SEROPREVALENCE OF EQUINE BRUCELLOSIS: FIRST REPORT IN BANGLADESH

M. K. S. A. Millat[1], N. M. Shafy[1], S. T. Sharmy[1], F. Yeasmin[1], M. F. Karim[1], M. A. Ehsan[1], R. R. Sarker[1, 2], F. Khatun[2], M. A. Wares[2], M. M. Hasan[1], I. Nishidate[2] and M. S. Rahman[1, 2]*

[1]Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, 2202, Bangladesh; [2]Graduate School of Bio-Application and Systems Engineering, Tokyo University of Agriculture and Technology, 2-24-16, Naka-cho, Koganei, Tokyo, 184-8588, Japan

ABSTRACT

Despite the endemicity of brucellosis, there is no report on the equine brucellosis in Bangladesh. The Rose Bengal Test (RBT) was used to determine the seroprevalence of *Brucella* antibodies amongst 112 horses from different areas of Bangladesh. The overall seroprevalence of equine brucellosis was 1.79%.The prevalence recorded in Ghatail area was 3.45% and there was no positive reactor in Shakipur and Savar areas. Sex wise prevalence showed that the prevalence was 3.08%in female and 0.00% in male horse. Only the adult (>3 years of old) horses showed the positive RBT reaction (2.35%), whereas young (<3 years of old) horses did not showed positive RBT reaction. The present study reports the first serological prevalence of *Brucella* infection in horses in Bangladesh. There is need for the inclusion of horses in brucellosis surveillance and control strategies in Bangladesh to safeguard people from high risk.

Keywords: Brucella, horse, RBT, Bangladesh.

INTRODUCTION

Brucellosis is a disease of sexually matured animals that has a great economic importance. Brucellosis is an enormous problem throughout the world; it is endemic problem in most countries including Bangladesh (Pappas *et al.*, 2006; Rahman *et al.*, 2011). It alternatively affects the production and reproduction potential of the animal in terms of abortion, infertility and reduction or no production of milk after abortion (Radostits *et al.*, 2000; Rahman *et al*., 2011, 2012; Roth *et al*., 2003). Brucellosis affects several mammalian species, including horses (Ribeiro *et al.*, 2008).Brucellosis in domestic and wild horses is caused by Gram-negative facultative intracellular coccobacilli (0.5 to 0.7µm wide and 0.6 to 1.5 µm long) bacteria of the genus Brucella (Lucero *et al.*, 2008; OIE, 2009). Three species of *Brucella* have been identified that's mainly infecting horses: *B. abortus*, *B. suis* and *B. canis*. However, *B. abortus* has been found to be the most common cause of brucellosis in rural horse populations (Ribeiro *et al.*, 2008). The disease is typically transmitted through contact with infected animals or materials, ingestion of raw milk or meat, aborted materials and aerosolized organisms (Alexander *et al.*, 1981; Godfroid *et al.*, 2004; OIE, 2009).Horses present nonspecific signs such as depression, intermittent fever, muscles stiffness, atlantal and carpal bursitis, tenosynovitis, osteomyelitis, osteoarthritis, and rare reproductive disorders (Ribeiro *et al.*, 2008). Equine brucellosis has also been associated with fistulous withers (Ocholi *et al.*, 2004; Cvetnic *et al.*, 2005).

Brucellosis has zoonotic potentiality. From public health view point, it is considered to be an occupational disease that mainly affects slaughter-house workers, butchers, and veterinarians or those people directly contract with the affected animals (Acha and Szyfer, 1987). The data regarding equine brucellosis appears to be exiguous unlike ruminants in Bangladesh. To the best of knowledge there is no published report of the sero-prevalence of brucellosis in horse in Bangladesh. Therefore, the following study was carried out for the diagnosis of brucellosis in horse with the objective of the estimation of seroprevalence of brucellosis in horse using RBT.

METHODS AND MATERIALS

A total of 112 blood samples were collected from horse populated areas of Dhaka and Tangail districts of Bangladesh. In Dhaka, 34 horse blood sample from Savar. In Tangil58 from Ghatail and 20 samples from Sokhipur were collected. Venous blood sample were conveniently and aseptically obtained from sexually matured horse. The epidemiological data regarding the age, sex, geographical location etc. were collected from the animal owner. All the blood samples were processed for sera followed by preservation in refrigerator and then tested by Rose Bengal Test (RBT) as a screening test. The study was conducted for a period of 6 months from January 2017 to June 2017 in the Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh.

Blood and sera samples collection

After controlling the animal blood was collected from jugular vein aseptically. About 5-7 ml of blood was collected from jugular vein of each horse with the help of sterile disposable syringe and needle. Then the blood was taken into test-tube and was kept undisturbed on jar with ice water for at least 1 hour at room temperature in slightly inclined position to facilitate clotting and separation of serum. After this period the sera sample were transferred to the sterilized labeled eppendorf tube and stored at -20°C until used.

RBT

The diagnostic antigen preparation and test was done according to the procedure of Manufacturer (Instituto de Salud Tropical Universidad de Navarra @ Edificio CIMA AvdaPioXII, 55 E-31008 Pamplona, Spain). Approximate number (112) of processed test sample (serum), sufficient antigen, positive and negative control sera for a day's testing were removed from refrigeration and kept for adjusting at room temperature(25°C) for 30 minutes.30µl of each serum to be tested was placed on a previously cleaned fine plastic plate circled approximately 1.5 cm in diameter. Then the bacterial antigen was shacked gently for proper mixing and 30µl of antigen put beside each of the sera on the plastic plate circle. The antigen and sera was mixed on the plate with a plastic stirrer and spread over the area enclosed by the circle. Then the plate was placed on a mechanical rotator at 90-100rpm for 3-5 minutes and the reading was taken immediately by the help of magnifying glass and also necked eye.

Data processing and Statistical analysis

The data obtained in the study with respect to area, age, sex of animals was processed by Microsoft Excel and analyzed by SPSS version 20.0 (Statistical Package for Social Services). Pearson chi-square test was done to determine the susceptibility of the sex (male/female), area and age wise prevalence to brucellosis. Significance was calculated between 1% and 5% level.

RESULTS AND DISCUSSION

Out of total 112 samples, 2 showed positive reactions and the overall seroprevalence of equine brucellosis was 1.79% (Table 1). The prevalence of equine brucellosis in present study is much more lower than the prevalence reported by Solmaz *et al.* (2004) and Wadood *et al.* (2009) who reported 20.7% and 60.56%, respectively by Rose Bengal Test. Area wise seroprevalence was 0.00%, 3.45% and 0.00% in Savar, Ghatail and Sokhipur, respectively (Table 2). The difference among various sources was not statistically significant. There was no positive reactor in Savar and Sokhipur area which may be due to smaller sample size from these two areas.

Table 1. Overall prevalence of brucellosis of horses based on RBT.

Total number of sample	Positive reactor	Negative reactor	% of positive reactor
112	2	110	1.79

Table 2. Prevalence of brucellosis of horses based on RBT.

Parameter	Total	Positive reactor	Negative reactor	% of positive reactor	Level of significance
Area					
Savar	34	0	34	0.00	
Ghatail	58	2	56	3.45	NS
Sokhipur	20	0	20	0.00	
Age					
Young (0 to <3 years)	27	0	27	0.00	NS
Adult (>3 years to above)	85	2	83	2.35	
Sex					
Male	47	0	47	0.00	NS
Female	65	2	63	3.08	

NS= Not significant (at 5% level of significance)

According to age, the all horses were divided into two groups: Young (0 to <3 years) and Adult (>3 years to above). Age wise prevalence showed that RBT is positive to only adult horses (>3 years to above) with the prevalence of 2.35% (2/85)but no young (<3 years of old) animal showed such type of positive reaction (Table 2).Difference in seroprevalence among various age groups was statistically non significant (P>0.05). This low prevalence in young animals may be explained on the basis that the animal may harbor the organism without expressing any detectable antibodies until their first parturition or abortion (Wadood *et al.*, 2009). In this study, a total of 47 male horses and 65 female horses were tested from different areas. Among them 2 female horses showed positive reaction to RBT with the prevalence of 3.08% whereas all male horses showed negative reaction to RBT (Table 2). There was no significant association between sex of horse which is similar with the findings of Muma *et al.* (2006) and Wadood *et al.* (2009) who reported that seroprevalence of brucellosis was not associated with sex. But the finding is differed from the findings of Ahmed and Munir (1995) and Solmaz *et al.* (2004) reported higher prevalence of brucellosis in females than in males. The higher prevalence in female may be due to the fact that females remain in close association with the infected mares, which can infect the healthy ones.

The current study determined the first information on the seroprevalence of *Brucella* antibodies among the horses in Bangladesh. In the present study, RBPT was conducted because it is widely used as a screening test and also can detect IgG and IgM (Omer *et al.*, 2007). The RBPT is easy to perform, cheap, rapid and highly sensitive but less specific than SAT (Gul and Khan, 2007). Sera negative for RBPT are not tested further. Regular sero-monitoring of the horse, culling of positive reactors from breeding program are important to eradicate or control of this zoonotic disease.

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