

Seroprevalence of *Babesia ovis* in sheep around Afyon*

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Received: 16.10.2002

Abstract: This study was carried out to determine the seroprevalence of *Babesia ovis* around Afyon between June and September 2000. During this period, blood samples were collected to obtain serum samples from total of 204 Akkaraman sheep in different age groups in 9 different localities. During sampling, peripheral blood smears were prepared from the punctured ear of each animal. Ticks were also collected from the animals sampled. The sera were tested for the presence of antibodies to *B. ovis* by ELISA. The blood smears were stained with 5% Giemsa stain solution and were examined microscopically. Antibodies against *B. ovis* were detected in 106 of the 204 sheep (51.96%) using ELISA. Under microscopic examination, *B. ovis* was detected in 1 of the 204 sheep (0.49%). Of the 62 adult ticks examined, 41 were male and 20 were female. During this period, 1 nymph was also collected from the sampled animals. The adult ticks were identified as *Rhipicephalus bursa*, *R. sanguineus* and *R. turanicus*. The nymph was identified as *Hyalomma* sp. The collected ticks were not examined for the presence of *Babesia* vermicules.

Key Words: *Babesia ovis*, ELISA, seroprevalence, sheep, tick

Afyon yöresinde koyunlarda *Babesia ovis*'in seroprevalansı

Özet: Bu çalışma, Haziran ve Eylül 2000 tarihleri arasında Afyon yöresinde *Babesia ovis*'in seroprevalansını belirlemek amacıyla yapılmıştır. Araştırma süresince dokuz ayrı yöreden, farklı yaşlarda toplam 204 Akkaraman koyundan serum için kan alınmış, herbir hayvanın kulak ucundan perifer kan frotileri hazırlanmış ve mevcut keneler toplanmıştır. Elde edilen serumlar ELISA ile *B. ovis* antikorları yönünden yoklanmıştır. Perifer kan frotileri % 5'lik Giemsa boya solusyonu ile boyanarak mikroskopta incelenmişlerdir. ELISA ile 204 koyunun 106'sında (% 51,96) *B. ovis*'e karşı antikor tespit edilmiştir. Mikroskopik muayenede 204 koyunun 1'inde (% 0,49) *B. ovis*'e rastlanmıştır. Enfeste koyunlardan 41'i erkek, 20'si dişi ve 1'i nimf olmak üzere toplam 62 kene toplanmıştır. Ergin keneler *Rhipicephalus bursa*, *R. sanguineus*, *R. turanicus*; nimf ise *Hyalomma* sp. olarak tanımlanmıştır. Toplanan keneler *Babesia* vermicülleri yönünden incelenmemiştir.

Anahtar Sözcükler: *Babesia ovis*, ELISA, seroprevalans, koyun, kene

Introduction

Babesia species are protozoan parasites that develop in the erythrocytes of vertebrate hosts and are transmitted transovarially and transstadially by Ixodid ticks (1,2). The severity of the disease caused by these parasites, namely babesiosis, is related to the vertebrate host and protozoan species (1). *Babesia ovis* and *B. motasi* are more frequently observed in sheep throughout the world compared to *B. crassa*. The vector ticks of *B.*

ovis and *B. motasi* are *Rhipicephalus bursa*, *R. turanicus*, *Hyalomma anatolicum excavatum*, *Haemaphysalis punctata* and *Ixodes ricinus*, whereas the vector of *B. crassa* is still unknown (2,3).

Babesiosis is the most important seasonal sheep disease and has been observed in all geographical regions in Turkey (4). *B. ovis* generally causes subclinical infection in local sheep in the region where the infection occurs. However, when susceptible sheep enter such regions,

* This study was supported by The Turkish Atomic Energy Authority, Ankara Nuclear Research Center in Agriculture and Animal Science (Project No: F.2.2.13).

untreated animals with several clinical symptoms die (1). Several serological tests such as IFAT (4-11), CF (12), ELISA (13-19) and PCR (20) have been used for the detection of latent babesiosis in sheep. It is claimed that ELISA is a more appropriate test than the others (15,21).

This study was carried out to detect the seroprevalence of *B. ovis* infections using ELISA in sheep around Afyon.

Materials and Methods

This study was carried out on Akkaraman sheep in 9 different localities of Afyon between June and September 2000. During this period, blood samples were collected to obtain serum samples from 204 sheep, of which 32 were less than 1 year old and 172 were over 1 year old. The serum samples were tested for the presence of *B. ovis* antibodies using ELISA. The positive reference serum required for ELISA was obtained from the Atomic Energy Organization, and the negative reference serum was obtained from CSIRO, Long Pocket Laboratories Australia. Pure *B. ovis* lysate was used as an antigen. Blood smears were prepared and stained with 5% Giemsa stain solution and examined for the presence of the piroplasms of *B. ovis*. The collected ticks from infested sheep were kept alive and identified as indicated by various investigators (22,23). The engorged nymph was kept in an incubator at 28 °C and 80% relative humidity for moulting. The pearson chi-square test was used for the statistical analysis of the data.

Results

As shown in Table 1, 106 (51.96%) of the 204 sampled sheep were seropositive against *B. ovis*. The seropositivity in the different study centers ranged from 22.22% to 78.57%. The differences between study centers were determined to be statistically significant ($P \leq 0.05$) (Table 1).

Seropositivity was 40.63% in the group under 1 year old and 54.07% in the group over 1 year old. No statistically significant difference ($P \geq 0.05$) was determined between the groups (Table 2).

In the microscopic examination of the smears, 1 (0.49%) of the 204 sheep was found to be infected with *B. ovis*. A total of 62 ticks collected from the sheep were identified as the adult stages of *Rhipicephalus bursa*, *R. sanguineus*, *R. turanicus*, their percentages being 24.19%, 70.97% and 3.23%, respectively. During this period, 1 nymph was also collected from the sampled animals. The engorged nymph was kept in an incubator but died prior to moulting and was identified as *Hyalomma* sp. (Table 3).

Discussion

For the diagnosis of sheep babesiosis in Turkey, early studies were mainly based on microscopic examinations (24-28). However, there has recently been increasing interest in the use of serological tests (8,17-19). The results of earlier studies showed that the rate of latent *B.*

Table 1. The state of *B. ovis* infection in sheep in Afyon province according to study centers.

Locality	Serum				
	No.	No. seropositive	%	No. seronegative	%
Çay (district)	13	6	46.15	7	53.84
Büyükçobanlar (district)	26	16	61.54	10	38.46
Çakır (village)	31	11	35.48	20	64.52
Kılıçarslan (village)	18	4	22.22	14	77.78
İğdeli (village)	33	22	66.67	11	33.33
Halımoru (village)	23	18	78.26	5	21.74
Belkaracaören (village)	14	11	78.57	3	21.43
Salar (town)	32	13	40.63	19	59.38
Akören (town)	14	5	35.71	9	64.29
Total	204	106	51.96	98	48.04

Table 2. The distribution of *B. ovis* infection according to age group.

Age groups	No. animals tested	No. ELISA seropositive	%
age 0-1	32	13	40.63
above the age of 1	172	93	54.07

Table 3. Ticks collected from the sampled sheep around Afyon.

Tick Species	Male	Female	Total	Percentage (%)
<i>Rhipicephalus bursa</i>	5	10	15	24.19
<i>R. sanguineus</i>	35	9	44	70.97
<i>R. turanicus</i>	1	1	2	3.23
<i>Hyalomma</i> sp. (nymph)	-	-	1	1.61
Total	41	20	62	100

ovis infection in sheep ranged from 0.41% to 27.35% (24-29) under microscopic examination. On the other hand, serological test results revealed the seropositivity rate to be 23.63%-91.02% using IFAT (4-8,30) and 41.02%-79% using ELISA (13-19,30). *B. ovis* infections were reported to be widespread. The results of this study, which was carried out for the first time around Afyon, indicated that the prevalence determined by ELISA of *B. ovis* in sheep was 51.96%. This value is similar to the values determined by some researchers (14-19) but lower than those determined by others (13,30). Studies have confirmed the close relationship between the incidence of sheep babesiosis and the geographical distribution of the vector tick *R. bursa* (9,31). This could be explained by the low distribution rate (24.19%) of the vector tick *R. bursa* around Afyon during the study period.

Previous studies in Turkey have shown that the seroprevalence of *B. ovis* determined by ELISA was lower in sheep under 1 year old than in older sheep (13,14,16,17). However, in some studies carried out using ELISA (15,19) and IFAT (7) *B. ovis* prevalence was found to be high in sheep under the age of 1. According to another study (18), there was no statistically significant correlation ($P \geq 0.05$) between *B. ovis* infection and age group. The results of this study carried out around Afyon showed that there was no statistically significant difference ($P \geq 0.05$) between age groups with regard to *B. ovis* infection. This may be due to the animals in different age groups exposed to vector tick infestation showing similar seropositivity.

This study has shown the presence of subclinical *B. ovis* infections around Afyon and also revealed the fact that Afyon may be among the regions at risk.

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