# COMPARISON OF SEROLOGICAL METHODS FOR THE DETECTION OF *BRUCELLA ABORTUS* ANTIBODIES IN SERA FROM INFECTED BOVINES

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## ABSTRACT

The efficacy of rose Bengal plate test (RBPT) , standard tube agglutination test (STAT) and indirect enzyme linked immunosorbent assay (i-ELISA) in detection of bovine brucellosis was evaluated. The serum samples of 74 animals (68 cattle and 6 buffaloes )from different locations in Maharashtra were investigated for presence of Brucella antibodies by RBPT , STAT and i-ELISA. Overall observation of results of three serological tests employed in the present investigation for detection of bovine brucellosis suggests superiority of i-ELISA (56.75 %) over RBPT (54.05 %) and that of RBPT over STAT (47.29 %) in detecting the Brucella antibodies.

**KEYWORDS :** Brucella, i-ELISA, STAT, RBPT.

## INTRODUCTION

Brucellosis, an important disease of livestock is one of the common bacterial zoonoses in the world. The disease attributes to serious economic losses by adversely affecting the reproductive and productive potential of the animals (Chahota *et al.* 2003). Isolation of the causative agent has been the most accepted tool for confirmatory diagnosis of brucellosis ,however , the procedure is time consuming and may take several days for precise identification of the agent. Moreover, the technique has reduced sensitivity in chronic infection and the culture materials need to be handled carefully as the organisms are class III pathogens (Alton *et al.*, 1988). The serological tests have been widely employed for screening the animals for brucellosis since they are inexpensive, rapid and sensitive. The different serological tests vary in their sensitivity and specificity and their diagnostic value may be questionable on individual basis because of cross-reacting antibodies (Debeaumont *et al.*, 2005). Despite of their limitations, the serological tests still remain the first choice for screening of herds. The present investigation was undertaken with view to evaluate comparative efficacy of three serological tests viz. rose bengal plate test (RBPT), standard tube agglutination test (STAT) and indirect enzyme linked immunosorbent assay (i-ELISA) in detection of brucellosis in bovines.

## MATERIALS AND METHODS

A total of 74 serum samples collected from 68 cattle and 6 buffaloes from different locations in Maharashtra were included in the study. All the investigated animals were derived from the herds with the history of abortion.

### Rose Bengal Plate Test (RBPT):

The coloured antigen required for RBPT was obtained from the Division of Biological products, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh and the test was performed as per the manufacturer's instructions.

### Standard Tube Agglutination Test (STAT)

The STAT was performed using Brucella abortus agglutinating antigen obtained from Division of Biological Products, Indian Veterinary Research Institute (I.V.R.I.); Izatnagar, Uttar Pradesh. The

test procedure was as per manufacturer's instructions. The values of titers obtained were converted into International Unit (I.U.) of Brucella antibody activity as recommended by the joint FAO/WHO expert committee on Brucellosis (Alton and Jones, 1967). The titers of 80 I.U. per ml or above were considered positive for brucellosis in cattle and buffaloes while those of 40 I. U. as doubtful.

**Enzyme Linked Immunosorbent Assay**

The enzyme linked immunosorbent assay (ELISA) for detection of anti-Brucella antibodies in the sera samples of cattle and buffaloes was performed using ELISA kit obtained from B. V. European Veterinary Laboratory, Netherlands. The iELISA test was performed as per the manufacturer's instructions (OIE, 2010). A sample was scored as negative when the OD value of the test was lower than 2 x OD of the negative control. A sample between 2X and 3X the OD of the negative control was considered as weak positive. A sample above 3X the negative OD was considered to be positive.

**RESULTS AND DISCUSSION**

The present investigation dealt with the evaluation of efficacy of the serological tests viz. Rose Bengal plate test (RBPT), Standard tube agglutination test (STAT), Indirect Enzyme Linked Immunosorbent Assay (i-ELISA) in diagnosis of Brucellosis in cattle and buffaloes. A total of 74 animals either with the history of abortion or in-contact with the infected animals from different locations in Konkan and Western Maharashtra region were included in the study. A total of 74 bovine serum samples were tested for detection of Brucella antibodies by RBPT of which 40 (54.05%) proved positive. The results of STAT performed on 74 animal sera indicated that, the proportion of sera samples reacting positively in STAT was relatively less (47.29 %) however, the trend of prevalence was similar to that of RBPT. All the 74 animal sera were also tested simultaneously by monoclonal antibody based *B. abortus* specific i-ELISA. It is evident from the results of i-ELISA that a total of 42 animals (56.75%) were positive for presence of antibodies against *B. abortus*.

Three serological tests viz. RBPT, STAT and i-ELISA were employed in the present investigation for detection of bovine brucellosis. The sensitivity, specificity and proportion of agreement between the different tests were estimated using Win Episcope 2.0 software. Considering i-ELISA as the gold standard, the sensitivity and specificity of RBPT were 80.95% and 81.25% respectively whereas the proportion of agreement between these tests was 81.08%. The values of sensitivity , specificity of STAT were 76.19% and 90.62% respectively while the proportion of agreement between STAT and i-ELISA was 82.43%.

An early and precise diagnosis of brucellosis is crucial in order to initiate appropriate control measures. The serological tests offer a simple and relatively inexpensive alternative. These tests are based on the reaction between Brucella antigen and antibodies produced in response to the infection. The classes and subclasses of antibody (isotypes) generated in infected animals could be of different types; the different serological tests therefore vary significantly in their ability to detect brucellosis. Several workers have evaluated the efficacy of different serological tests in detection of brucellosis. Among the various serological tests, RBPT and ELISA have been most extensively used for screening animals for Brucellosis (Abuharfeil and Abo-Shehada, 1998; Nielsen, 2002 and Gall and Nielsen, 2004). Extensive review of literature on serological tests showed that no individual test is perfect for diagnosis of brucellosis; however the error could be minimized using the most reliable test. In the present investigation we found i-ELISA was most sensitive and could detect 56.75% positive animals. The RBPT was second in order with rate of detection of 54.05%. The STAT could detect relatively low numbers of positive animals. Despite of a number of disadvantages, serological tests continue to be the most widely used tool for diagnosis of brucellosis. Several studies conducted for efficacy evaluation of serological tests indicate that there is a marked variation in sensitivity and specificity of different tests. The finding of present study of higher sensitivity of i-ELISA over RBPT and STAT is supported by the work of many researchers who recorded a better

sensitivity of ELISA compared to STAT and RBPT. Chakraborty *et al.*, (2000) while investigating 141 bovine sera for brucellosis recorded highest proportion (56.02%) of positive samples by i-ELISA. Similarly, Mishra *et al.* (2005) during their investigations on brucellosis in buffaloes observed higher sensitivity of iELISA over STAT. Our finding is in agreement with the observations of Barbuddhe *et al.* (2004), Singh *et al.* (2004). It can therefore be inferred from the results of the present study that the i-ELISA has a greater sensitivity in detection of brucellosis and could be used as an adjunct to RBPT and STAT in detection of brucellosis.

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