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Seroprevalence Study of Brucella Infection in and around Rewa District, India

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A B S T R A C T

The present work was conducted to study the seroprevalence of Brucella infection amongst the cattle population in and around Rewa district (Madhya Pradesh). Seroprevalence study was conducted using Rose Bengal Plate Test and SVANOVIR Brucella Antibody c-ELISA kit. C-ELISA is OIE referred gold standard test for detecting Brucella antibodies. Around 50 serum samples were screened for the presence of Brucella antibody using both Rose bengal plate test (RBPT), and Competitive Enzyme Linked Immunosorbent assay (c- ELISA) kit. Out of 50 samples tested with Rose Bengal Plate Test and c-ELISA, 42% and 20% were detected to be positive respectively. Rose Bengal Plate Test was not sensitive enough to detect weak positive sample, which were detected by c-ELISA, considering it as gold standard test for seroprevalence study of Brucella infection.

Introduction

Bovine Brucellosis is a serious disease of livestock that has significant animal, public health and international trade consequences. Considering the damage done by the infection in animals such as death losses, decreased milk production, weight loss, infertility and lameness, this disease is a formidable threat to livestock. The fact that this disease can spread rapidly and be transmitted to humans makes it all the more serious. *Brucella* species are small, Gram-negative, non-motile, non-spore-forming, rod-shaped (cocco-bacilli) bacteria. *Brucella abortus* is the principal cause of Brucellosis in cattle. Once exposed, the likelihood of an animal becoming infected is variable, depending on age, pregnancy status, and other intrinsic factors of the animal, as well as the number of bacteria to which the animal was exposed (Radostitis *et al.*, 2000). World Organisation for Animal Health (OIE) considers the disease as a threat of public health importance and a boundary for international trade of animals (OIE, 2004).

Specific antibodies against bacterial lipopolysaccharide and other antigens can be detected by the Standard Agglutination Test (SAT), Rose Bengal plate test, 2-

mercaptoethanol (2-ME), Antihuman Globulin (Coombs), Indirect Enzyme Linked Immunosorbent Assay (ELISA) and Competitive Enzyme Linked Immunosorbent Assay. The Buffered Antigen Plate Agglutination test (BPAT) (Angus and Barton, 1984) and the Rose Bengal test (RBT) (Morgan *et al.,* 1969) has been widely used. This test is considered as suitable screening tests for Brucellosis, followed by confirmatory testing. ELISA typically uses cytoplasmic proteins as antigens. It measures IgM, IgG and IgA with better sensitivity and specificity than the SAT in most recent comparative studies (Mantur *et al.,* 2010). The specificity of the competitive enzyme immunoassay is very high; however, it is slightly less sensitive than the indirect enzyme immunoassay. This assay is not only an excellent confirmatory assay for the diagnosis of brucellosis in most mammalian species, but it is also capable of discriminating between Brucella infected animals and animals vaccinated with Brucella strain 19 in cattles.

Materials and Methods

Study area

Seroprevalence study of Brucella infection was carried out in cattle population of district Rewa (Madhya Pradesh), India. Rewa district situated in the north- eastern part of Madhya Pradesh. The climate of the district is humid subtropical with cold, misty winters, hot summer and humid monsoon season.

Samples

Total 50 serum samples of cattle from Laxman Bagh Gaushala, village- Paharkha and Silpara and from small organized farm of Rewa district were collected. Samples were stored at -20^{o}C until they were used. All the serum samples were tested for the presence of anti Brucella antibodies by using RBPT and c- ELISA kit.

Rose Bengal plate test

The standard technique suggested by Alton *et al.,* (1975) was adopted in the present study to perform RBPT for cattle serum sample. The RBPT antigen was procured from Department of Veterinary Microbiology, College Of Veterinary Science and Animal Husbandry, Ludhiana, India. To perform the test antigen and serum were brought to the room temperature. Homogenous suspension of antigen was made by shaking the bottle containing antigen. Then, one drop (0.03ml) of serum sample and antigen was taken on the same slide using different micropipette and mixed thoroughly using a spreader. The slide was incubated with rotation for 4 min and immediately observed for results. A result was considered as positive when a noticeable agglutination was observed after 4 minutes.

Competitive ELISA(c- ELISA)

Competitive ELISA was performed using the SVANOVIR Brucella Ab c-ELISA kit using *Brucella abortus* smooth lipopolysaccharide (s-LPS) coated wells on microtiter plate. In order to perform the test reagents, samples and plate (s) were equilibrated to room temperature before use. 45µl of sample dilution buffer was dispensed into each well as per layout plan for serum sample, serum controls and conjugate control. After that 5µl of positive, weak positive and negative serum control was added into each of the appropriate well. 5µl of sample dilution buffer were added into conjugate control wells. Diluted test serum samples were dispensed at last. The sides of the plate were tapped to ensure even distribution of the antibody over the bottom of each well. Reconstituted 50µl of monoclonal antibody solution were dispensed into each wells, used for control and samples.

Plate was covered with aluminium foil/lid and reagents mixed thoroughly for 5 minutes by tapping the sides of the plate. Plate was incubated at room temperature (18-25^{o}C) for 30 minutes. After incubation plates/ strips was washed four times with PBS-Tween buffer and tapped hard to remove all remains of fluid. After washing 100µl of conjugate solution was dispensed into each well. Again plate was covered with aluminium foil/lid and incubated at room temperature for 30 minutes.

After incubation plate/ strip was washed as above mentioned. After washing 100µl of substrate solution added to each well and incubated at room temperature for 10 minutes. Reaction stopped by adding 50µl of stop solution to each well and mixed thoroughly. Immediately after adding the stopping solution the plate was read in the ELISA plate reader at 450 nm wavelength.

Mean optical density (O.D.) value for each of the controls and samples were calculated. Percent Inhibition (PI) values for controls as well as samples were calculated as follows-

$PI = 100 - (OD_{sample} \text{ X } 100) / OD_{conjugate\ control(Cc)}$

PI value for positive control, weak positive control and negative control were 80-100, 30-70, and (-10) to-15 respectively. Sample that gave more than 30% PI value were considered as positive, while below 30% were considered as negative. If sample showed PI value 30% then it was retested.

Results and Discussion

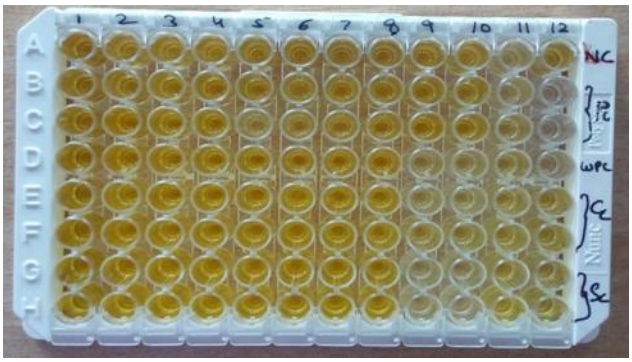
In cattle out of 50 samples tested, 21(42%) were positive by RBPT, whereas 10(20%) were found to be positive by SVANOVIR c-ELISA kit (Table no. 1) (Figure 1). Further 6 (28.57%) out of 21 RBPT positive serum samples were found to be positive, however 15 (71.42%) were found negative by c-ELISA which showed positive result by RBPT. Out of total 10 samples found to be positive by SVANOVIR c-ELISA kit; four samples were such which were shown negative by RPBT. Considering the fact that by c-ELISA is highly specific test the overall positive cases detected is 20 percent.

Brucellosis is an important zoonosis and serological surveillance is essential for its control (Erdenebaatar *et al.,* 2004). Although many countries have eradication programs for controlling brucellosis, economic losses can be heavy due to abortion and infertility and subsequent culling, so herds should be monitored for the presence of infection. Despite eradication programs, including vaccination, testing and slaughter, brucellosis remains a major zoonosis worldwide (WHO, 1986; Baek *et al.,* 2003) and the disease has remained prevalent in many areas in the world.

Table.1 Seroprevalance of Brucella antibodies in cattle by RBPT and c- ELISA

Study Area	No.of serum samples tested	RBPT positive samples	c- ELISA positive samples
In and Around Rewa District	50	21 (42%)	10 (20%)

Fig.1 Microtiter plate showing the results of Competitive ELISA



The results are in accordance with Mai *et al.,* (2012) where they tested 4745 samples by RBPT out of which 1735 (36.6%) were positive for Brucellosis. Out of 1735, 1137 (65.5%) were confirmed to be seropositive for brucellosis upon further testing by c-ELISA. The competitive ELISA (c-ELISA) using monoclonal antibody (MAb) specific for one of the epitopes of the *Brucella sp*. OPS has been shown to have higher specificity but lower sensitivity than the I-ELISA (Macmillan *et al.,* 1990; Munoz *et al.,* 2005; Neilson *et al.,* 1995; Stuck *et al.,* 1999 and Weynants *et al.,* 1997). This is accomplished by selecting a MAb that has higher affinity than cross-reacting antibody. However, it has been shown that the c-ELISA eliminates some but not all reactions due to cross-reacting bacteria (Munoz *et al.,* 2005; Neilson, 2002).The c-ELISA is also capable of eliminating most reactions due to residual antibody produced in response to vaccination with S19. The choice of MAb and its unique specificity and affinity will have a distinct influence on the diagnostic performance characteristics of the assay. As with any MAb-based assay, the universal availability of the MAb or the hybridoma must also be considered with respect to international acceptance and widespread use. The results indicate that however the RBPT can be used as initial screening method for Brucella infection, the results should be confirmed by c-ELISA. The overall positive cases detected by c-ELISA is 20 percent, revealing that there is significant prevalence of Brucella antibodies in cattle population in Rewa district, depicting the presence of Brucella infection in population and warranting the need for continued sero-surveillance of the disease posing a threat for disease condition.

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