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ORIGINAL ARTICLE

Seroprevalence of Equine Brucellosis in Southeast Turkey

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SUMMARY

In this study, it was aimed to determine the seroprevalence of brucellosis in equine in southeast Turkey. A total of 1954 sera samples were collected from donkeys (n=1172), horses (n=782) raised in Sanliurfa and Diyarbakir provinces. The sera were tested for the presence of anti-Brucella antibodies by using Rose Bengal Plate test (RBPT). Positive or doubtful sera by RBPT were further examined by Serum Agglutination test (SAT) for confirmation. The seroprevalence of brucellosis in horses was 13.68% and 0.51% by RBPT and SAT, respectively. The seroprevalence of brucellosis in donkey was 6.05% and 0.25% by RBPT and SAT, respectively. In Sanliurfa and Diyarbakir provinces 99 (18.71%) and 8 (3.16%) of the horses, respectively, were defined as seropositive by RBPT, while 9 (5.69%) and 62 (6.11%) of the donkeys from Sanliurfa and Diyarbakir provinces, respectively were defined as seropositive by RBPT. On the other hand, 4 (0.75%) of the horses in Sanliurfa region and 3 (0.29%) donkeys in Diyarbakir region were defined as seropositive by SAT. Consequently, the results indicated that brucellosis is not widely distributed among horses and donkeys raised in Sanliurfa and Diyarbakir provinces. However it can threat health of other sensitive animals, and humans. Including horses and donkeys in this region into brucellosis control program may be beneficial for public health.

Key Words *Brucellosis, Horse, Donkey, Seroprevalence*

Türkiye'nin Güneydoğusundaki Tektırnaklı Hayvanlarda Brusellozisin seroprevalansı

ÖZET

Bu çalışmada, Türkiye'nin güneydoğusundaki tektırnaklı hayvanlarda brusellozis seroprevalansının belirlenmesi amaçlandı. Sanliurfa ve Diyarbakır bölgesinde bulunan eşekler (n=1172) ve atlardan (n=782 at) 1954 serum örneği toplandı. Serumlar, Brucella antikorlarının varlığı yönünden Rose Bengal plate test (RBPT) ile incelendi. RBPT ile test edilerek pozitif veya şüpheli bulunan serumlar, serum aglutinasyon testiyle (SAT) ile incelendi.. RBPT ile test edilerek pozitif veya şüpheli bulunan serumlar SAT ile incelendi. Brusellozis seroprevalansı atlarda RBPT ile %13.68, SAT ile %0.51, eşeklerde RBPT ile %6.05 ve SAT ile %0.25 olarak bulundu. Sanliurfa bölgesinde 9 (%5.69), Diyarbakır bölgesinde 62 (%6.11) eşek RBPT ile pozitif olarak bulunurken, Sanliurfa bölgesinde 99 (%18.71), Diyarbakır bölgesinde ise 8 (%3.16) at RBPT ile pozitif olarak saptandı. Ayrıca, Sanliurfa'da 4 (%0.75) at ve Diyarbakır'da 3 (%0.29) eşek SAT ile seropozitif olarak belirlendi. Sonuç olarak, brusellozisin Sanliurfa ve Diyarbakır bölgesinde yaşayan at ve eşekler arasında yaygın bir infeksiyon olmasa da, diğer duyarlı hayvanlar ve insan sağlığı bakımından risk oluşturabileceği düşünüldü. Bu nedenle, kamu sağlığı açısından bruselloz kontrol programına tektırnaklıların da dahil edilmesinin yararlı olabileceği sonucuna varıldı.

Anahtar Kelimeler *Brusellozis, At, Eşek, Seroprevalans*

INTRODUCTION

Brucellosis is an important zoonotic disease worldwide causing serious human health problems and substantial economic losses for the livestock industry (Corbel, 1997). Among domesticated species, cattle, sheep, pigs and goats are most commonly affected. Infection in horses is uncommon. Brucellosis is generally asymptomatic in horses. Fistulous withers and poll evil are the most common clinical manifestations in the horse, and infection in horses were associated with a variety of clinical manifestations, including vertebral osteomyelitis (Collins et al. 1971; Cohn et al. 1992), fistulous withers, poll evil or fistulous bursitis (Cohn et al. 1992), abortion, infertility (Denny, 1972) and arthritis (Carrigan et al. 1987).

Equine infection most frequently involves *Brucella abortus*. Because of the difficulty that may be encountered in attempts to culture *B. abortus* from horses with fistulous withers, concomitant serologic testing for detection of specific antibodies is recommended. Serological surveys have indicated that many horses may be exposed to *B. abortus* without developing clinical signs of the disease (Göz et al. 2007). For serodiagnosis of horse brucellosis, many serologic tests such as Rose Bengal plate test (RBPT), serum agglutination test (SAT), complement fixation test (CFT), mercaptoethanol agglutination, agar gel diffusion and coombs tests have been commonly used (Hutchins and Lepherd, 1968; Denny, 1972; Mac Millan,

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1985).

B. abortus infection in horses is important not only as clinical existence but also as a source of infection for man and other animals. Studies concerning brucellosis have been conducted on cattle, sheep, and goats, but most have focused on cattle. However, a small number of surveys have been carried out to determine the epidemiologic role of horses and donkeys. Therefore, the aim of this study was to detect the seroprevalence of brucellosis in horses and donkeys raised in Diyarbakir and Sanliurfa provinces, in southeast region of Turkey.

MATERIALS and METHODS

Animals

Between November 2009 and March 2010, a total of 1954 serum samples were randomly collected from healthy horses (n:782) and donkeys (n:1172) raised in Diyarbakir and Sanliurfa provinces, of Turkey (Table 1). Horses and donkeys ranged in age from 1 month to 20 years old and horses in represented a variety of breeds (Thoroughbred, Arabian and half-bred). At the time of sample collection, none of the animals had any history of clinical signs of brucellosis. Serum samples were obtained by venous puncture and stored at 4°C, until analysis under field laboratory conditions. Serum samples were tested using RBPT and SAT for brucellosis.

Table 1. Numbers of animals randomly sampled for determining the seroprevalence rate of equine brucellosis southeast region of Turkey

Equine	Diyarbakir (n:1267)			Sanliurfa (n:687)		
	Male	Female	Total	Male	Female	Total
Horses	91	162	253	80	449	529
Donkey	598	416	1014	81	77	158

RBPT

The RBPT test was carried out according to the method described by Alton et al. (1988) with *B. abortus* antigen obtained from the Refik Saydam National Public Health Agency (RSHC), Ankara, Turkey. 30 µl of serum was mixed with equal volume of antigen on a clean glass slide. The mixture was rocked gently for 4 min at room temperature. Mixtures formed agglutination was considered positive. Serum samples found to be positive or suspected by RBPT and were further examined by SAT.

SAT

In SAT, serum samples were diluted at 1:10, 1:20, 1:40 and 1:80 with *B. abortus* antigen. The results were evaluated after incubation at 37°C for 12-24 hours. A titer of 1:40 or higher is considered positive according to Denny (1972).

RESULTS

In this study 107 (13.68%) and 71 (6.05%) of the samples from horse and donkey were determined as seropositive by RBPT, respectively (Table 2). By using SAT 4 (0.51%) and 3 (0.25%) of the sera form horse and donkey, respectively showed a titer value of 1/40 or above. Seropositivity was obtained in 8 (3.16%) of 253 and 62 (6.11%) of 1014 sera of horses and donkeys in Diyarbakır region by RBPT respectively. Also 99 (18.71%) of 529 horse sera and 9 (5.69%) of 158 donkey sera were obtained as seropositive in Sanliurfa region. In addition as a result of SAT investigation of sera found positive by RBPT, in Sanliurfa region 86 (95.5%) of 90 horse sera, and 3 (33.3%) of 9 donkey sera were obtained as between 1/10, and 1/20 titer while in 15 sera any titer was not observed. Also in Diyarbakır region 3(37.5%) of 8 horse sera, and 46 (74.1%) of 62 donkey sera, which were found seropositive by RBPT, had titer between 1/10, and 1/20 values and any titer was not observed in 18 sera. The titer values 1/40, or above, which were accepted as seropositive, were obtained in 4 (0.75%) horses in Sanliurfa region, and 3 (0.29%) donkeys in Diyarbakır region (Table 3).

Table 2. Results of RBPT in horse and donkey from southeast Turkey

Province	RBPT Positive (%)	
	Horse (n:782)	Donkey (n:1172)
Diyarbakir	8 (1.02)	62 (5.29)
Sanliurfa	99 (12.66)	9 (0.76)
Total	107 (13.68%)	71 (6.05%)

Table 3. SAT titers of the RBPT positive sera

Equine	Diyarbakir (n:1267)						Sanliurfa (n:687)					
	Positive sera		SAT titers				Positive sera		SAT titers			
	RBPT	SAT	1/10	1/20	1/40	1/80	RBPT	SAT	1/10	1/20	1/40	1/80
Horse	8	3	2	1	-	-	99	90	47	39	2	2
Donkey	62	49	34	12	1	2	9	3	1	2	-	-

DISCUSSION and CONCLUSION

Brucellosis, a zoonotic disease, is an important threat to human health and causes substantial economic losses to agricultural industry (Nicoletti, 2007). In the previous studies prevalence of seropositivity in horses have been reported as between 0-20.7% by RBPT and 0-17.7% by SAT (Hutchins and Lepherd 1968; Denny, 1972; Omer et al. 2000; Acosta-González et al. 2006; Wadood et al. 2009; Tahamtan et al. 2010; Ehizibolo et al. 2011). In this study 13.68% of the horses were found to be seropositive by RBPT while 0.51% the horses showed a titer value 1/40 or higher, which was considered seropositive by using SAT. In Turkey Izgur et al. (1998) found 42.40%, and 1.89% of horse sera as seropositive by Plate test and RBPT, respectively and 29% of those sera have shown a titer value between 1/10-1/20. Göz et al. (2007) performed a study on 74 horses in Hakkari region and reported that 9.5% horses had 1/40 or higher titers while the rest of the horses had titer values between 1/10-1/20 by SAT. Solmaz et al. (2004) reported a seroprevalence of 60.59% in horses raised in Van province of Turkey, by using RBPT. In

this study any titer value was not obtained in 13.01% of horse sera, which were positive by RBPT test, while in 86.9% of those samples titer values between 1/10 and 1/20 were obtained. The lower titers obtained in this study might be due to separation of living places of horses from that of other ruminants. The prevalence of seropositive samples obtained in this study by using SAT was similar to the findings of Izgur et al. (1998) while it was lower than that reported by Solmaz et al. (2004) and Göz et al. (2007). Abo-Sheda (2009) has reported that brucellosis seropositivity rate would increase depending on sheltering the horses with brucellosis positive cows especially in the regions where the brucellosis is endemic.

The majority of donkeys in the region is an integral part of the husbandry of small ruminant herds and employed for the transportation of the shepherd and his necessities while grazing the flock. Thus, the close association between donkeys and small ruminants exposes donkeys to many small ruminant pathogens including *Brucella* species, resulting in the seropositivity found in this study. Seropositivity 6.05% by RBPT and 0.25% by SAT found among donkeys in this study was lower than those reported by Hamoda and Montaser (1998) and Abo-Shehada (2009) among donkeys in Egypt (16.5%) and Jordan (7.4%) by using CFT, respectively. The higher ratios found by these authors might be due to use of samples from the donkeys rising together with the cows showing brucellosis symptoms (Abo-Shehada, 2009). In this study higher seropositivity ratios were found by RBPT in Sanliurfa region than Diyarbakır region. This difference can be resulted from brucellosis ratios in cows and the conditions of enterprices and sheds (Ehizibolo et al. 2011)

Consequently, the results indicated that brucellosis is not widely distributed among horses and donkeys raised in Sanliurfa and Diyarbakir provinces. However it can threat health of other sensitive animals, and humans. Therefore horses and donkeys should not be sheltered with ruminants. Including horses and donkeys in this region into brucellosis control program may be beneficial for public health.

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