INVESTIGATION OF PREVALENCE OF *BABESIA EQUI* (LAVERAN, 1901) AND *BABESIA CABALLI* (NUTTALL, 1910) IN HORSES BY SEROLOGICAL METHODS IN ELAZIĞ AND MALATYA PROVINCE*

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ABSTRACT

The aim of this study was to determine the prevalance of *Babesia equi* and *Babesia caballi* by serologic (IFAT and CFT) and microscopic examinations in Elazığ and Malatya provinces. For this purpose, 78 sera and 90 microscopic slides were prepared and examined from horses randomly chosen from these cities in this region in year 2003. Microscopic slides stained with Giemsa were examined for *Babesia* species, and the agents were not observed in microscopic preparations. In the serological tests performed, in Malatya seropositivity was found by IFAT to be 20.5% for *B. equi*, and 2.5% for *B. caballi*; while CFT revealed a prevalence between 18% for *B. equi*, and 0% for *B. caballi*. In Elazığ seropositivity was found by IFAT and CFT to be 0 % for *B. equi* and *B. caballi*.

The differences between the results of the tests were evaluated statistically, no difference existed between IFAT and CFT.

Anahtar Kelimeler:*Horse, Babesia, Equi, Babesiacabelli, IFAT, CFT.*

ÖZET

Elazığ ve Malatya İllerinde Atlarda Babesia equi (Laveran, 1901) ve Babesia caballi (Nuttall, 1910) Türlerinin Yayılışının Serolojik Yöntemlerle Araştırılması

Bu çalışma, Elazığ ve Malatya'da bulunan Babesia türlerinin tespiti ve serolojik olarak IFAT ve CFT yöntemiyle *Babesia equi* ve *Babesia caballi* seroprevalansının saptanması amacıyla yapılmıştır.Bu amaç için, 2003 yılında bu bölgedeki illerden rastgele seçilen atlardan 78 serum ve 90 froti hazırlanmış ve incelenmiştir. Giemsa ile boyanan frotiler Babesia türleri yönünden muayene edilmiş ve hiçbir preparatta etken görülememiştir. Yapılan serolojik testlerde seropozitiflik Malatya'da IFAT ile *B. equi* yönünden %20.5, *B. caballi* yönünden %2.5; CFT ile *B.equi* yönünden % 18, *B. caballi* yönünden % 0 olarak saptanırken, Elazığ'da IFAT ve CFT ile *B. equi* ve *B.caballi* % 0 olarak saptanmıştır.

Testler arasındaki sonuçların farklılığı istatistiksel olarak değerlendirilmiş, IFAT ve CFT arasında önemli bir fark bulunamamıştır.

Key Words: *At, Babesia, Equi, Babesiacabelli, IFAT, CFT.*

INTRODUCTION

Babesiosis is a protozoan disease caused by *Babesia* species in vertebrated animals. The disease is caused by *Babesia equi* and *B. caballi* in horses (1). It was reported that *B. equi* was much more pathogenic than *B. caballi*, as *B. equi* damaged the blood pattern more severely, and it took longer for this pattern to turn normal than it did for *B. caballi* (1, 2). The studies on babesiosis in horses in Turkey involved only microscopic examination of blood, however no serological study was reported. The aim of this study was to microscopically and serologically detect the presence and prevalence of *Babesia* species in the horses in Elazığ and Malatya provinces.

MATERIALS and METHODS

The material of this study consisted of 78 horses sera and 90 microscobic slides supplied from Elazığ and Malatya province within the year of 2003. Peripheric blood microscopic slides were prepared from tail tips of each randomly chosen horse, and the blood samples were collected from the jugular veins into vacuumed tubes, as described for the technique. The sera were then isolated in the laboratory, and stored at -20°C until use. Antigens (12-well IFA substrate slide) Lot BK-12- 030320, used in the Indirect Fluorescent Antibody Test (IFAT), were acquired from Fuller Laboratories Fullerton, CA USA. Anti-horse IgG (whole-molecule) FITC conjugate, no. F3762, by Sigma was used as

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conjugate, with a 1:32 dilution. The Complement Fixation Test (CFT) was performed according to the USDA National Veterinary Services Laboratories Testing Protocol (3) and as Alton et al. (4) described. Statistical significance of the differences between the results of the tests was examined by X^2 (chi square) test, and their accordances by Kappa test.

RESULTS

Blood microscopic slides from total 90 horses in Elazığ and Malatya cities were examined microscopically for *Babesia spp.* parasites, and 78 sera samples were examined by IFAT and CFT, for antibodies against *B. equi* and *B. caballi.*

In this study, no parasites were detected in microscopic examinations. A total of 64 horses from Malatya province showed 20.5% (16 horses) seropositivity for *B. equi* and 2.5% (2 horses) seropositivity for *B. caballi* according to IFAT. In CFT, 14 (18%) horses showed seropositivity for *B. equi* and no seropositivity was detected for *B. caballi*.

A total of 14 horses sera from Elazığ province did not show seropositivity for the both agents examined microscopically IFAT and CFT.

Fourteen sera from Malatya province showed seropositivity both IFAT and CFT for *B. equi* and none of them for *B. caballi*.

The results of the two tests were statistically evaluated, no difference existed between IFAT and CFT($P > 0.05$).

DISCUSSION

It was reported that the best and the most reliable method for detection of the causative agent in horses was microscopic examination, but that the chance of detect the cause in subclinical infections decreased (1). In this study, no parasites were detected in microscopic preparations. This situation is in accordance with the ideas suggested by some investigators (5-9).

All the studies in Turkey involved microscopic examination of blood microscopic slides (2, 10-14). It is known that use of serological tests are common in especially detection of carrier horses, besides blood microscopic slides for diagnosis of piroplasmosis in single clawed animals (9, 15, 16). *B. equi* infections are reported to be more common in the world, compared to *B. caballi* infections (1). Some investigators (9, 17) have used IFAT and CFT in diagnosis of *Babesia* infections in horses. Although they have detected seronegativity by CFT on from 2-3 months following experimental infections, they have reported that these horses were still positive when they were studied by IFAT. Furthermore, they have observed that *B. equi* antibodies remained in high titer by IFAT and CFT, compared to *B. caballi* antibodies.

In an IFAT study in Mongolia, 88.2% positivity for *B.equi*, and 84.5% positivity for *B.caballi* was observed (7). In the study with 23 horses which appeared healthy in Argentina, all were detected to carry specific antibodies for *B. equi*, and 18 of them were found to be seropositive for *B. equi* by CFT (8). In Israel, 361 sera samples collected from 361 horses were examined by IFAT, and one third of the horses were observed to be seropositive for *B. equi* (18).

In this study, although prevalence of antibodies specific to *B. equi* was detected to be 16 (20.5%) and that for *B. caballi* detected to be 2 (2.5%) by IFAT, and although CFT revealed prevalence of antibodies specific to *B. equi* to be 14 (18%) and that for *B. caballi* to be 0 (0%) in Malatya. No sera from Elazığ province showed seropositivity for the agents examined microscopically IFAT and CFT.

Both serological methods indicated that *B. equi* was more prevalent than *B. caballi* in Malatya province.

Some investigators (5, 6, 9, 15-17) have reported that, starting from 2-3 months following the acute infection, particularly the antibodies against *B. caballi* became undetectable by CFT, but that these horses remained positive by IFAT for a long period. This study showed that there was no difference between IFAT and CFT results .

This result was not supported by earlier studies (6, 7, 9, 15-17).

All the studies on horses performed in Turkey to date were carried out solely by the microscopical diagnosis method, and no other studies which applied microscopic examination and serological tests together existed. As this study was the first in Turkey, it was evaluated according to the results of microscopic examinations performed before. When the data we acquired as a result of serological tests in this study were compared with the results from other microscopic investigations (2, 10, 12-14), IFAT and CFT findings revealed that subclinical and chronic infections were relatively more prevalent.

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