SEROEPIDEMIOLOGY OF BABESIA BIGEMINA IN CATTLE IN THE KONYA PROVINCE, TURKEY: ENDEMIC STATUS

OZLEM DERINBAY EKICI AND FERDA SEVINC
Department of Parasitology, Faculty of Veterinary Medicine, University of Selcuk, 42100 Konya, Turkey
derinbay@selcuk.edu.tr

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Abstract

This study was designed to determine the seroepidemiology of Babesia bigemina in cattle in the Turkish province of Konya. Between April 2006 and March 2007, a total of 770 cattle were randomly selected, assigned to different age groups (i.e. 0 to 3 months, 3 to 6 months, 6 to 9 months, 9 to 12 months, 12 to 24 months, 24 to 36 months, and older than 36 months) and coming from the Kadinhanı, Çumra, and Beyşehir districts and in Konya city centre. B. bigemina was diagnosed by microscopic examination of blood smears and indirect fluorescent antibody testing. Parasites were observed in 1.95% of blood smears and 42.9% of animals were positive for B. bigemina antibodies. The seropositivity rate of B. bigemina in cattle in Kadinhanı, Çumra, Beyşehir, and Konya was 33.52%, 44.3%, 37.9%, and 54.8%, respectively. The rates of seropositivity according to age group were 5.3%, 14.3%, 35.4%, 74.8%, 44.0%, 39.8%, and 46.9%, respectively. The endemic status of the disease was determined by calculating the inoculation rate \( h \) of each age group. The \( h \) value was detected to be lower than 0.005 in each group and the endemic status of B. bigemina was found to be unstable. Research centres were visited regularly each month and the animals were examined for tick infestations. On examination, 101 ticks were collected and their species identified. The identified tick species were Hyalomma anatolicum anatolicum, Hyalomma anatolicum excavatum, Hyalomma marginatum marginatum, Ixodes ricinus, Dermancetor marginatus, Rhipicephalus bursa, Rhipicephalus turanicus, Rhipicephalus sanguineus, Ornithodoros lahorensis, Haemaphysalis parva, Haemaphysalis sp. (nymph) and Hyalomma sp. (nymph).

Key words: cattle, Babesia bigemina, seroepidemiology, Turkey.

Babesiosis is a protozoal disease seen in tropical and subtropical regions, which is transmitted transovarially by vector ticks of the Ixodidae family (10, 23). At the acute stage, diagnosis is based on clinical symptoms and the detection of parasites in blood smears. Serological diagnosis is applied for carriers, and chronic or latent infection (6). In regions where the disease is endemic, pathognomic symptoms, such as high fever, haemolytic anaemia, jaundice, and haemoglobinuria are frequently seen in animals and vector ticks are found to exist on these animals (20, 28). The ultimate diagnosis of the disease is confirmed by observing the protozoa in blood smears.

A characteristic property of protozoal and rickettsial agents is that animals that recover from the illness become hosts (26). Such host animals remain a source of infection for vector ticks and cannot be clinically distinguished from those animals that are not infected. The incidence of such animals in the cattle population depends on the epidemiology of the disease. For this reason, the determination of carrier animals is one of the most important steps in the control and identification of babesiosis. However, usually only a small number of parasites are in the blood of carrier animals and they cannot be easily detected. Various serological and molecular methods are employed for the detection of the agent in such animals. The most commonly-used test in the diagnosis of babesiosis is indirect fluorescent antibody test (IFAT) (29).

In regions where babesiosis is endemic, the most reliable method of protecting animals from blood parasite infection is vaccination. Vaccination reduces the economic cost of the disease by achieving the immunity of susceptible animals to field challenge. The initial step of deciding whether a vaccination protocol is required is to assess the endemic status of the disease. Vaccination is usually only required in countries where the endemic status of the disease is unstable. Seroprevalence of the disease in animals of different ages should also be established to estimate the endemic status of the disease.

Turkey is located in a subtropical zone and babesiosis is an endemic disease. Ticks transporting Babesia species exist in almost all regions of the country and the disease is seen every year during seasons when ticks are active (27). ‘Endemic’ is an epidemiological term that has frequently been debated and defines herd
immunity. The rate of herd immunity is generally measured using serological tests, and is expressed by the term ‘inoculation rate’. The inoculation rate is directly related to the frequency of tick infection and infection in animals. When the inoculation rate of Babesia is at such a level as to infect cattle that are protected by natural and colostral immunity, clinical disease is at a limited rate, and endemic stability is reached. In contrast, if the inoculation rate is low, and natural and colostral immunity of calves are not properly formed, endemic instability emerges, resulting in clinical cases (12). The method developed by Mahoney and Ross (22) is commonly used throughout the world for the calculation of the inoculation rate. The objectives of the present study were to identify seroepidemiology of Babesia bigemina in cattle in the province of Konya and to determine whether babesiosis in the region is endemically stable or not.

**Material and Methods**

Between April 2006 and March 2007, a total of 770 cattle were randomly selected, assigned to different age groups (i.e. 0 to 3 months, 3 to 6 months, 6 to 9 months, 9 to 12 months, 12 to 24 months, 24 to 36 months and older than 36 months). The cattle came from Kadmham, Çumra, and Beyşehir districts and from Konya city centre.

**Clinical evaluation.** Clinical examinations (rectal temperature, the appearance of mucous membranes, respiration and pulse rates, the presence of haemoglobinuria) were performed for all the animals.

**Microscopic examination of blood smears.** Peripheral blood smears of the cattle were examined microscopically. Blood samples were taken from the tip of the ear and thin blood smears were prepared. The smears were fixed in methyl alcohol and stained for 30 min with Giemsa solution. The stained smears were washed with tap water, dried at room temperature, and examined under a light microscope with a 100x objective lens.

**Serum samples.** Blood samples of 8 ml were collected into vacuum tubes from the jugular vein. The samples were transferred in a transport medium at 4°C and then centrifuged at 4,000 rpm for 10 min on the same day. The sera were distributed in 1.5 ml aliquots and stored at -20°C until they were used in IFAT.

**Indirect fluorescent antibody test.** IFAT was performed according to the manufacturer's recommendations (Babesia bigemina IFA IgG Antibody Kit, Fullerton, USA). The slides were examined using a fluorescence microscope (Olympus BX51, Olympus Optical Co. Ltd., Japan). Serum samples that showed fluorescence at a dilution rate of 1:80 were regarded as positive.

**Parasites.** The ticks collected from the animals were put into tubes containing 70% ethanol and were examined under a stereo microscope. Thus their species was identified (4, 18).

**Endemic status.** In order to identify the endemic status of the region, inoculation rates of the cattle in each age group were calculated using the following formula by Mahoney and Ross (22): $h = (-1)[\ln(1-I)] / t (7)$ where: $h =$ inoculation rate; $I =$ the rate of infected animals (%); and $t =$ arithmetic mean of the age of the animals in days (calculated using $t$ test). In endemically-stable situations, the inoculation rate ranges from 0.005 to 0.05 (22), which corresponds to a 75% and 100% infection rate, respectively (7, 11).

**Results**

In the present study, samples from 770 cattle from 74 barns were examined microscopically and serologically for B. bigemina. Based on these examinations, 15 (1.95%) cattle were found to be positive for B. bigemina and 331 (42.9%) cattle were diagnosed as positive for B. bigemina antibodies. Five from 15 samples positive for these antibodies in microscopic examination were also positive in serology. These animals showed specific clinical symptoms (fever, haemoglobinuria, pale mucous membranes, haemolytic anaemia) of acute bovine babesiosis. B. bigemina was detected microscopically in six (5.83%) of 103 cattle in the group of 24 to 36-month-olds and in nine (4.95%) of 192 cattle aged over 36 months. B. bigemina was observed more frequently in August (17.5%), than in September (4.6%) and April (1.5%). B. bigemina could not be identified in any of the blood smears analysed in the other time periods. The proportion of animals displaying acute signs of infection was quite low. B. bigemina antibodies were detected at the lowest rate in four (5.3%) of the 76 cattle in the 0 to 3-month-old group, and the highest rate was seen in 92 (74.8 %) cattle of 123 allocated to the group aged 9 to 12 months. Their serology results were used to calculate inoculation rates. Inoculation rates were calculated for each age group to determine whether the endemic status of the region is stable or unstable (Table 1).

The seropositivity rates in each of the age groups were different. According to age, the seroprevalence values of B. bigemina were determined as 5.3%, 14.3%, 35.4%, 74.8%, 44.0%, 39.8%, and 46.9%, respectively. As noted in Table 1, the $h$ value was found to be lower than 0.005 in all groups. Therefore, the endemic status of the cattle was found to be unstable in the locations where the study was conducted.

In the study, 101 ticks were collected from the animals examined and the following species were detected: Hyalomma anatolicum anatolicum, Hyalomma anatolicum excavatum, Hyalomma marginatum marginatum, Ixodes ricinus, Dermacentor marginatus, Rhipicephalus bursa, Rhipicephalus turanicus, Rhipicephalus sanguineus, Ornithodoros lahorensis, and Haemaphysalis parva. In particular seasons the following species were determined: in spring - H. a. anatolicum, H. a. excavatum, I. ricinus, D. marginatus, R. turanicus, and O. lahorensis, in the summer - H. a. anatolicum, H. m. marginatum, R. bursa, R. sanguineus,
The inoculation rates of *Babesia bigemina* in cattle divided into different age groups

<table>
<thead>
<tr>
<th>Age groups (month)</th>
<th>Number of animals examined</th>
<th>Mean age (±SEM) Arithmetic average age (l)</th>
<th>Positivity % (l)</th>
<th>Inoculation rate (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–3</td>
<td>76</td>
<td>51.9 ± 3.34</td>
<td>5.260</td>
<td>0.0009</td>
</tr>
<tr>
<td>3–6</td>
<td>35</td>
<td>158 ± 4.14</td>
<td>14.29</td>
<td>0.0009</td>
</tr>
<tr>
<td>6–9</td>
<td>82</td>
<td>241 ± 3.71</td>
<td>35.37</td>
<td>0.0020</td>
</tr>
<tr>
<td>9–12</td>
<td>123</td>
<td>339 ± 2.59</td>
<td>74.79</td>
<td>0.0040</td>
</tr>
<tr>
<td>12–24</td>
<td>159</td>
<td>656 ± 8.23</td>
<td>44.03</td>
<td>0.0008</td>
</tr>
<tr>
<td>24–36</td>
<td>103</td>
<td>1,069 ± 4.14</td>
<td>39.81</td>
<td>0.0004</td>
</tr>
<tr>
<td>&gt;36</td>
<td>192</td>
<td>2,008 ± 53.4</td>
<td>46.88</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

SEM = standard error of the mean.

*R. turanicus* and *D. marginatus*, in autumn - *H. a. anatolicum*, *H. a. excavatum*, *H. m. marginatum*, and *Haemaphysalis parva*, and in winter – *Haemaphysalis*. The most common tick species found were *R. turanicus* and *H. a. anatolicum*. The number of ticks collected in central Konya, Çumra, Beyşehir, and Kadinhanı was 43 (42.6%), 11 (10.9%), 16 (15.8%), and 31 (30.7%), respectively.

### Discussion

Bovine babesiosis is an major significant disease, which is spread by ticks in tropical and subtropical areas and causes significant economic loss (19). As in many countries in the world, it threatens cattle raised in Turkey (15, 24).

The agent is widely seen where pasture ticks are present, especially in countries in tropical and subtropical zones (19, 23). *B. bigemina* is economically significant, especially in Asia, Africa, middle and South America, southern Europe, and Australia (9). It has been declared that tick-borne diseases, especially theileriosis, babesiosis, and anaplasmosis, are quite prevalent in Asian countries. The methods employed to control the disease include chemotherapy, vaccination, and anti-tick control strategies (1). According to original data from serological studies conducted in different regions of the world, seroprevalence of *B. bigemina* varies between 40% and 93% (2, 3, 5, 13, 25, 30, 31).

Since the first babesiosis study on cattle in 1955 in Turkey (24), data on the prevalence of the disease based on microscopic examinations have been collected from different regions. The results of these studies have reported the prevalence of the disease to be between 0.6% and 32.2% (15, 16, 24, 27). In the present study, *B. bigemina* was microscopically observed in 15 (1.95%) of 770 cattle older than two years. During the study period, *B. bigemina* was observed more frequently in August (17.5%), than in September (4.6%) and April (1.5%). *B. bigemina* was not identified in any of the blood smears analysed in the other periods. It was observed that data derived from microscopic examination of blood smears are generally compatible with those from previous studies conducted in Turkey.

In the present study, the proportion of animals displaying acute signs of infection was quite low. This was probably because this kind of animal was treated with Imidocarb dipropionate (1.2 mg/kg b.w.) by their owners before the study.

Serological tests are critically important when determining the epidemiology of the disease. According to results from serological studies in Turkey, the seroprevalence of *B. bigemina* has been identified to vary between 0.93% and 100% (15, 17, 27).

Determining the endemic status of *B. bigemina* in the Konya province was one of the objectives of the present study. The results indicate that the area is endemically unstable for *B. bigemina*, as *h* values calculated were lower than 0.005, which is the minimum level required for the stability. The inoculation rate calculated for the cattle aged between 9 and 12 months (74.8%) was close to the minimum inoculation rate (0.004) needed for endemic stability. This may indicate that endemic stability was partly present in this age group and the probability of acute disease is low in the animals infested with infected ticks. Although the endemic status of the animals in the first three age groups was determined to be unstable, the probability of clinical infection was low because of maternal antibodies and non-specific age resistance. In contrast, this is not true for cattle over the age of one year. In all three groups of cattle over this age, the seropositivity rate was under 75%, which is the limit to show endemic stability. In such animals, vaccination against haemoparasites in all calves is strongly recommended to ensure herd immunity. Therefore, if the cattle in Konya province are vaccinated when they are 9 to 12 months of age, they can be protected during the affected seasons.

The age of the animal is one of the most important factors that affects bovine babesiosis. The incidence of the disease increases in proportion to the age of the animal. Calves younger than six-month-old are generally resistant to acute infections because of innate immunity gained from colostrum and from the non-specific natural resistance they have. If calves come into contact with an infested tick during this period of their life, they gain resistance against reinfection. In contrast, if infected tick invasion is not encountered during this period, the calves’ resistance gradually decreases.
decreases from the 6th to the 9th month of life (12, 21). Until the animals are 9 to 12 months old, the seropositivity rate increases in correlation with age. After this period, it gradually decreases. In this study, the highest seropositivity rate was observed in the group of animals aged between 9 and 12 months. This result indicates an important fact for the epidemiology of babesiosis in Konya province. The decrease in seropositivity rate in cattle can be explained by the reduction in antibody titres by that age. Additionally, the decrease in antibody titres can most probably be influenced by the season.

In this study, Boophilus annulatus, the principal vector of B. bigemina, was not observed. However, tick species other than B. annulatus could be vectors for transferring B. bigemina. The number of ticks collected from cattle in this study was comparatively low compared with figures reported in previous studies (14, 16). The barns and animals used in the current study were regularly medicated every few seasons.

In order to determine the epidemiology of tick-borne diseases, it is crucial to know the seasonal activities of the ticks. In the present study, the tick species found on infested cattle changed according to the season. The tick species most commonly encountered were R. turanicus and H. a. anatolicum. This result is in agreement with other studies (8, 14).

In conclusion, to the best of the authors’ knowledge, this study reports for the first time that decrease in antibody titres can most probably be reduction in antibody titres by that age. Additionally, the seropositivity rate in cattle can be explained by the decrease in antibody titres by that age. Moreover, the decrease in the seropositivity rate was also observed in animals aged between 9 and 12 months. This result indicates an important fact for the epidemiology of babesiosis in Konya province. The decrease in antibody titres indicates an important fact for the epidemiology of babesiosis in Konya province. The decrease in antibody titres by that age. Additionally, the seropositivity rate in cattle can be explained by the decrease in antibody titres by that age.

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References