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Seroprevalence of brucellosis among livestock in Hyderabad, India

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Abstract
Brucellosis is a highly infectious zoonotic disease and an economically important infection of humans and livestock with a worldwide distribution. It is a major veterinary and human public health problem in most parts of the world. The main pathogenic species worldwide are *B. abortus*, responsible for bovine brucellosis; *B. melitensis*, the main etiologic agent of ovine and caprine brucellosis; and *B. suis*, responsible for swine brucellosis. Hence, in the present study we have aimed at finding out the seroprevalence of Brucellosis in and around Hyderabad region. In the current study, a total of 105 (unvaccinated against Brucellosis) serum samples were collected from breeding bulls, calves more than 6 months of age, dams with a history of at least one abortion in dairy farms, and from animals presented at clinics suspected of Brucellosis. All the serum samples were subjected to Rose Bengal Plate Agglutination Test (RBPT), and Standard Tube Agglutination Test (STAT). The seroprevalence of brucellosis in the present study by RBPT, STAT was found to be 42% and 60%, respectively. Indirect ELISA when performed on samples collected from unorganized farms (N=33) revealed that 75.75% were positive among the sera samples tested and proved to be equally sensitive with STAT. Out of the 17 sera samples from breeding bulls, 6 samples were found to be seropositive for Brucellosis. Milk samples (28) were also collected from the above herds and tested for Brucellosis by Abortus Bang Ring (ABR) test, out of which 20 were found positive. Moreover, 7 workers in the tested herds were also found to be seropositive for Brucellosis. The present study thus reveals high incidence of *Brucella spp* antibodies in the herds which could be due to presence of infected bulls, carrier dams, and or poor management practices in herds. These findings suggest strict management practices like vaccination frequent screening and prompt culling of infected animals for effective disease control in herds.

Keywords: brucellosis, milk ring test, rose bengal plate agglutination test, standard tube agglutination test, indirect ELISA.

Introduction
Brucellosis is one of the world's major zoonotic disease, caused by bacteria of the genus *Brucella*. This world's most widespread zoonosis mainly affects cattle, sheep, and goats, pigs leading to abortions, infertility, and low milk yields. It is a highly contagious disease of dairy animals and humans in many parts of the world, including India causing significant morbidity and enormous economic losses (Singh *et al.*, 2002; McDermott *et al.*, 2015) [7, 3]. The disease causes abortions in the last trimester of pregnancy, premature births followed by retention of placenta, metritis, decreased milk production and lameness as a common sequel to infection in dairy animals (Megid *et al.*, 2010) [4]. Humans acquire Brucellosis from direct contact with livestock or from drinking unpasteurized milk. *Brucella spp*. is also considered as the most common laboratory-acquired pathogens. Several serological tests are being widely used for diagnosis of *Brucella* such as Rose Bengal plate test (RBPT), Standard tube agglutination test (STAT), complement fixation test (CFT), and enzyme linked immunosorbant assay (ELISA) Raheela Aktar *et al.* (2010). Besides these, polymerase chain reaction (PCR) based identification and typing, fluorescence polarization assays (FPA) are also important diagnostic tools (Silva *et al.* (2000; Tittarelli *et al.* 2006) [6, 8]. The worldwide economic losses due to brucellosis are extensive. Although a number of successful vaccines are being used for immunization of animals no satisfactory vaccine against human brucellosis is available yet (Yasmin Bano and Sajad Ahmad Lone 2015) [15].

The varied symptoms which brucellosis presents make it troublesome for clinical diagnosis. The conventional diagnosis is microbiological confirmation by means of isolation of bacteria from the blood or from other body fluids. The isolation rate of *Brucella* is poor due to its slow growth rate, quantity of circulating viable bacteria, culture medium, and blood culture

techniques employed as well as presence of antibiotics that inhibits growth (Yagupsky 1999). Blood culture provides definite proof of brucellosis (Al Dahouk *et al.*, 2002), but may not provide a positive result for all clinical cases even under ideal conditions. The demonstration of antibodies generated against *Brucella* by serological tests remains a viable alternative to culture, and several serological tests like Standard Tube Agglutination Test (STAT) and Rose Bengal Plate Agglutination Test (RBPT) are the most popular serological tests used in the field for the diagnosis of brucellosis (Magee, 1980) [2]. Periodical surveillance of serological scenario helps in understanding the disease prevalence in different regions. Hence, in the present study, we have aimed at looking for the seroprevalence of brucellosis in dairy farms in and around Hyderabad, a southern region of India.

Materials and Methods

Sample Collection

Milk

The udder was thoroughly washed and cleaned with potassium permanganate solution (1:1000) and dried with clean cloth. Teat openings were disinfected with 70% of ethyl alcohol. After discarding few drops of milk, approximately 10 ml from each quarter was collected in two sets of sterile screw capped plastic vials and transported in ice pack to the laboratory. One set was used for cultural isolation and another was used for Milk Ring Test (MRT).

Blood

About 9 ml of blood was collected aseptically from the jugular vein of individual animal in a vaccutainer with sodium citrate anticoagulant (BD vaccutainer) and transported to the laboratory on ice.

Milk Ring Test (MRT)

The Abortus Bang Ring Antigen (ABR Antigen) obtained from Bangalore College of veterinary science was used for the test. The test was performed according to the manufacturer's guidelines. MRT was performed on individual milk samples. Antigen and milk samples were brought to the room temperature prior to performing the test. About 30-50 µl of antigen was added to the 2 ml of milk in a narrow test tube and mixed thoroughly. The tubes then were incubated at 37°C for 1 h together with positive and negative working standards. A strong positive reaction was indicated by formation of dark blue ring above a white milk column. The test was considered to be negative if the blue colour is the underlying milk and doesn't exceed that of the cream layer.

Serological Tests

Rose Bengal Plate Test

The Rose Bengal Plate Test antigen obtained from the Indian Veterinary Research Institute (I.V.R.I.), Izatnagar, Uttara Pradesh was used for the test. One drop (0.03 ml) of serum was taken on a glass slide by micropipette. The antigen bottle was shaken well to ensure homogenous suspension and then one drop (0.03 ml) of the antigen was added to the serum. The antigen and serum were mixed thoroughly with the spreader and then the slide is slightly tilted for 4-5 times. The result was read immediately after four min. definite clumping/agglutination was considered as positive reaction, whereas no clumping/agglutination were considered as negative.

Standard Tube Agglutination Test

The antigen obtained from the I.V.R.I., Izatnagar was used for the test. Five agglutination tubes were placed in a rack. 0.8 ml of 0.5 % phenol saline was taken in a first tube and 0.5 ml in rest of the tubes. 0.2 ml of serum was added in the first tube, mixed well and transferred 0.5 ml of diluted serum to the second tube. The process was continued up to the fifth tube and 0.5 ml was discarded from the last tube after mixing. 0.5 ml antigen was added to each tube and mixed thoroughly. This provided a final dilution of 1:10, 1:20, 1:40, 1:80 and 1:160 and so on. Considering the special significance of 50 per cent end point, a control tube was set up to simulate 50 per cent clearing by mixing 0.5 ml antigen with 1.5 ml of 0.5 % phenol saline in an agglutination tube. All the tubes were incubated at 37ºC for 20 h before result was read. The degree of agglutination was judged by opacity of the supernatant fluid. The highest serum dilution showing 50 per cent or more agglutination (50 % clearing) was considered as the titre of the serum. The titre so obtained was expressed in unit system by doubling of the serum titre as International Unit (I.U.) per ml of serum. 40 I.U. per ml or above was considered positive for brucellosis in cattle as well as buffaloes.

Elisa

Brucella abortus Antibody Test Kit, (Brucellose Serum) was procured from IDEXX CHEKIT., USA and the test was performed as per manufacturer's guidelines. The test was considered valid when mean OD of the negative control does not exceed 0.300 and the OD of the positive control does not exceed 2.000. The difference between the positive and negative control must be ≥ 0.300.

Observation of Result

SP Ratio was calculated as follows: SP = [(Sample OD - Mean NC) ÷ (Mean PC - Mean NC)] x 100 Where: SP = Sample/Positive Control Ratio Sample, OD = OD value of sample, MeanNC = Mean OD value of Negative, Mean PC = Mean OD value of Positive Control. Samples producing SP ratio <80% were considered negative, whereas with SP ratios $\geq$ 80% were considered positive.

Results and Discussion

A total of 105 serum samples were screened for the presence of *Brucella* antibodies by RBPT and STAT. Results showed that STAT had higher sensitivity (60%) when compared to that of the RBPT (42%). Furthermore, it was found that a higher prevalence (75.75%) was seen among the unorganized farms followed by clinical cases (74.28%) and organized farms (32.43%) (Table1). 6 out of the 17 breeding bulls tested were found to be seropositive for Brucella sp. Intrestingly, 7 out of 19 people working in the farm were also found to be seropositive for breucellosis by STAT. When the sensitivity of the STAT was compared with the iELISA, our findings showed that STAT and I ELISA are equally sensitive (Table 1).

When milk ring test was performed on 28 milk samples collected from the unorganized farms, 20 (71.4%) (Fig1) were found to be positive. This test serves as an easier test as simple milk sample can be used for the detection of the pathogen. Findings from our study strive for an urgent need for the prophylactic approach for the prevention of spread of this zoonotically important disease to animals and humans. Though the existence of RB51 calfhood vaccination seems to be in availability, our interaction with both the organized and

the unorganised farms led to a very sadfull conclusion that none of the animals tested in the current study were vaccinated. This situation suggests that though we are able to detect at an earlier stage, the lack of awareness of vacination among the farmers makes the situation even more worse, all of which can be overcome by educating the farmers about the importance of vaccination and its zoonotic importance.

Table1: Prevalence of Brucellosis

TESTS	Organized Farms(N=37)	Unorganized Farms(N=33)	Clinical Samples(N=35)
Rose Bengal Plate Agglutination Test	5(13.51%)	23(69.69%)	17(48.57%)
Standard Tube Agglutination Test	12(32.43%)	25(75.75%)	26(74.28%)
IELISA	-	25(75.75%)	-

Unorganized farms showed the highest prevalence of brucellosis, followed by clinical samples and organized farms. Sensitivity was found to be similar for the tube agglutination test and I ELISA when performed on samples collected from unorganized farms.

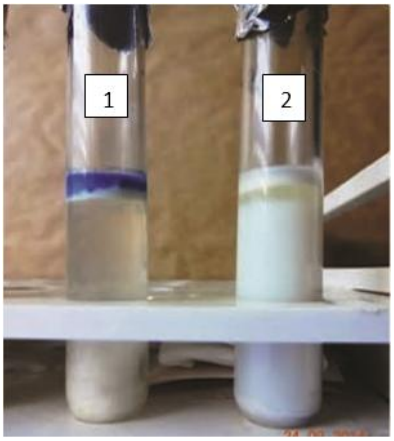

Fig 1: Milk Ring Test for Detection of *Brucella* Sp

Tube1 shows a dark blue ring above the white milk column indicating it as positive for Milk Ring Test. Tube2 shows that the blue column underlying of the milk is homogenously dispersed which is an indicative for negative Milk Ring Test.

Conclusion

Brucellosis was found to be widely prevalent in livestock around Hyderabad, with major prevalence in the unorganized farms. Indirect ELISA and STAT were found to have equal sensitivity on the limited number of samples tested. Though slide agglutination was found to be least sensitive it was highly convenient in its ease of performance. Prevalence of brucellosis in farm associated workers indicate the high risk of contracting the infection. This finding reflects the seriousness of the problem and hence control measures have to be taken upon urgent basis. The epidemiology of brucellosis is complex and criteria other than test results are needed in order to guarantee the success of an eradication program. The S19 and Rev. 1 vaccines are still the cornerstones of control and eradication programs. In the absence of a human brucellosis vaccine, prevention of human brucellosis depends on the control of the disease in animals. Serology is the first tool in detecting subclinical infections and hence continuous and periodical surveillance shall help in better understanding of the disease prevalence for efficient planning of control and treatment strategies.

Conflict Of Interest

The authors declare that they have no competing interests.

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