *T. annulata* and *B. bigemina* by IFAT. Antibodies were detected against *T. annulata* in 138 (72.25%) and *B. bigemina* in 84 (43.97%) sera of the tested 191 cattle.

**Key words:** *Theileria annulata*, *Babesia bigemina*, IFAT, seroprevalence, cattle, Sanliurfa

## INTRODUCTION

*Theileria* and *Babesia* sp. are tick-borne haemoprotozoan parasites of vertebrates that have a major impact on livestock production, mainly cattle and small ruminants, in tropical and subtropical areas (Sevinc *et al*., 2001; Kaya *et al*., 2006; Ica *et al*., 2007).

The protozoan parasite *Theileria annulata* is the causative agent of tropical theileriosis and endemic in the area around the Mediterranean and reaches the Middle East and the Southern of Asia. Tropical theileriosis is a protozoan infection seen in cattle, buffola, zebu and bison. It causes severe infection especially in cattle. The parasite is transmitted from cattle to cattle by ticks of the genus *Hyalomma*. In Turkey, *T. annulata* is considered to be a major threat to the cattle breeding since the disease causes mortality and economic losses, particularly in crossbred cattle (Mimioglu *et al*., 1971; McCosker, 1981; Blood and Radostits, 1989).

Diagnosis of the disease is based on clinical findings and Microscopic Examination (ME) of blood and lymph node smears stained with Giemsa in acute cases. Frequently serological methods are employed in determining subclinical infections. Since morphological structures of piroplasm form *Theileria* sp. similar to each other, differential diagnosis of specific species is difficult (Aktas *et al*., 2001a; Vatansever and Nalbantoglu, 2002).

Studies focusing on prevalence of tropical theileriosis in Turkey routinely utilized ME of peripheric blood and lymph node smears (Goksu, 1970; Tuzer, 1981; Dumanli and Ozer, 1987). Recently, indirect fluorescence antibody test has been reported to be used for the same purpose (Eren *et al*., 1995; Sayin *et al*., 2002; Aktas *et al*., 2002; Dumanli *et al*., 2005).

Babesiosis is one of the more common diseases of farm animals worldwide and is gaining increasing attention as an emerging tick-borne zoonosis in humans. Bovine babesiosis, caused by the tick-transmitted protozoan *Babesia bigemina*, *B. bovis* and *B. divergens*, is considered one of the most frequent and important tickborne diseases of cattle worldwide. Babesiosis is considered to be another major threat to the cattle industry since it causes mortality and economical losses in cattle farms as well (Cakmak, 1987; Inci, 1992; Eren, 1993; Tanyuksel *et al*., 2002; Ica, 2004).

There are four *Babesia* sp. in cattle in Turkey, which are *Babesia bigemina*, *B. bovis*, *B. divergens* and *B. major*. The greatest economic losses occur due to *B. bigemina* and *B. bovis*. In many areas of Turkey both

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species of *Babesia* occur concurrently, transmitted by one vector, *Rhipicephalus annulatus* (formerly *Boophilus annulatus*). The vectors of *B. bigemina* are coincided with babesiosis in various regions of Turkey (Cakmak, 1987; Dincer *et al*., 1991; Inci, 1992; Eren, 1993; Aydin and Bakirci, 2007).

The diagnosis of babesiosis is done by microscopical examination of blood smears and observing of clinical symptoms. Microscopic diagnosis is easier in acute form than in subclinical infections. Consequently, various serological tests are used in the diagnosis of subclinical infections. Many serological techniques have been developed for the detection of antibodies against the piroplasms in the last decades (Blood and Radostits, 1989; Cakmak, 1987; Eren, 1993; Sevinc *et al*., 2001).

The objective of the present study was to determine the seroprevalence of *Theileria annulata* and *Babesia bigemina* in cows in and around Sanliurfa by microscopic examination and IFAT.

## MATERIALS AND METHODS

**Area of the study and animals:** The study was conducted in Sanliurfa province located in the South-Eastern of Turkey. A total of 191 cattle were sampled between March to October 2008. Blood samples were collected from randomly selected healthy cattle, in 5 different locations of namely Central (44), Siverek (35), Viransehir (30), Akcakale (38), Birecik (44). The owners of the animals were questioned about animal management and age; then obtained information was recorded. In the present study, the majority of the cattle raised at small-scale farms (4-19 heads per farms). Of these, 127 were 1-2 years old and 64 were between 3-5 years old.

**Sample collection:** The thin blood smears prepared from ear capillaries were fixed in methanol for 5 min and stained with 5% Giemsa solution for 30 min and the presence of *Theileria* and *Babesia* piroplasms were examined microscopically. At least 50 microscopical areas were carefully examined for *Theileria* and *Babesia* sp. piroplasms under the oil immersion lens. The presence of even a single piroplasm was considered positive.

For serum samples, 10 mL blood was collected in plain test tubes by jugular vene puncture. After collection, blood samples were stored in ice box for 4-6 h. The sera were seperated from the blood within 24 h by centrifugation at 2500 rpm for 10 min. Collected sera were aliquoted in 2 mL labelled tubes and kept at -20°C, until analysed by IFAT as described (Pipano and Cahana, 1969).

**Serological examination:** The IFAT, using both the schizont and piroplasm stage of the *T. annulata* and *Babesia* sp. piroplasm stages as antigens, was used to examine serum samples for the presence of appropriate specific parasite antibodies. *T. annulata* and *B. bigemina* antigens and control sera (positive-negative) were prepared in the Parasitology Department, Faculty of Veterinary Medicine, Ankara University used to detect antibodies to *T. annulata* and *B. bigemina*. Anti-bovine IgG, FITC Conjugate was obtained from SIGMA (Cat.No. F-7887). Slides were examined in dark room following the IFAT procedure using a flourescein microscope (Zeiss) with Neoflaur objective (40x).

**Statistical analysis:** The $\chi^2$-test was applied to compare the rates of seropositivity between age groups, study sites and gender. Statistical significance was defined as $p<0.05$. All statistical analyses were performed using SPSS.

## RESULTS AND DISCUSSION

Out of 191 smears examined microscopically, 19 (9.94%) were positive for *T. annulata* and 17 (8.90%) *Babesia* sp. piroplasms. Of all the smears, three showed a mixed infection with piroplasms of *Theileria* and *Babesia* sp.

As shown in Table 1, antibodies to *T. annulata* were found in 138 (72.25%) and *B. bigemina* 84 (43.97%) of 191 cattle sera based on IFAT test results. In this study, antibodies to *T. annulata* and *B. bigemina* were determined in five districts in Sanliurfa. The prevalence of antibodies to *T. annulata* and *B. bigemina* for the five districts is presented by Table 1. As shown in Table 1, the

Table 1: Prevalence of antibodies to *T. annulata* and *B. bigemina* in cattle in districts of Sanliurfa by the IFAT and microscopic examination

| | Age | | Sex | | Origin | | | | |
|---|---|---|---|---|---|---|---|---|---|
| The number of percentage | 1-2 (127) | 3-5 (64) | Male (103) | Female (88) | Centre (44) | Siverek (35) | Viransehir (30) | Akcakale (38) | Birecik (44) |
| Microscopic examination for *T. annulata* | 14 (11.02) | 5 (7.81) | 10 (9.71) | 9 (10.22) | 5 (11.36) | 4 (11.42) | 4 (13.33) | 2 (5.26) | 4 (9.09) |
| Seropositive animals for *T. annulata* | 94 (74.01) | 44 (68.75) | 76 (73.78) | 62 (70.45) | 28 (63.63) | 31 (88.57) | 17 (56.66) | 32 (84.21) | 30 (68.18) |
| Microscopic examination for *Babesia* sp. | 7 (5.51) | 10 (15.62) | 8 (7.76) | 9 (10.22) | 5 (11.36) | 3 (8.57) | 1 (3.33) | 3 (7.89) | 5 (11.36) |
| Seropositive animals for *B. bigemina* | 49 (38.58) | 35 (54.68) | 44 (42.71) | 40 (45.45) | 13 (29.54) | 10 (28.57) | 17 (56.66) | 20 (52.63) | 24 (54.54) |

highest seroprevalence for *T. annulata* was observed in Siverek (88.57%) and the lowest seropositivity was found in Viransehir (56.66%). The seropositivity rates for Birecik (68.18%) and Centre (63.63%) were found as similar. The difference between the rates according to districts was statistically significant for *T. annulata* ($p = 0.011$).

The highest seroprevalence for *B. bigemina* was observed in Viransehir (56.66%) and the lowest seropositivity was found in Siverek (28.57%). The seropositivity rates for Birecik (54.54%) and Akcakale (52.63%) were found as similar. The difference between the rates according to districts was also statistically significant for *B. bigemina* ($p = 0.016$).

A total of 127 sera samples from 1-2 years old cattle and 64 serum samples from 3-5 years old cattle were examined for *T. annulata* and *B. bigemina*. Antibodies to *B. bigemina* were found in 49 (38.58%) cows at the age of 1-2 and 35 (54.68%) at the age of 3-5. Besides, antibodies to *T. annulata* were found in 94 (74.01%) cows at the age of 1-2 and 44 (68.75%) cows at the age of 3-5. A statistically significant difference between the age groups was observed for antibodies *B. bigemina* ($p = 0.034$). On the other hand, there is no statistically significant difference in seropositivity rates for *T. annulata* among the age groups, although the seropositivity rate was higher for the 1-2 years age group than the other age group ($p = 0.443$).

Anti-*T. annulata* antibodies were detected in 76 (73.78%) of 103 male cows and 62 (70.45%) of 88 female cows. Although, the seropositivity rate for the male cows was higher than the rate for the female cows, the difference between the rates was not found statistically significant ($p = 0.608$). From the 44 (42.71%) out of 103 male cows and the 40 (45.45%) out of 88 female cows were detected anti *B. bigemina* antibodies. There was no statistically significant difference in seropositivity between gender ($p = 0.704$).

Tick-transmitted diseases such as babesiosis and theileriosis are economically important globally (Uilenberg, 1995). These animals have an important role in the transmission of the infection by ticks (Brown, 1990). The diagnosis of piroplasm infections are based on clinical findings and microscopic examination of Giemsa-stained blood smears. However, this method is not sensitive enough or sufficiently specific to detect chronic carriers, particularly when mixed infections occur. Serological tests are frequently used for diagnosis of latent infections. Nevertheless, it is difficult and time consuming to identify piroplasmic forms within the erythrocytes from carrier animals. Serological tests based on determination of antibodies developed against agent causing disease are applied (Pipano and Cahana, 1969; Vatansever and Nalbantoglu, 2002).

Aktas *et al.* (2001a) examined microscopically of peripheral blood smears for *Theileria annulata* and observed in 42.8% (285), 17.1% (292) and 34.8% (164) cattle in Elazig, Malatya and Tunceli, respectively. Sevinc *et al.* (2001) found *T. annulata* in 15 of 157 smears (9.55%) in Konya. Acici (1995) examined blood smears of 184 cattle suspected of theileriosis and found as positive, 26 (17%) for *T. annulata*. The prevalence of *B. bigemina* by the examination of Giemsa stained blood smears were found between 0.6 and 54.96% of cattle according to the studies performed in various regions of Turkey (Mimioglu, 1955; Goksu, 1959; Ozcan, 1961; Hoffman *et al.*, 1971; Tuzer, 1981; Dincer *et al.*, 1991; Dumanli and Ozer, 1987; Eren, 1993; Ozer *et al.*, 1993; Acici, 1995; Aktas *et al.*, 2001b; Sevinc *et al.*, 2001; Ica *et al.*, 2007).

In Turkey IFAT was first used to detect the seroprevalence of *Babesia* sp. and *Theileria annulata* in cattle by Cakmak (1987). Cakmak (1987) detected the seropositivity at the rates of 4.8 and 9.7% against *B. bigemina* and *B. bovis* in cattle in Beytepe village of Ankara, respectively. In the following studies, the seroprevalence of *B. bigemina* were found between 49.2 and 100% in cattle at various regions of Turkey (Cakmak, 1987, 1993; Dincer *et al.*, 1991; Inci, 1992; Cakmak and Oz, 1993; Eren, 1993; Eren *et al.*, 1995; Acici, 1995; Sayin *et al.*, 1997; Aktas *et al.*, 2001b; Inci *et al.*, 2002; Karatepe *et al.*, 2003; Dumanli *et al.*, 2005; Ica, 2004; Sayin *et al.*, 2004; Ica *et al.*, 2007).

The prevalences of *T. annulata* were determined very high in different regions. The highest prevalences were found in Southeastern Anatolia, 91.40% and Central Anatolia, 92.65% (Dincer *et al.*, 1991; Cakmak, 1993; Eren *et al.*, 1995; Sayin *et al.*, 1997; Aktas *et al.*, 2001a; Vatansever and Nalbantoglu, 2002; Dumanli *et al.*, 2005). High seroprevalence (81.20%) was found in Sanliurfa by Dumanli *et al.* (2005). Cakmak and Oz (1993) found *T.annulata* seropositivity as 10.68% in Adana. Acici (1995) carried out IFAT on serum samples collected from 76 cattle in Samsun; 48 cattle had antibodies to *T. annulata*. Kaya *et al.* (2006) found *T. annulata* 24 of 214 (11.21%) cattle in Hatay.

In this study, the prevalence of *Babesia* sp. and *Theileria annulata* by microscopic examination were respectively found to be 19 (9.94%) and 17 (8.90%). However, IFAT screening resulted with 138 (72.25%) *T. annulata* and 84 (43.97%) *B. bigemina* seropositive out of 191 animals.

## CONCLUSION

The prevalence of *T. annulata* is widespread in cattle of Sanliurfa Province. Urgent measures such as

anti-*Theileria* vaccines, chemotherapy, chemoprophylaxis and vector control should therefore be taken prevention of theileriosis.

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