# Seroprevalence of Brucellosis in Horses in Kars and Ardahan Provinces of Turkey Where Ruminant Brucellosis is Endemic and Prevalent

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## **Summary**

The prevalence of brucellosis was investigated in horses in Kars and Ardahan Districts of Turkey between 2008-2010. In order to achieve this, a total of 361 horse serum samples from 23 different villages were examined for Brucella antibodies by Rose Bengal Plate Test and Serum Agglutination Test. Of the 361 sera obtained from horses, 48 (13.29%) were determined positive by RBPT and 52 (14.40%) by SAT. The positive titers varied between 1/40 and 1/320 for brucellosis by SAT. Among the positive samples, the titers were 1/40 in 24, 1/80 in 6, 1/160 in 18 and 1/320 in 4 samples.

Keywords: Brucellosis, Horse, Seroprevalence, RBPT, SAT

# Ruminant Brusellozisinin Endemik ve Yaygın Olduğu Kars ve Ardahan Yörelerinde Atlarda Brusellozisin Seroprevalansı

## Özet

Kars ve Ardahan yörelerinde 2008 ile 2010 yılları arasında atlarda brusellozisin seroprevalansının araştırılmasının amaçlandığı bu çalışmada, 23 farklı yerleşkeden elde edilen toplam 361 at serum örneği Rose Bengal Plate Test (RBPT) ve Serum Aglütinasyon Testi (SAT) ile incelendi. İncelenen 361 at serum örneğinin 48 (%13.29)' i RBPT ve 52 (%14.40)' si SAT ile pozitif saptandı. SAT ile pozitif saptanan serum örneklerinde antikor titrelerinin 1/40 ile 1/320 arasında değişkenlik gösterdiği (24 örnek 1/40, 6 örnek 1/80, 18 örnek 1/160 ve 4 örnek 1/320 olarak) belirlendi.

Anahtar sözcükler: Brusellozis, At, Seroprevalans, RBPT, SAT

# INTRODUCTION

Brucellosis is an important zoonotic disease worldwide and endemic in many regions of Turkey. Although several control and eradication measures have been established, the disease continues to produce large economic losses especially in cattle and small ruminants and cause a serious public health problem in Turkey <sup>1</sup>. Naturally acquired Brucella infection in horses associated with infected cattle (*B. abortus*) and swine (*B. suis*) horizontal transfer has been demonstrated <sup>2</sup>. Brucellosis is uncommon and generally asymptomatic in horses. But in some cases it is associated with diseases, inflammation of the supraspinal bursa (fistulous withers) and atlantal bursa (poll evil), and also tenosynovitis,

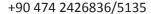
osteomyelitis or osteoarthritis and, rarely, abortion and infertility <sup>3-5</sup>. The horses infected with *B. abortus*, even they do not show signs of illness, they excrete the organism in purulent discharges, milk and urine <sup>4</sup>. So that horse brucellosis may be a potential source of infection in human beings and other animals.

National Control and Eradication Programme for Brucellosis in domestic animals run by the Ministry of Food, Agriculture and Animal Livestock has been implemented in Turkey since 1984. However, the prevalence of brucellosis still ranges from region to region throughout Turkey. Kars











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and Ardahan regions have about 650.000 cattle, 230.000 sheep, 17.000 goats and 12.000 horses population mainly raised on family-operated farms and human population in these regions is 406.000 and 75% of them deal with animal husbandry. It can be expressed that almost every farmer has also a horse kept together with the farm animals in these regions used especially for purposes of harvesting and transporting of crops in July and August. In addition, they are commonly used in racing games and for other recreational purposes for the rest of the year during which no agricultural activities are carried out 6. The prevalence of brucellosis in cattle, Turkey was investigated previously and the most prevalent figures with 20.8% and 7.9% <sup>7</sup> were reported from Kars and Ardahan Provinces, respectively that located in the Northeast Anatolia Region of Turkey. However, no research has been conducted in horses from these two regions.

In this study, we aimed to determine seroprevalence of brucellosis in horses in the Kars and Ardahan district by RBPT and SAT, where ruminant brusellosis is endemic and prevalent.

#### MATERIAL and METHODS

#### **Horse Serum Samples**

This study was carried out between January 2008 and January 2010. A total of 361 horse serum samples from 23 different villages were evaluated for brucellosis in Kars and Ardahan Provinces in Northeast Anatolia Region of Turkey (Fig. 1). The animals were treated according to the Animal Care and Use Regulation (European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purpose). Horses are traditionally raised together with cattle, sheep and other animals in the same stable in these provinces. Native breed horses investigated in

this study aged between 3-8 years and approximately 10 ml of blood samples from each horses were taken and transported to the Laboratory of Microbiology Department, Faculty of Veterinary Medicine, Kafkas University. The serum samples were kept at -20°C until tested.

#### Serological Detections of Brucella Antibodies

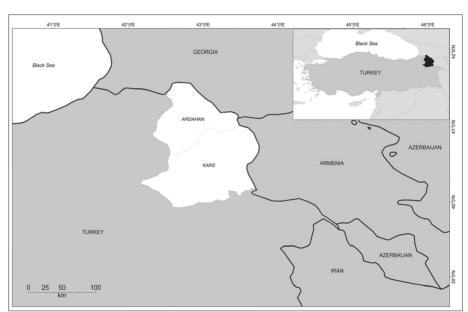
Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT) were used to detect Brucella antibodies for all serum samples. *Brucella abortus* antigen used in the study for both RBPT and SAT was obtained from Pendik Veterinary Control and Research Institute, Istanbul. The RBPT and SAT were carried out according to the method described by Alton et al.<sup>8</sup>. In RBPT, 25 µl of antigen was mixed with an equal volume of horse serum on a clean glass slide and examined for agglutination after 4 min. In SAT, 0.5 ml of Brucella SAT antigen was added to 0.5 ml of each serum sample serially diluted in 1:10, 1:20, 1:40, 1:80, 1:160 and 1:320. The mixture was incubated at 37°C overnight. The agglutination ++ and stronger, observed in sera at dilution 1:40 and higher, was considered to be positive <sup>3</sup>.

## RESULTS

Of the 361 sera obtained from horses, 48 (13.29%) were determined positive by RBPT and 52 (14.40%) by SAT. The positive titers varied between 1/40 and 1/320 for brucellosis by SAT. Among the positive samples, the titers were 1/40 in 24, 1/80 in 6, 1/160 in 18 and 1/320 in 4 samples. Number and results of the distribution of the samples according to regions are given in *Table 1*.

#### DISCUSSION

Brucellosis is prevalent in some middle-eastern countries such as Iran, Iraq, Saudi Arabia, Egypt, Syria, Pakistan and



**Fig 1.** Map of Turkey and geographical location of Kars and Ardahan Districts

**Şekil 1.** Türkiye haritası ve Kars ile Ardahan illerinin coğrafi konumu

<b>Table 1.</b> Province	ces, number and re	esults of the hor	se serum sampl	es analyzed by	RBPT and SAT				
<b>Tablo 1.</b> At serum örneklerinin RBPT ve SAT sonuçları									
Province	Number of Samples	Assay and Numbers of Positive and Negative Serum Samples							
		RBPT				SAT			
		Positive		Negative		Positive		Negative	
		n	%	n	%	n	%	n	%
Kars	225	31	13.77	194	86.23	33	14.66	92	85.33
Ardahan	136	17	12.50	119	87.50	19	13.97	117	86.02
Total	361	48	13.29	313	86.71	52	14.40	309	85.60

some south-eastern European countries. Turkey borders with several of these countries and is situated within this geography. Therefore, Turkey lies within the risky area between Middle East and Europe.

Turkey has a relatively large area and is divided into seven geographical regions. Various prevalence rates of brucellosis have been reported for human, cattle and sheep population from different parts of Turkey. However, there are few researches conducted for the determination of the prevalence of brucellosis in horses. In a study from Hakkari region, Eastern part of Turkey, 9.5% Brucella seropositivity was reported for horses with RBPT and presence of anti B. abortus antibodies was confirmed by SAT 9. Solmaz et al. 10 reported a seroprevalance of 60.59% (123/203) in horses raised in Van province of East Turkey by RBPT and out of these 123 positive serum samples 98 (79.67%) showed 1/40 or higher titers by SAT. The result of another study on horse brucellosis in Southeast Turkey was 13.68 and 0.51% by RBPT and SAT, respectively 11. If we take a look at the situation in neighbouring countries; in a study conducted in North-East of Iran, the seroprevalence of brucellosis was determined as 2.5% in horses by RBPT and SAT 12. In another study seroprevalence of brucellosis on 393 sera of horses in Iraq has been found to be 16.28% by RBPT but nine of them were found positive by SAT 13. Wadood et al. 14 determined the disease's seroprevalence in horses in and around Fisalabad- Pakistan, as 20.7 and 17.7% by RBPT and SAT, respectively. In another study, Abo-Shehada 15 determined the corresponding figures for horses and donkeys in Jordan as 1% and 8.5%, respectively.

In the current study, the seroprevalence of brucellosis was determined to be 48 (13.29%) by RBPT and 52 (14.40%) by SAT in 361 horse serum samples. The results of our study and the studies mentioned above show that horse brucellosis is very common in both the some regions of Turkey and the Middle East which is also a common case for other animal species like cattle and sheep. In previous studies performed in our laboratory in the same region, the seroprevalence of brucellosis was determined to be 32.92% by RBPT and 34.64% by SAT in 407 serum samples of cattle collected from the herds with a history of abortions and 32 (13%), 35

(14.22%) and 44 (17.88%) of the farmers' sera were found positive for brucellosis by RBPT, SAT and ELISA, respectively 1. In addition, the culture isolation of the organism supported these serological findings. As the previous results in the region from our laboratory, Sahin et al. 16 isolated B. abortus from 48 (32.21%) of 149 lung samples and stomach contents of the aborted cattle fetuses. Therefore, these findings in horses were not surprising. Since no clinical or microbiological examination was performed on the horses, the ratio of active infection on seropositive animals cannot be determined. However there are several reports about isolation of *B. abortus* from infected horses <sup>17,18</sup>. The results of this study show that there is a need for further clinical and microbiological studies on horse brucellosis and a possible clonal relationship among different B. abortus isolates from these two regions should be verified by molecular typing methods. Nonetheless, when considering the possibility of risk for Brucella transmission from horses to humans (especially horse riders, caretakers, farmers and veterinarians) as well as to other animals, horses should be avoided to raise together with other farm species. These issues are suggested to be taken into account for determining strategies for Brucella control and eradication programs.

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