Prevalence of *Babesia bigemina* in cattle

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**SUMMARY**

This study was carried out to determine the prevalence of *Babesia bigemina* infection in cattle by microscopic and serologic methods in centre villages of Konya between April and October 1999. Peripheral blood was examined microscopically using thin smears. In 18 of 157 cattle (11.46 %) *Babesia sp.* and in 15 cattle (9.55 %) *Theileria annulata* were observed. The serum samples of 277 cattle were examined using the IFA test. The antibodies against *B. bigemina* were detected in 147 of 277 cattle (53.07 %) by IFA test.

**KEY-WORDS :** *Babesia bigemina* - bovine.

1. Introduction

Babesiosis is a tick-borne disease which is very widespread in Turkey. The prevalence of the disease is very high in the high yielding breeds of exotic cattle, especially. Mortality rate of acute babesiosis is frequently high. In animals even if recovering from acute babesiosis, losses in meat and milk production, temporary infertility of male animals and abortions as a result of high fever occur. Consequently, babesiosis of cattle in Konya province is of serious importance on the breeding of cattle and economy of country.

There are four *Babesia* species 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 species of *Babesia* occur concurrently, transmitted by one vector, *Boophilus microplus*. The vectors of *B. bigemina* are coincided with babesiosis in various regions of Turkey [2, 13, 14, 17, 22, 24, 26, 31].

*Babesia bigemina* is a large babesia species, the pyriform bodies are 4 - 5 µm long. In acute babesiosis due to *B. bigemina*, rectal temperature rises in parallel with the increase in parasitemia and reaches a maximum of 41° to 42°C in two and three days. Haemoglobinemia and haemoglobinuria then occur, followed by jaundice. The feces are dry and blood stained. Animals not treated die. The most of cattle recovering from acute infections remain chronic carrier [4, 18, 20, 21, 25].

The diagnosis of babesiosis is done by microscopical examination of blood smears and observing of clinical symptoms. Microscopic diagnosis is easier in acute 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 [6, 29, 30, 32, 34]. The indirect immunofluorescence test (IFAT) is one of the simplest, most sensitive and effective techniques used for this purpose [3, 5, 11, 16, 30].
The objective of the study was to determine the prevalence of *B. bigemina* antibodies in bovine sera by IFAT in Konya province.

### 2. Materials and methods

**A) MICROSCOPIC EXAMINATION**

The peripheral blood smears of 157 cattle was examined microscopically between April and October 1999. Blood samples were taken from the tip of ear of each cattle, and thin blood smears were done. The smears were fixed in methyl alcohol and stained for 45 min by Giemsa solution. The stained smears were washed by tap water, dried at room temperature and examined with a light microscope with a X 100 objective.

**B) ANTIGEN PREPARATION**

Whole blood, with a parasitaemia of 10% *B. bigemina* from an naturally infected calf, was drawn directly into heparinized injectors. The blood cells were washed 3 times by centrifugation of blood in PBS (pH 7.4) and the buffy coat was removed during this processes. The volume was made up to that of the original blood by the addition of PBS. The parasitised blood samples in the volume of 10 µl were dropped on every well of multitest slides (15 well, ICN- 6041505), and dried at room temperature. Slides were wrapped in paper in groups of 5 to 8, placed in nylon film bags, and stored at -70°C [7, 11, 16, 19, 30].

**C) POSITIVE AND NEGATIVE CONTROL SERA**

Positive and negative control sera were provided from University of Ankara, Faculty of Veterinary Medicine, Department of Protozoology and Entomology, Ankara, Turkey.

**D) CONJUGATE**

Rabbit anti-bovine-IgG-conjugate (Sigma F-7887) labelled with fluorescein isothiocyanate (FITC) was used and diluted at 1/32 in PBS pH 7.4.

**E) FIELD SERA**

Ten ml of blood samples were collected from the jugular vein of 277 cattle into vacuum tubes. The blood samples were transferred in a transport medium at 4 °C and centrifuged in the same day at 2000 X g for 10 min. The sera were distributed in aliquots of 1.5 ml and stored at -20 °C.

The sera were tested using two-fold dilutions in PBS pH 7.4. Six dilutions from 1 / 32 to 1 / 1024 were made for the negative control sera, positive control sera and field sera. The control sera were tested in the each test performed.

**F) THE APPLICATION OF IFA TEST**

The packets of antigen were maintained for 30 min at room temperature before removing of nylon bag. The slides were placed in a moist chamber and 10 µl of each serum dilution was pipetted on each thick smear in a standard sequence. After the incubation for 30 min at 37 °C, the slides were rinsed in PBS three times for 5 min. The slides were placed again in the moist chamber, covered by the conjugate dilution, incubated for 30 min at 37 °C, rinsed as mentioned before and mounted with buffered glycerine for the examination [7, 11, 16, 19, 30].

The slides were examined at a magnification of about 725 X with dark field illumination and blue-light fluorescence filters (Leitz Orthoplan microscope).

### 3. Results

*Babesia bigemina* in 5, *Babesia sp* in 13, *Theileria annulata* in 15 cattle were detected in the examination of Giemsa stained blood smears. The sera of 4 cattle in which *B. bigemina* detected microscopically had also antibodies against to *B. bigemina* antigen, however, antibodies against the same antigen were not found in one of these cattle sera.

Antibodies against to *B. bigemina* were detected in 147 (53.07 %) of a total of 277 cattle sera by IFA test. The titers of positive reactions was between 1 / 64 and 1 / 1024 dilutions.

The appearance of smears with positive reactions for *B. bigemina* detected by IFA test is shown on Fig. 1.

### 4. Discussion

Babesiosis is a disease which cause an important economical loss in cattle of Turkey [1, 9, 13-15, 24, 26, 33] and various countries [3, 12, 28, 35]. The prevalence of *B. bigemina* by the examination of Giemsa stained blood smears were found between 0.6 and 32.21 % of cattle according to the studies performed in various regions of Turkey [1, 9, 13, 14, 24, 26, 33].

Indirect immunofluorescence test was first used to detect the seroprevalence of *B. ovis* by ÖZKOÇ [27]. The same test was used on the serodiagnosis of *B. bigemina*, *B. bovis*, *B. divergens* and *T. annulata* in cattle of Turkey by ÇAKMAK [7]. ÇAKMAK [7] 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 [8-10, 15]. In this study, it was observed that the prevalence of *Babesia sp* and *Theileria annulata* by microscopic examination were respectively found to be 11.46 % and 9.55 %. The seroprevalence of *B. bigemina* were detected as 53.07 % by IFAT.

The results of this study showed that babesiosis was a quite widespread disease in cattle of Konya province. So, the prevention of disease by anti-Babesia vaccines, chemotherapy, chemoprophylaxis and vector control is urgent.

5. References


