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Seroprevalence of *Babesia ovis* in Awassi sheep in Urfa, Turkey

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Abstract: Seroprevalence of *Babesia ovis* in Awassi sheep was studied in Urfa and environs between November 1997 and October 1998. A total of 607 serum samples were collected from sheep in 12 different localities, and tested for the presence of antibodies to B. ovis by using an indirect ELISA. Blood smears were also prepared from 110 sheep.

The overall prevalence of *B. ovis* infection was 41.02%. *B. ovis* was only detected in two blood smears examined (1.82%).

A total of 86 ticks were collected from the sheep during the study. Of the ticks examined, 17.44% were *Rhipicephalus turanicus*, 54.65% were *Haemophysalis sulcata*, 11.63% were *Hyalomma anatolicum excavatum* and 16.28% were other species (5.81% *Haemophysalis parva* and 10.47% *Rhipicephalus sanguineus*).

Key Words: *Babesia ovis*, ELISA, seroprevalence

Urfa Yöresi İvesi Koyunlarında *Babesia ovis*'in Seroprevalansı Üzerine Araştırmalar

Özet: Bu çalışma Urfa ve yöresinde *Babesia ovis* enfeksiyonunun seroprevalansını araştırmak amacıyla Kasım 1997 - Ekim 1998 tarihleri arasında yürütülmüştür. Oniki ayrı yöreden toplanan toplam 607 ivesi koyun serumunda ELISA ile *B. ovis* antikorları aranmıştır. Toplam 110 kan frotisi *B. ovis* parazitleri yönünde incelenmiştir. Urfa ve yöresinde *B. ovis*'in prevalansı %41,02 olarak bulunmuştur. Kan frotilerinin mikroskobik incelemesinde sadece iki frotide *B. ovis* parazitlerine rastlanmıştır. (%1,82).

Toplanan 86 adet kenenin %17,44'ü *Rhipicephalus turanicus*, %54,65'i *Haemophysalis sulcata*, %11,63'ü *Hyalomma anatolicum excavatum* olarak identifiye edilmiştir. Kalan %16,28'nin diğer türlerden olduğu görülmüştür (%5.81 *Haemophysalis parva*, %10.47 *Rhipicephalus sanguineus*).

Anahtar Sözcükler: *Babesia ovis*, ELISA, seroprevalans

Introduction

Babesia ovis is an intraerythrocytic protozoan parasite that causes babesiosis in sheep and goats, a disease of economic importance in subtropical and tropical regions of the world (1,2). Since the Enzyme Linked Immunosorbent Assay (ELISA) has become available for a serological diagnosis and survey of babesiosis, it is possible to test a number of serum samples in a short period of time with high sensitivity. Furthermore, ELISA is a reliable technique for the determination of low grade or subclinical cases (3,4).

A number of epidemiological surveys on ovine babesiosis have been reported using IFA and ELISA techniques in Turkey (5-9). However, the literature on ovine babesiosis in the eastern part of Turkey is very limited (10,11).

The purpose of this study is to document the presence of *B. ovis* in Urfa and environs in order to provide information on the prevalence and intensity of infection in sheep.

Materials and Methods

Sampling: Six hundred and seven ovine serum samples were collected from Awassi sheep located in the villages around Urfa for a period of one year from

November 1997 to October 1998. The animals sampled from each flock were selected randomly. Information on age, breed and sex was recorded. Sera were separated by centrifugation and stored at -20ºC.

ELISA: The serum samples were tested for antibodies against B. ovis by ELISA using methods previously described by Düzgün et al. (12). The antigen was produced in our laboratories, and positive sera were obtained from mature sheep with a history of clinical babesiosis from different parts of Turkey. Negative sera were obtained from CSIRO, Australia. Five positive and ten negative reference sera were included in each plate to control inter- and intra-assay variation. The mean plus two standard deviations of the mean of the ten negative sera were taken as the negative threshold point for each test. All values over that figure were regarded as positive.

Blood smears: Thin blood smears were prepared from the blood of 110 sheep. The smears were air dried, fixed in methanol and stained for 30 min in a 5% dilution of Giemsa solution in PBS, pH 7.2. The slides were examined with oil immersion.

Ticks: Ticks were collected from the animals sampled and examined at the University of Ankara, Faculty of Veterinary Medicine, Department of Protozoology and Entomology. The sex, species and the results of the examination of the haemolymph and ovaries were also added to the data.

Results

A total of 607 ovine serum samples collected from 12 locations in Urfa and environs were tested for the presence of B. ovis in antibodies; 249 samples were found to be positive and the overall prevalence was estimated to be 41.02%. Table 1 shows the number of animals tested and the proportions reacting positively to the ELISA of *B. ovis* antibody.

The distribution of seropositive cases according to month showed that the infection peaked in both March and July (Fig. 1). The prevalence of infection between months was found to be statistically significant ($p<0.01$, Fisher chi-square test).

The distributions according to sex and ages of animals tested are shown in Tables 2 and 3 and Fig. 2 respectively.

The examination of Giemsa stained blood smears revealed the presence of *B. ovis* in only two out of 110 sheep (1.82%).

Eighty-six ticks were examined for the determination of sex and species, and 47 were found to be female. In terms of species, 17.44% were *Rhipicephalus turanicus*, 54.65% were *Haemophysalis sulcata*, 11.63% were *Hyalomma anatolicum excavatum*, and 16.28% were other species (5.81% *Haemophysalis parva* and 10.47% *Rhipicephalus sanguineus*).

Table 1. The prevalence of *Babesia ovis* infection in Urfa and environs.

Location	No. animals tested	Seropositive		Seronegative	
		No.	%	No.	%
Küçükler village (Piroz)	13	8	61.54	5	38.46
Küçükler village (Kadıkenti)	37	18	48.65	19	51.35
Küçükler village (Göklüce)	52	22	42.31	30	57.69
Paşabağı (centre)	62	34	54.84	28	45.16
Yeşildirek (centre)	90	22	24.44	68	75.56
Yeşildirek (Alagöz)	50	10	20.00	40	80.00
Esenkulu village (centre)	50	17	34.00	33	66.00
Esenkulu village (Taşlı)	50	29	58.00	21	42.00
Paşabahçe (centre)	50	25	50.00	25	50.00
Fatmakuyu village	50	33	66.00	17	34.00
Sultantepe village	50	28	56.00	22	44.00
Yakınyurt village	53	3	5.66	50	94.34
Total	607	249	41.02	358	58.98

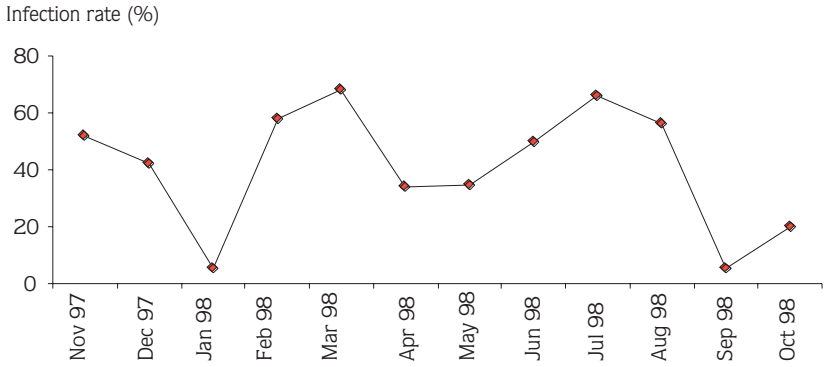

Fig. 1. The distribution of *Babesia ovis* seropositive cases throughout the year.

Table 2. The distribution of *Babesia ovis* infection according to sex. (p>0.50, Fisher chi-square test)

	ELISA			
	Seropositive		Seronegative	
	No.	%	No.	%
FEMALE	228	41.38	323	58.62
MALE	21	37.50	35	62.50

Table 3. The distribution of *Babesia ovis* infection according to age group.
(p>0.05, Fisher chi-square test)

ELISA	age 0-2	age 2-4	age ≥ 4
Seropositive	67 (41.10%)	142 (43.83%)	40 (33.33%)
Seronegative	96 (58.90%)	182 (56.17%)	80 (66.67%)

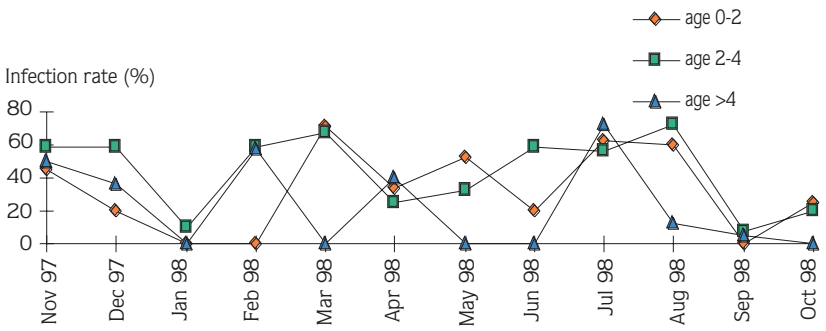

Fig. 2. The prevalence of antibody to *Babesia ovis* versus age groups.

No parasitic forms were seen in Giemsa stained preparations of haemolymph and ova.

Discussion

The use of serological tests for the detection of antibodies to *Babesia* sp. is necessary in order to learn the epidemiological status of babesiosis and for its control. ELISA is a sensitive, specific and easy to perform test which has been developed to measure immune response to several species of Babesia (3,13). In this study also, it was possible to detect latent infections as well as acute infections following antibody production by using ELISA.

The present study showed that antibody activity against *B. ovis* antigen was high with an overall prevalence of 41.02% in Awassi sheep in Urfa and environs. This is the first value calculated for this region, and it is rather comparable to the prevalences reported for eastern Turkey (10,11). Papadopoulos et al. (14) found an infection rate of 52.1% in sheep and 36.4% in goats in Macedonia by using the IFA technique; however, the literature on ovine babesiosis in sheep and goats is very limited.

This survey has demonstrated that the foci of babesiosis occur subclinically even in areas where ticks are widespread. In such areas, silent foci of infection may become endemic and losses of animals could be prevented by controlling the tick population. A serodiagnostic service for the detection of silent foci would be practicable in order to control the infection.

A total of 86 ticks collected from nine locations were examined and no parasitic forms were seen in preparations of haemolymph and ova. This observation also indicates that ovine babesiosis is subclinical in Urfa and environs and endemic babesiosis may occur at any time.

In general, the distribution of the parasite is correlated with the distribution of tick vector species. Friedhoff (15) reported that *Rhipicephalus turanicus* and *Hyalomma anatolicum excavatum* may be a vector for *B. ovis* infection. The present data supports this report.

In conclusion, the present study presents evidence that sheep infected with *B. ovis* were almost ubiquitous in Urfa and environs. The present control measures, such as vector tick control, are inadequate. Future control strategies for ovine babesiosis would be facilitated if the epidemiology and distribution of this infection were known for this area of Turkey.

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