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# SEROPREVALENCE OF BRUCELLOSIS IN HORSES IN AND AROUND FAISALABAD

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## ABSTRACT

Rose Bengal Plate test (RBPT) and Serum Agglutination test (SAT) were used to monitor the seroprevalence of brucellosis in horses in and around Faisalabad, Pakistan. Sera were screened by RBPT and positive or doubtful sera were further processed by SAT for confirmation. The overall seroprevalence of brucellosis in horses was 20.7 and 17.7% by RBPT and SAT, respectively. Source wise seroprevalence of brucellosis was 19.8, 25.5, 2.9 and 0% in horses of Remount Area Faisalabad, Remount Area Toba Tek Singh, private and Livestock Management Department University of Agriculture Faisalabad, respectively. Sex wise seroprevalence in horses was 9.67 and 17.7% in male and female, respectively. In relation to age, seroprevalence was 12.9, 16.5, 14.8 and 20.6%, in horses of 1-5, 6-10, 11-15 and above 15 years of age, respectively. Highest seroprevalence was recorded in horses of above 15 years of age. Depending upon the body condition, the seroprevalence was 9.7, 13, and 20% in poor fair, and good body conditioned horses, respectively. Seroprevalence of brucellosis on the basis of parity was 19.2, 20.9, 18.7, 16.6, and 21.1% in 0, 1, 2, 3 and above 3 foaling females, respectively. Prevalence of brucellosis in different breeds of horses was 22.4, 17.1, 25.7 and 0.0% in Desi, Thoroughbred, Crossbred and Arabian horses, respectively. However, statistically, in relation to various factors like source, sex, body condition, parity and breed of horses, a non significant difference was observed among various groups. Statistically a significant difference ($P<0.001$) in seroprevalence was observed with respect to age, only.

**Key words**: Brucellosis, seroprevalence, horses.

## INTRODUCTION

Brucellosis is a disease of great economic importance, as it adversely affects the productive and reproductive potential of the animal in terms of loss of young ones, infertility and reduction or complete cessation of milk after abortion (Radostits *et al.,* 2000). Brucella is an infectious and contagious gram-negative coccobacilli. Main natural hosts of this organism are horses, cattle and humans. From public health view point, brucellosis is considered to be occupational disease that mainly affects slaughter-house workers, butchers, and veterinarians (Acha and Szyfer, 1987). Transmission typically occurs through contact with infected animals or materials.

Serological based testing and culling is carried out at Government farms for the eradication of brucellosis. However, desired objectives can not be achieved until the disease status in other domestic animals is also known (Ahmed and Munir, 1995a). In Pakistan, seroprevalence of brucellosis on the basis of serum agglutination test was 5.05 and 5.45% in cattle and buffaloes (Ahmed and Munir, 1995a), and 1.46 and 1.93% in sheep and goats (Nasir *et al.,* 2000), respectively. There is, however, relatively less information about the disease status in horses. Taking into consideration the thick population of equidae in the Faisalabad division and the importance of horses in our society, the seroprevalence of brucellosis in horses has been described in the present paper.

## MATERIALS AND METHODS

### Experimental animals

For this study, 300 horses of both sexes were selected randomly from periphery of Faisalabad city and university clinics. For each animal, information about age, parity, body condition and history of abortion if any, was noted. Horses were divided in to four age groups i.e. 1-5, 6-10, 11-15 and above 15 years. Sex wise distribution of horses was also done. Animals were also divided on the basis of body condition (fair, poor and good), parity (0, 1, 2, 3 and above 3) and breed (Desi, Thoroughbred, Crossbred and Arabian). Blood samples were collected from these horses without anticoagulant, serum was harvested and stored at $-20^0$C for serodiagnosis.

### Serodiagnosis

For serodiagnosis, Rose Bengal Plate test (RBPT) was performed as a screening test. For this purpose, a drop of serum was mixed with a drop of antigen containing *Brucella abortus* on a clean glass slide and examined for agglutination after 4 minutes. Hyperimmune sera were raised in rabbits. For this purpose, 3 rabbits were injected intravenously in the ear

vain with known *Brucella abortus* antigen procured from the Veterinary Research Institute Lahore, Pakistan. Dose schedule for raising hyperimmune sera was: 0.1 ml on day 1, 0.2 ml on day 3, 0.4 ml on day 5, 0.6 ml on day 7, 0.8 ml on day 9 and 1.0 ml on day 11.

Blood without anticoagulant was collected by slaughtering the animals on day 25 and serum was extracted. Serum titer of these known sera against *B. abortus* was determined by SAT and titer was 1:160. Hyperimmune sera raised in rabbits were used to run a control positive and control negative test along with the serum samples for both RBPT and SAT.

Samples found positive or doubtful by RBPT were subjected to SAT, following the procedure described by Hussain (2002). Briefly, five conical tubes were placed in a rack. Phenol saline solution (0.8 ml) was added to the first test tube and 0.5 ml in the remaining four test tubes. Test serum (0.2 ml) was added to the first test tube and a serial two-fold dilution (1:5 to 1:160) from first upto the $5^{th}$ test tube was made. A 0.5 ml of concentrated antigen was added to all test tubes. After mixing, test tubes were incubated at $60^0$C for 1 hour and sedimentation was noted.

Chi-square was applied to know the difference in seroprevalence of brucellosis among various groups, recorded through SAT.

## RESULTS AND DISCUSSION

The seroprevalence of brucellosis in horses was recorded as 20.7 and 17.7% by Rose Bengal Plate test and serum agglutination test, respectively. Solmaz *et al*. (2004) noted prevalence of 60.56% in horses which was higher than our results. 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. Sera negative for RBPT are not tested further (Gul and Khan, 2007)

According to source wise, seoprevalence was 19.8, 25.5, 2.9 and 0% by SAT in horses of Remount Area Faisalabad, Remount Area Toba Tek Singh, private animals and Livestock Management Department, University of Agriculture, Faisalabad, respectively (Table 1), the difference among various sources was statistically non significant. These results do not support previous results by Bandara and Mahipala (2002), who noted a significant difference in seroprevalence on the basis of source variation. The said researchers have noted difference in managemental conditions as the reason for source wise variations in the prevalence of this disease.

Seroprevalence of brucellosis was found to be 9.6 and 17.7% by SAT in stallions and mares, respectively. However, difference among males and females was statistically non significant (Table 1). These results are in accordance to Muma *et al.* (2006), who reported that seroprevalence of brucellosis was not associated with sex. However, Ahmed and Munir (1995b) and Solmaz *et al.* (2004) reported higher prevalence of brucellosis in females than in males. The higher prevalence in mares was attributed to the fact that females remain in close association and discharges passed after abortion or parturition by infected mares, which can infect the healthy ones. Moreover, females experience comparatively greater physiological stress during pregnancy and lactation due to which they are more susceptible to infection.

Regarding age, seroprevalence of brucellosis was 12.9, 16.5, 14.8 and 20.6% in horses up to 5, 6-10, 11-15 and above 15 years of age, respectively (Table 1). Difference in seroprevalence among various age groups was statistically significant ($P<0.01$). Agab (1997), Ahmed and Munir (1995b) and Kazi *et al.* (2005) also noted that the antibody titer against *Br. abortus* appears to be associated with age, as low prevalence in young stock has been reported than the adults. 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. It may be possible that after entry, the organism localizes itself in the regional lymph nodes and enjoy there without provoking antibody production until the animal is conceived and start secreting erythritol which stimulates and supports the growth of Brucella organisms (Keppie *et al*., 1965).

In this study, prevalence of brucellosis on the basis of body condition was 9.7, 13.0 and 20.0% in poor, fair and good conditioned animals by SAT, respectively (Table1). However, difference among three groups was non significant. Ahmed and Munir (1995b) also observed that there was no relationship of body condition with the seroprevalence of brucellosis.

On the basis of parity, seroprevalence of brucellosis was 19.2, 20.9, 18.7, 16.6 and 21.1% by SAT in 0, 1, 2, 3, and above 3 foalings, respectively (Table 1). However, difference observed in seroprevalence was statistically non significant. Similar observations were made by Berhe *et al.* (2007). A non significant difference in seroprevalence was observed on the basis of different breeds of horses. Seroprevalence was 22.4, 17.1, 25.7 and 0% by SAT in Desi, Thoroughbred, Crossbred and Arabian horses, respectively (Table 1).

Being a zoonotic disease, brucellosis has been eradicated from most of the developed countries through test and culling policy. Vaccination in dairy animals against this disease has been used successfully in most countries of the world including Pakistan to prevent brucellosis, but there is a dire need to vaccinate non dairy animals which may serve as source of infection for human beings as well as for other animals.

**Table 1: Seroprevalence of brucellosis in horses**

| Parameter | | Total | SAT positive cases | | Chi-square value | P value |
|---|---|---|---|---|---|---|
| | | | No | % | | |
| Source | Army Remount Faisalabad | 126 | 25 | 19.8 | 6.199 | 0.102 |
| | Army Remount T. T. Singh | 134 | 35 | 25.5 | | |
| | Private | 34 | 2 | 2.9 | | |
| | UAF | 6 | 0 | 0.0 | | |
| Sex | Male | 31 | 3 | 9.6 | 0.918 | 0.338 |
| | Female | 269 | 47 | 17.7 | | |
| Age (years) | 1-5 | 64 | 8 | 12.9 | 18.770 | 0.001 |
| | 6-10 | 133 | 22 | 16.5 | | |
| | 11-15 | 74 | 11 | 14.8 | | |
| | Above 15 | 29 | 6 | 20.6 | | |
| Body condition | Poor | 72 | 7 | 9.7 | 3.336 | 0.189 |
| | Fair | 78 | 10 | 13.0 | | |
| | Good | 150 | 30 | 20.0 | | |
| Parity | 0 | 52 | 10 | 19.2 | 0.520 | 0.972 |
| | 1 | 81 | 17 | 20.9 | | |
| | 2 | 64 | 12 | 18.7 | | |
| | 3 | 38 | 8 | 16.6 | | |
| | Above 3 | 65 | 16 | 21.1 | | |
| Breed | Desi | 129 | 29 | 22.4 | 3.858 | 0.277 |
| | Thorough bred | 123 | 21 | 17.1 | | |
| | Cross bred | 35 | 9 | 25.7 | | |
| | Arabian | 13 | 0 | 0.0 | | |

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