

Full Length Research Paper

Standard serological tests for diagnosis of bovine brucellosis in Mathura district of Western Uttar Pradesh, India

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A total of 56 serum samples from suspected cattles and 5 from brucellosis-free non- vaccinated cattle herd were collected from various livestock farms in Mathura district of Western Uttar Pradesh, of which 16 serum samples found to be positive were subjected to all the three serological tests that is Rose Bengal plate test (RBPT), Standard tube agglutination test (STAT) and Dot- enzyme linked immunosorbant assay (ELISA) respectively. The isolation of *Brucella abortus* from cervical swab samples was vital in the confirmation of *Brucella* infection and epidemiological evaluation of the herd. High titer of 1:320 and 1:640 was observed in the present investigation. Two (12.5%) isolates out of 16 samples were positive by STAT and all the 6 RBPT positive samples (100%) exhibited negative results by STAT. However, it was observed that all the 16 samples (100%) which earlier revealed to be negative for RBPT and STAT exhibited positive results with Dot- ELISA. In the present study though Dot-ELISA proved to be the most sensitive of the 3 tests used, it can, however be suggested that a combination of RBPT and Dot ELISA should be used, especially in case of those samples that are found to be negative by either RBPT or STAT used alone or in combination.

Key words: Brucellosis, Rose Bengal plate test (RBPT), Standard tube agglutination test (STAT), Dot enzyme linked immunosorbant assay (ELISA).

INTRODUCTION

Brucellosis is an important disease of livestock (Nasir et al., 2005) which still remains as a widely prevalent zoonotic disease of public health and economic importance to livestock owners as well as to a nation (Schelling et al., 2003). The common serological tests however still widely used for diagnosis of *Brucella* are Rose Bengal plate test (RBPT) based on agglutination of colored particulate antigen (killed *Brucella* organisms) by the antibodies present in the patient's serum and Standard tube agglutination test (STAT) (Chachra et al., 2009). Besides these, there are various serological tests like complement fixation test (CFT), enzyme linked immunosorbant assay (ELISA) which are promptly used today though ELISA

has claimed to be a good screening test whether used alone or in combination with the RBPT (Jacques et al., 1998). Generally RBPT has been considered to be less sensitive than other tests like STAT, CFT and ELISA but is still widely used test for screening of brucellosis in many specified countries. However, serological cross-reactions have been observed between *Brucella* sp. and other bacteria such as *E. coli*, *Salmonella*, *Staphylococcus*, etc. (Otto et al., 2000). Thus to overcome the possibility of wrong diagnosis in the fields and livestock farms, the present study was undertaken to further confirm the RBPT and STAT tests when performed alone by ELISA test for serodiagnosis of brucellosis and prevent the heavy revenue losses occurring to the livestock owners and its comparison to conventional serological tests. At present, fewer studies have been conducted based on the comparative

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sensitivity of the 3 tests, (Chachra et al., 2009) particularly the negative tests exhibited by RBPT and STAT when used alone need to be further re-confirmed by tests like ELISA so as to avoid any possibility of wrong diagnosis owing to false negative reactions by these tests. Hence, the present study was therefore, undertaken to diagnose this aspect of serodiagnosis of brucellosis.

MATERIALS AND METHODS

During the course of study, 56 serum samples from suspected cattles and 5 from brucellosis-free non-vaccinated cattle herd were collected from various livestock farms in Mathura district of Western Uttar Pradesh, of which 16 serum samples included in this study found to be positive were subjected to all the three serological tests that is, RBPT (Alton et al., 1975), STAT and Dot-ELISA respectively. Samples observed positive by all the three tests were taken as positive controls. Also the samples exhibiting negative results by RBPT and / or STAT but found to be positive by Dot-ELISA test were recorded as positive as ELISA has been acclaimed of good screener when used alone. A total 10 numbers of cervical swabs were further collected randomly from suspected cattles and subjected for culturing of *Brucella* organisms on Brucella Selective agar (HI-MEDIA, India) under microaerophilic condition at 37°C for 5 to 7 days. Bacterial identification was done on the basis of morphological, cultural and biochemical tests (Cruickshank et al., 1975). The disk diffusion test for sensitivity to different antibiotics was conducted as per Bauer et al. (1966) Cruickshank et al. (1975). Positive test was indicated by zones of inhibition which were measured by using the zone size interpretative tables provided by the manufacturer of the discs. The antibiotic disks used were of HI-MEDIA Laboratories, Mumbai, India, consisting of the following- Ampicillin (10 µg), Trimethoprim (1.25 µg), Ciprofloxacin (5 µg), Gentamicin (30 µg), Tetracycline (30 µg), Doxycycline hydrochloride (30 µg) Norfloxacin (10 µg), Bacitracin (10 µg).

RESULTS

From the present study it was observed that out of 56 serum samples collected from cattles suspected of brucellosis, 7 (43.75%) samples were found to be positive while other 9 (56.25%) were found to be negative by RBPT. Hence, only 2 (12.5%) out of 16 samples were positive by STAT and all the 7 RBPT positive samples (100%) exhibited negative results by STAT. However, it was observed that all the 16 samples (100%) which earlier revealed to be negative for RBPT and STAT exhibited positive results with Dot- ELISA. Also further it was observed that among the RBPT negative samples, 5

out of 7 (71.42%) showed negative results by STAT while 2 out of 7 (28.57%) showed a high titer of 1:320 and 1:640. All the serum samples from normal healthy cattle were negative by RBPT, STAT and Dot-ELISA. Two isolates of *B. abortus* colonies were obtained at day 5. Beside these 2 *B. abortus* isolates, 4 isolates of *Staphylococcus* and 3 isolates of *E. coli* were also obtained. An overall analysis of drug sensitivity test revealed that the 2 isolates of *B. abortus* were sensitive to tetracycline, streptomycin, norfloxacin and ciprofloxacin. The *E. coli* isolates were highly sensitive to ciprofloxacin, norfloxacin and gentamicin and resistant to ampicillin. *Staphylococcus* isolates were highly sensitive to bacitracin, ampicillin, gentamicin, and resistant to tetracycline and trimethoprim.

DISCUSSION

The isolation of *B. abortus* from cervical swab samples was vital in the confirmation of *Brucella* infection and epidemiological evaluation of the herd (Ewalt et al., 1989). High titer of 1:320 and 1:640 was observed in the present investigation. Workers like Vaishali et al. (2005) and Rathore et al. (2002) have also reported a significantly higher titer and seroprevalence rate which is in concordance with our study. Several serological tests need to be used for surveillance study to determine variable sensitivity and specificity rate as single individual test is not enough to confirm the vaccinated and out bred animals due to interference with serological tests leading to diagnostic errors (Neilson, 2002). Present study has revealed that with the advancement in newer diagnostic techniques like CFT, ELISA and PCR though the diagnosis of brucellosis has become easier but practical application of these techniques in field service is difficult and not economical. Though in comparison to ELISA sensitive test, RBPT and STAT are less sensitive but these tests still have their own importance and prove to be effective for screening of brucellosis in many countries. However, the findings of the present study are in agreement with those stated by Chopra et al. (2009) and Chachra et al. (2009), who described RBPT to be more reliable and useful than STAT for screening of brucellosis although a combination of RBPT and ELISA would be more useful in cases of samples found negative either by RBPT or STAT used singly or in combination. The frequencies of bacterial strains resistant to antimicrobial agents have increased dramatically in the environment as a consequence of the wide spread use of drugs (Kruse and Sorum, 1994). A significant public health concern and the possibility of transfer of resistant genes between bacteria in the natural habitats have attracted attention (Sharma et al., 2010). In the present study, antimicrobial susceptibility pattern against 8 antibiotics was studied for 2 isolates of *B. abortus*, 4 isolates of *Staphylococcus* and 3 isolates of *E. coli*.

The results were interpreted according to the diameter of the zone of inhibition as per the manufacturer's (HI-MEDIA Laboratories, Mumbai) instructions. Differences in the frequency of resistance observed may very well be related to the source of isolates and the frequency and type of antimicrobial agents prescribed for treating infections in different geographical areas. Brucellosis is considered to be worldwide economically devastating diseases, causing great losses and health problems in both urban and rural populations (Taleski et al., 2002). Thus eradication of the disease is a necessary step to control the infection being passed to humans (OIE, 2008).

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