COMPARATIVE SEROLOGICAL STUDIES IN MILK AND SERUM AND EVALUATION OF EFFICACY OF BRUCELLA ABORTUS S99

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Received 23-12-2013 Accepted 23.3.2014

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ABSTRACT

A study was conducted to compare serological characteristics of milk and serum from brucellosis suspected cows. The serological tests like Rose Bengal Plate Test (RBPT), Milk Ring Test (MRT), Standard Tube Agglutination Test (STAT) and Enzyme Linked Immune Assay (ELISA) were conducted for the detection of antibodies in serum and milk and the results were compared. Hyper immune serum (HIS) against Brucella abortus S99 was raised in rabbits and was tested against RBPT and plain antigen by STAT. The HIS showed an antibody titre of 1: 2560.

KEYWORDS: MRT, RBPT, ELISA, HIS, STAT

INTRODUCTION

Brucellosis is an important re-emerging zoonosis with a worldwide distribution. Many serological tests have been proposed for the diagnosis of brucellosis, ELISA using S-type Lipopolysaccharide (S-LPS) extracts or its O-chain have been extensively studied (Nielson. 2002) and may replace the MRT, RBPT and STAT.

Serum agglutination tests have been used as standard diagnostic method for detection of Brucellosis (OIE, 2005). Agglutination tests will detect antibodies in serum, milk (Hamdy et al., 2002), whey and semen. STAT is useful in detecting the antibody titre of serum or level of infection in an individual animal and can be used as confirmatory test. Hence the persent work was undertaken to asses efficacy of brucella abortus S99 in cows.

MATERIALS AND METHODS

Collection of serum and milk sample

The blood and milk samples were collected from 50 cows suspected for Brucellosis from government and private organized farms. Serum was seprated from blood by centrifugation. The serum samples were subjected to Rose Bengal Plate Test (RBPT), Standard Tube agglutination test (STAT) and ELISA and the milk samples were subjected to Milk Ring Test (MRT) and ELISA as per standard method in use.

Preparation and Inoculation of Brucella antigen for HIS:

Sterile B.abortus culture was inoculated in 100 ml of the tryptose broth. The cell pellet suspension was inactivated and adjusted to 4 per cent Biomass or cell mass by Haemocrit PCV tube and Biuret Kit method. Four healthy rabbits were procured from IAH&VB. Rabbits were screened for sero negativity. 0.5 ml inactivated cell suspension was inoculated into the rabbit by intramuscularly for hyper immunization. Booster doses of four injections were given at an interval of four days.

Determining the titre value of the HIS

Serial dilution of serum was done. 0.2 ml of serum in 0.8 ml of phenol saline and serially diluted to 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280, 1:2560, and 1:5120. 0.5 ml of phenol saline and 0.5ml plain standard antigen was added to all the tubes. The tubes were incubated at 37ºC for 48 hours.

Preparation of standard serum

After hyper immunization the rabbits were bled and serum was separated and Thiomersol was added as preservative at 1:10,000 final concentrations of the sera. This serum was further filtered by Seitz filter. The filtered serum was freeze dried.

RESULTS AND DISCUSSION

Brucellosis is one of the major zoonotic diseases and is being controlled by employing eradication programs. Serology is the best method to detect the prevalence of the disease in a herd (Hussain et al., 2000 and Kumar et al., 1997). In this context an attempt was made to compare the sensitivity and specificity of RBPT (Corbel, 1972), MRT, and STAT with ELISA (Ganesan and Anuradha, 2006). The sensitivity and specificity was calculated as follows :

$$\text{Sensitivity} = \frac{\text{No. of true positive}}{\text{No. of true positive + No. of false negative}}$$

$$\text{Specificity} = \frac{\text{No. of true negative}}{\text{No. of true negative + No. of false positive}}$$

Table. **1. Results showing Sensitivity and Specificity of MRT, RBPT, STAT and ELISA**

Sample	MRT		RBPT		STAT		ELISA	
Result	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
Milk	5	45	-	-	-	-	3	47
Serum	-	-	4	46	3	47	3	47
Sensitivity (in percent)	60.00		75.00		100		100	
Specificity (in percent)	95.91		97.91		100		100	

No. of samples n=50

Based on the findings it can be concluded that MRT and RBPT can be used initially as tests for screening and monitoring (Isloor *et al*., 1998) the herd. Subsequently to confirm the disease ELISA can be effectively utilized considering its high specificity and sensitivity (100 per cent). For detecting the level of infection in a given herd STAT can be employed. In MRT immunoglobulins present in the milk will attach to the fat globules through Fc portion. If antibody to Brucella is present, agglutination will take place resulting in a purple band at the top of the milk (Huber and Nicoletti 1986). If no antibody is present, the fat layer will remain as buff colour and the purple antigen will be distributed throughout the milk. The milk ring test is prone to false positive reactions caused by abnormal milk derived from mastitis, colostrums and milk from late lactation cycle (Sutra *et al.,* 1986). In spite of these problems, it may be used as an easy and inexpensive screening test

In the present study, we made an attempt to evaluate efficacy of *Brucella abortus* S99 strain by producing HIS in rabbits. The HIS was found to have a titre of 1:2560 by STAT. The HIS was used

as a positive control in RBPT, MRT, STAT and ELISA. The hyper immune serum also have many other applications like therapeutic use and used in various serological tests (Rogers *et al*., 1989). In future its use may be harnessed in various areas.

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