

## COMPARATIVE STUDY OF STANDARD AND MODIFIED SERUM AGGLUTINATION TESTS FOR THE DIAGNOSIS OF BRUCELLOSIS IN ANIMALS

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### ABSTRACT

A total of 500 serum samples collected from cattle and buffaloes from various livestock farms in Punjab were analysed for brucella antigens. Out of these 500 sera, Rose Bengal Plate Test (RBPT) recorded 79(15.8%) positive. These RBPT positive samples were subjected to routine serum agglutination (SAT) and modified SAT (EDTA added) tests. The SAT showed 18(22.7%) positive, 42(53.1%) doubtful and 19(24%) negative samples, whereas modified SAT recorded 15(18.9%) positive, 28(35.4%) doubtful and 36(45.5%) negative samples. Results of modified SAT were observed to be more clear and easy to read than routine SAT. It was concluded that modified SAT may be used as an alternate to routine SAT in the diagnosis of brucellosis which may reduce the chances of cross reaction.

**Key words:** Brucellosis, serum agglutination test, modified serum agglutination test.

### INTRODUCTION

Brucellosis is an important disease of livestock and is characterized by abortion, retained placenta and infertility and remains a leading zoonotic disease of public health and economic importance (FAO, 1971). The standard serum agglutination test (SAT) is still widely used for the diagnosis of brucellosis and is specified in many countries. However, serological cross reactions have been demonstrated between *Brucella* species and other bacteria e.g. *E. coli* O:116 and O:157, *Francisella tularensis*, *Salmonella* serotypes of Kauffman-White group N, *Pseudomonas maltophilia*, *Vibrio cholerae* and *Yersinia enterocolitica* serotype O:9 (Otto *et al.*, 2000). To overcome this problem, the standard SAT was modified and its comparative efficacy has been described in this paper.

### MATERIALS AND METHODS

A total of 500 serum samples were collected from cattle and buffaloes from various livestock farms in Punjab. All the samples were screened for the presence of brucella antigens by Rose Bengal Plate Test (RBPT). Out of these, 79 sera that gave positive reaction with RBPT were further subjected to the standard serum agglutination test (SAT) by using method of Stemshorm *et al.* (1985) and a modified SAT, as described below (OIE, 1996):

#### Modified serum agglutination test

This test was performed in clear glass or plastic tubes of approximately 2 ml total volume by placing 0.8

ml of phosphate buffer saline solution (PBSS, sodium chloride 8.0g/L, potassium chloride 0.2g/L, di sodium hydrogen phosphate 1.15g/L, potassium di hydrogen phosphate 0.2g/L) with 10 millimole ethylene tetra acetate having pH 7.2 i.e., 3.72g/L, into the first tube and 0.5 ml volumes of PBSS in the remaining tubes of a series of five or ten tubes. A 0.2 ml serum was added to the first tube, mixed and then 0.5 ml was transferred to the next tube. Further volumes of 0.5 ml were transferred to subsequent tubes to give a series of doubling dilutions. An equal volume (0.5 ml) of standard *B. abortus* agglutination suspension, diluted to working strength in phenol saline (1:10) was then added to each tube, and the tubes were incubated at 37°C for 20 hours. The results of agglutination in SAT test were determined by reading the degree of clearing and sedimentation of the tubes. A titre of 1:40 (i.e. 50% agglutination at 1:40) or more was indicative of infection, whereas 50% or above reaction in titre of 1:20 was considered a suspicious. A titre of 1:10 was treated as negative.

### RESULTS AND DISCUSSION

Out of 79 RBPT positive serum samples, SAT recorded 18(22.7%) positive, 42(53.1%) doubtful and 19(24%) negative as compared to modified (EDTA added) SAT where 15(18.9%) samples were positive, 28(35.4%) were doubtful and 36(45.5%) were negative. Thus, modified SAT showed 3(3.8%) less positive, reduced doubtful by 14(17.7%) and showed 17(21.5%) more negative as compared to SAT (Table 1).

**Table 1: Comparison of SAT and modified SAT**

Tests	No. of samples	Antibody titers		
		1:10 (negative)	1:20 (doubtful)	1:40 or more (positive)
SAT	79	19(24.0%)	42(53.1%)	18(22.7%)
SAT (modified)	79	36(45.5%)	28(35.4%)	15(18.9%)
Difference	----	+ 17(21.5%)	-14(17.7%)	-3( 3.8%)

A similar study was conducted by Carin and Trap (1984), who found that EDTA added SAT did not result in a decrease in the antibody of infected cattle but it decreased the titre of non specific reactions and was preferable to the serum agglutination test. Trap *et al.* (1985) found that EDTA reduced the reaction rate of samples in infected herds from 82.6 to 80.7%, while heating reduced the rate by 10%. Romakhov *et al.* (1990) studied the value of EDTA test for classifying doubtful reactions in comparison to the standard agglutination test and confirmed by tests on serum samples from healthy, infected and vaccinated cattle. Corbel (1985) reported that the sera in which the agglutinating activity was entirely attributable to EDTA-labile agglutinins, a complete or almost complete loss of titre occurred in the presence of a chelating agent.

The present findings are in accordance with those of Otto *et al.* (2000), who reported that non specific reactions with brucella could be reduced by addition of EDTA to the diluent. Macmillan and Cockrem (1985) stated that agglutination reaction was sufficiently affected by the action of EDTA.

The results of this study indicate that modified (EDTA added) SAT may be used as an alternate to routine SAT in the diagnosis of brucellosis, which may reduce the chances of cross reactions.

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