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Short Communication

Prevalance of *Theileria equi* and *Babesia caballi* in Donkeys from Eastern Turkey in Winter Season

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ABSTRACT

The aim of this study was to determine the prevalence of *Theileria equi* and *Babesia caballi* by the examination of Giemsa-stained blood smears and c-ELISA method in donkeys, selected randomly from Erzurum, the largest province of Eastern Turkey. The specimens were consisted of 92 thin blood-smears and 75 sera during winter season. As result of microscopic examination no parasite was detected. Of the 75 sera, 3 (4%) and 1 (1.33%) samples were positive for the presence of *T. equi* and *B. caballi* antibodies, respectively. We couldn't detect mix infection with both parasites. This study indicated the prevalence of *T. equi* and *B. caballi* in donkeys for the first time from Eastern Turkey in winter season.

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INTRODUCTION

Equine piroplasmosis, an OIE List disease, caused by tick borne protozoa *T. equi* and *B. caballi*, is responsible for important economic losses in the equine industry (Kuttler, 1988). The disease is endemic in tropical and subtropical regions of the world, including Southern Europe, Africa, The Middle East, Asia, South America and Central America (Kuttler, 1988). Disease can occur in peracute, acute and chronic forms. In infected animals there is fever, anemia, jaundice, haemoglobinuria and in some cases death can occur (OIE, 2008).

Donkeys usually remain asymptomatic carriers with positive antibody titre throughout life. The clinical form of the disease is diagnosed by peripheral blood smear examination, but in carrier donkeys it is very difficult to demonstrate the parasite in stained blood smears as the parasitaemia is extremely low. For diagnosis of such low grade infection or carrier animals, serological tests and molecular diagnostic techniques are used (Kumar *et al*., 2009). Currently, IFAT and c-ELISA are the primary tests used for qualifying horses for importation. CFT has been replaced by the IFAT and c-ELISA because of its low sensitivity and specificity (OIE, 2008).

Information on the prevalence of equine piroplasmosis in horses and donkeys is very limited in Turkey (Balkaya and Erdogmus, 2006; Acici *et al*., 2008; Piskin *et al*., 2008; Sarı *et al*., 2010). The aim of this was to determine the microscopic and serologic situation of *T. equi* and *B. caballi* in donkeys from Erzurum province of eastern Turkey in winter season.

MATERIALS AND METHODS

A total of 92 blood samples were taken from the jugular vein using vacutainers with and without anticoagulant in February 2009 from 55 female and 37 male donkeys randomly selected from Erzurum, the largest province of eastern Turkey. Age of the donkeys varied from 1 to 15 years old. All of the donkeys were clinically healthy. Blood samples with anticoagulant were used to prepare smears. The smears were stained with the Giemsa's method and examined under light microscopy by using the immersion objective.

Sera (n=75) were obtained from 92 blood samples without anticoagulant and stored at –20°C until analyzed. Antibodies to *T. equi* and *B. caballi* were detected by using a commercially available competitive enzyme linked immunosorbant assay (c-ELISA) kit (VMRD, USA). The test was performed following the instructions of manufacturer. The mean optical density (OD) at 630 nm was determined for all wells using a microplate reader (ELx 800 UV, Universal Microplate Reader, Bio-Tec Instruments, Inc). The percent inhibition for each test sample was determined using the below mentioned formula:

Inhibition (%) = 100- [(Sample O.D. X 100) ÷ (Mean Negative Control O.D.)]

The samples with values of ≥ 40% inhibition were regarded as positive and those with the values < 40% inhibition were regarded as negative. Kluskal Wallis H test of non-parametric statistical tests was employed.

RESULTS AND DISCUSSION

Equine piroplasmosis is a tick-borne protozoal disease of horses, mules, donkeys and zebra, it causes economic losses to the equine industry. Infected animals may remain carriers of these parasites for long period and act as sources of infection for ticks, which act as vectors. The introduction of carrier animals into areas where tick vectors are prevalent can lead to an epizootic spread of the disease (Kuttler, 1988).

Indirect fluorescent antibody test and c-ELISA are the primary tests used for qualifying horses for import. These tests have been proved more effective in detecting long-term infected animals and animals treated with antiparasitic drugs but these animals may be CFT negative but still be infected. The IFAT and c-ELISA have been shown highly specific for each of the two species of piroplasmosis agents involved (OIE, 2008).

In the present study, all slides were examined for the presence of *Theileria spp.* and *Babesia spp*. under light microscope and no parasite was detected. As shown in Table 1, of the 75 sera, 3 (4%) and 1 (1.33%) samples were positive for the presence of *T. equi* and *B. caballi* antibodies, respectively. We couldn't detect mix infection with both parasites. *Theileria equi* and *B. caballi* seropositivity were found higher in female donkeys compared to male donkeys and this difference between gender was considered statistically significant (P<0.01).

Serologic data about equine piroplasmossis is very limited in Turkey. Acici *et al.* (2008), studied on 38 donkeys in Black Sea region of Turkey. Seropositivity rates by IFAT were 13.1% for *T. equi* and 36.8% for *B. caballi*. Chahan *et al.* (2006) studied on 93 donkey sera in Western Xinjiang region of China. The ELISA test results revealed 9% for *T. equi* and 36% for *B. caballi* and they detected the co-infection rate as 2.2%. Turnbull *et al.* (2002) studied on 62 feral donkeys with IFAT in United Arab Emirates and found the seropositivity rates 9.68% and 1.61% for *T. equi* and *B. caballi*, respectively. In this study, 3 (4%) and 1 (1.33%) samples were positive for the presence of *T. equi* and *B. caballi* antibodies, respectively.

In general, *T. equi* infection is more prevalent than *B. caballi* infection in horses (Balkaya and Erdogmus, 2006; OIE, 2008; Piskin *et al*., 2008). Although results reported by Turnbull *et al.* (2002) and results of our study are in concordance with this general belief, however, results reported by Acici *et al.* (2008) and Chahan *et al.* (2006) opposite than from this general faith.

We think that further studies are required to determine the epidemiologic situation of equine piroplasmosis in donkeys in different provinces of Turkey and different countries of the world. Additionally control of equine piroplasmosis must include seromonitoring of carrier donkeys, effective tick control and treatment of sick animals.

Table 1: Prevalance of *Theileria equi* and *Babesia caballi* (c-ELISA based) in donkeys in from Erzurum province of Eastern Turkey.

Sex	Number of Animals Examined (n)	*T.equi* Positive Animals	Percent Prevalance of *T.equi* %	*B.caballi* Positive Animals	Percent Prevalance of *B.caballi* %
Female	48	2	4.16	1	2.08
Male	27	1	3.70	-	-
Total	75	3	4.00	1	1.33

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