COMPARATIVE STUDY ON EFFICACY OF ROSE BENGAL PLATE TEST (RBPT) AND SERUM AGGLUTINATION TEST (SAT) FOR DETECTING THE INCIDENE OF BRUCELLOSIS IN BUFFALOES (*Bubalus bubalis*)

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ABSTRACT

Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT) were applied for the diagnosis of brucellosis in 240 buffaloes maintained at organized livestock farms in Punjab, to measure their comparative efficacy. Based on RBPT and SAT, 11.25 (n=27) and 10.42 percent (n=25) buffaloes were found seropositive, 11.67 (n=28) and 4.58 percent (n=11) animals showed doubtful results, while 77.08 (n=185) and 85 percent (n=204) animals were found seronegative, respectively. Rose Bengal Plate Test detected higher percentages of seropositive, doubtful and seronegative cases than those detected by Serum Agglutination Test which showed lower percentages of seropositive, doubtful and seronegative cases. This study indicated that SAT is more sensitive and reliable diagnostic test for the detection of *Brucella abortus* antibodies in buffaloes.

INTRODUCTION

The importance of brucellosis is primarily due to its public health significance. Besides zoonotic significance, brucellosis adversely affects the fertility of livestock, resulting in appreciable economic losses to the livestock industry (Anonymous, 1971). The incidence of the disease is associated with demographic and geographical factors. Sero-prevalence of the disease has been reported to vary from 3.25 to 4.4 per cent in different areas of Pakistan (Ahmad *et al.*, 1990; Naeem *et al.*, 1990). There are several serological tests available for diagnosis of disease in buffaloes (*Bubalus bubalis*). The present study was conducted to compare the efficacy of two serological tests for the diagnosis of brucellosis in buffaloes maintained at four organized livestock farms in Punjab.

MATERIALS AND METHODS

Two hundred and forty sera samples were collected from 240 buffaloes maintained at Government Livestock Experiment Stations in Punjab. Fifty buffaloes each from Livestock Experiment Stations Chak Katora (Distt. Bahawalpur), Haroonabad (Distt. Bahawalnagar) and Khushab while 90 buffaloes from the Livestock Experiment Station, Rakh Ghulaman (Distt. Bakkar) were included in this study. These buffaloes had one or the other reproductive disorder, e.g., abortion, retained placenta, metritis and repeat breeding. These sera samples were subjected to two serological tests namely Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT) and were processed according to the methods prescribed by Ahmed *et al.* (1989). *Brucella abortus* antigens for these tests were procured from Veterinary Research Institute (VRI) Lahore.

Rose Bengal Plate Test

Rose Bengal antigen and sera both were brought to room temperature before testing. To one drop of each serum sample, one drop of antigen was added on a clear glass slide. The contents were thoroughly mixed. The reaction for the presence or absence of agglutination was observed after four minutes. The chances of false positives and false negatives were minimized by using known positive and negative serum controls. Complete agglutination was considered as positive, partial as doubtful and no agglutination at four minutes was recorded as negative.

Serum Agglutination Test

The phenol saline (0.85% NaCl solution containing 0.5% phenol) was used to prepare two fold serial dilutions of the test sera samples starting from 1:10 up to 1:640 (volume 0.5 ml). Each sample was tested in duplicate. The *Brucella abortus* antigen was diluted in sterile physiological saline according to the instructions of manufacturer and an equal amount (30µl) was added to each serum sample tube. After thorough mixing the contents of tubes were incubated at 37°C for 18-24 hours. The degree of agglutination and titre of serum were determined by the degree of clearing without shaking the tubes. Known negative and positive sera were used as controls. Complete agglutination and sedimentation with 100 percent clear supernatant was marked as four plus (++++) similarly 75, 50 and 25 per cent were marked as three, two and one plus, respectively. No agglutination and no clearing was considered as negative. The highest serum dilution

showing 50% clearing (+ +) was considered as titre of that serum. A titre of 1:40 or higher was considered as positive as per recommendations of FAO/WHO Expert Committee on brucellosis (Alton *et al.*, 1975).

RESULTS AND DISCUSSION

Based on RBPT and SAT, 11.25 (n=27) and 10.42 per cent (n=25) buffaloes were found seropositive, 11.67 (n=28) and 4.58 per cent (n=11) animals showed doubtful results, while 77.08 (n=185) and 85 per cent (n=204) animals were found seronegative, respectively (Table-1).

Results of two serodiagnostic tests indicated that RBPT detected higher percentages of seropositive, doubtful and seronegative cases than those detected by SAT. It is evident from Table 1 that SAT gave more clear cut results as it declared less number of doubtful cases (4.58%) as compared to RBPT (11.67%). These findings are in agreement with other research workers (Al-Delaimi and Ali, 1990; Akram, 1991; Roohi, 1992; Lodhi *et.al.*, 1995; Rahman *et al.*, 1997). However, these are not in agreement with the observations of Ahmad and Abd. El. Aal (1997), who reported higher percentage of seropositive cases detected by SAT than those detected by RBPT in bovine brucellosis. The findings of this study are also not in agreement with the observations of Rehman *et al.* (1983), who reported that 6.9% buffaloes were positive by RBPT while 2.4% were positive by SAT. Similarly, the findings of this study are not in agreement with the observations of Nicoletti (1980), who reported that several tests are available for diagnosis of brucellosis in cattle but these two tests had varying degree of sensitivity, specificity and limitations.

The RBPT and SAT are quantitative measurements of antibodies and are affected by many factors. In these studies, RBPT showed low specificity which is in agreement with the findings of Flade (1983), who reported that RBPT is rapid, simple and sensitive but it has low specificity. Rehman *et al.* (1990) reported that SAT detected higher percentage of doubtful cases (61.5%) as compared to RBPT (38.40%) which is not in agreement with this study. It is evident from this study that SAT is more sensitive and reliable than RBPT as a diagnostic test for detection of *Brucella abortus* antibodies in buffaloes.

Table 1: Comparative efficacy of two serological tests for the diagnosis of brucellosis in buffaloes

Place of Sampling	No. of Samples	Particulars	Diagnostic Tests					
			Rose Bengal Plate Test			Serum Agglutination Test		
			Positive	Doubtful	Negative	Positive	Doubtful	Negative
Livestock Experiment Station Chak Katora	50	No. of Samples	4	6	40	5	5	40
		Percentage	8.00	12.00	80.00	10.00	10.00	80.00
Livestock Experiment Station Haroonabad	50	No. of Samples	4	21	25	6	4	40
		Percentage	8.00	42.00	50.00	12.00	8.00	80.00
Livestock Experiment Station Khushab	50	No. of Samples	5	1	44	2	2	46
		Percentage	10.00	2.00	88.00	4.00	4.00	92.00
Livestock Experiment Station Rakh Ghulaman	90	No. of Samples	14	--	76	12	--	78
		Percentage	16.00	--	84.00	13.00	--	87.00
Over all	240	No. of Samples	27	28	185	25	11	204
		Percentage	11.25	11.67	77.08	10.42	4.58	85.00

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