JKIMSU, (2012), Vol. 1 (1) 55-60
ISSN 2231-4261

ORIGINAL ARTICLE

Seroprevalence of Brucellosis among Blood Donors of Satara District, Maharashtra

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Abstract:

Backgroind: In endemic area for brucellosis background levels of antibodies are found in healthy populations. *Aims & Objectives:* The aim of this study was to determine the seroprevalence of anti-brucella antibodies and base line titres among the blood donors. *Materials and Methods:* 321 blood samples were collected from blood donors. Serological tests - Rose Bengal Plate Agglutination Test (RBPT), Standard Tube Agglutination Test (STAT) and 2-Mercaptoethanol Agglutination Test (2ME), were carried out on all the blood samples. *Results:* **S**eroprevalence of brucellosis in blood donors was 5.91%, 3.11 % and 0.62% by RBPT, STAT and 2ME respectively. History of animal exposure and occasional raw milk ingestion was observed in 137 individuals residing in rural area. *Conclusion:* Brucellosis might be common in rural population who come in contact with infected animals and or consume raw milk. For the diagnosis of brucellosis RBPT test should be used only for screening purpose and the positive results should be confirmed by STAT and 2ME tests. Titres of 160 IU and above for STAT and 80 IU and above for 2ME test can be considered significant for diagnostic purpose.

Key Words: Brucellosis, Rose Bengal Plate Agglutination Test, Standard Tube Agglutination Test, 2Mercaptoethanol Test

Introduction:

Brucellosis is a major zoonosis in the developing countries including India. It is acquired by direct or indirect contact with infected animals or their products. It manifests as an acute, sub-acute or chronic disease involving any organ or organ systems. Signs and symptoms of brucellosis are protean in nature and clinical diagnosis is often difficult. Isolation of *Brucellae* from clinical specimens remains the gold standard in diagnosis of the disease [1, 2]. As culture facilities are not available in most of the laboratories, the diagnosis is mainly based on serological tests. Commonly employed tests include Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT) and 2Mercaptoethanol Test (2ME) [2]. These agglutination tests are based on the detection of antibodies against smooth lipopolysaccharide antigen of *Brucella abortus* strain 99.

The RBPT is used as a screening test and positive results are confirmed by the STAT [3, 4]. To exclude the cross-reacting IgM

antibodies and measure *Brucella* specific IgG antibodies, 2ME test is done [2]. However interpretation of the serological tests is often difficult in endemic areas where a high proportion of the population have antibodies against *Brucellae* [5]. Hence estimation of normal baseline titers of *Brucella* agglutinins in healthy individuals residing in endemic areas and the cutoff values are necessary for the serodiagnosis of brucellosis.

Since there are no documented reports on human brucellosis from Satara district of western Maharashtra, a study was undertaken at Krishna Institute of Medical Sciences, Karad, to determine the baseline antibody titers of healthy individuals in this area.

Materials and Methods:

A total of 321 blood samples were collected from healthy blood donors, through camps, organized by Blood Bank Department of KIMS, Karad and private blood banks at Satara. The age of the donors ranged between 18 to 50 years. Among the blood donors 319 were males and 2 females. Health screening of the donors was done and individuals positive for Hepatitis B, Syphilis and HIV were excluded. About 2 ml of blood was taken from the tubes of each bag, the serum was separated, labeled and stored at - 20° C in a sterile screw capped bottle until further processing. The samples were analyzed by RBPT, STAT and 2ME tests. The antigens for the tests were procured from Division of Biological Products, Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh.

Rose Bengal Plate Test (RBPT) [6]:

It is a rapid agglutination test commonly employed as a screening test [7]. RBPT antigen is a suspension of Rose Bengal stained pure smooth culture of *Brucella abortus*, strain 99 in buffer at PH 3.6 in phenol saline.

Procedure: The test was performed according to the manufacturer's instructions. The sera and the antigen were brought to the room temperature. The antigen was shaken well before use to ensure homogenization of suspension. In brief equal quantities (0.03 ml) of serum and the antigen were placed on white enameled slide, mixed and spread to an area of about 2.5 cm diameter. The plate was manually rotated for 4 minutes and read immediately. A known positive and negative control sera were included with each set of test.

Interpretation: The test was observed for agglutination in bright light. Any degree of agglutination was taken as positive and no agglutination was taken as negative [8].

Standard Tube Agglutination Test (STAT) [9]:

This is the most widely used test for the detection of *Brucella* antibodies in humans [10]. It measures the total quantity of agglutinating antibodies i.e. IgM and IgG. The antigen used for agglutination test is an unstained suspension of pure, smooth *Brucella abortus* strain 99 in phenol saline [9].

Procedure: Doubling dilution of the test serum was done with 0.5% phenol saline starting from 1:10 to 1:1280. Equal amount of (0.5 ml) *B. abortus* plain antigen was added to each tube. The contents of the tube were mixed and the tubes were incubated at 37^0 C for 20 hours ± 1hour in a water bath. A set of 5 antigen control

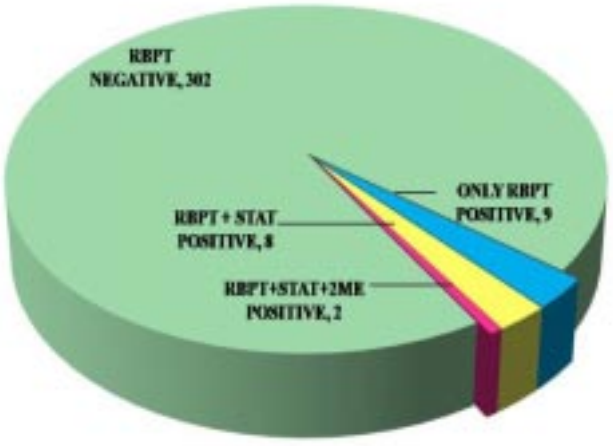
tubes with opacity corresponding to 0, 25, 50, 75 and 100 percent in 0.5% phenol saline were included for comparing the result of the test samples. A known positive and negative control sera also were included along with the samples.

Interpretation: The tubes of test series were compared with antigen control tubes for degree of opacity of the supernatant fluid. The maximum dilution that exhibited 50% agglutination was considered as the end point of serum activity and recorded as the titer of antibodies present in the individual against *Brucella*.

2-Mercaptoethanol Test (2-ME) [11]:

The 2-mercaptoethanol agglutination test was performed similar to the standard tube agglutination test, except for the addition of 2ME in place of phenol saline to a final concentration of 0.05M in each tube. The 2ME inactivates IgM antibodies by disrupting disulfide bonds, hence permitting agglutination by IgG antibodies that are resistant to 2-ME [11].

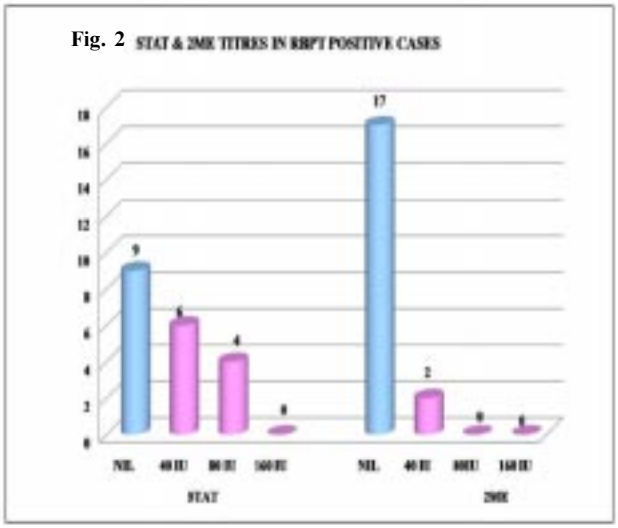
Fig. 1 COMPARATIVE RESULTS OF RBPT, STAT & 2ME TESTS



Results:

Of the 321 blood samples screened, 19 were positive by RBPT (5.91 %). Of these 19 RBPT positive samples 10 showed agglutination by STAT and 2 by 2-ME as shown in (Fig.1). Amongst the 10 STAT positives, 06 showed agglutination at dilution 40 IU/ml and 04 at 80 IU/ml with Mean ± SD of 56 ± 20.65. No sample was found positive at dilutions of 160 IU/ml and above. Two samples showed agglutination with 2-ME test at 40 IU/ml dilution (Fig. 2).

Fig. 2 STAT & 2ME TITRES IN RBPT POSITIVE CASES



The age of the donors ranged between 18 to 50 years with Mean age and SD being 26.88 ±5.73. There was male preponderance with only 2 female donors.

Of the 321 blood donors 42.67 % resided in rural area and had the history of animal exposure at home or in neighborhood and had consumed raw milk infrequently.

Discussion:

Definitive diagnosis of human Brucellosis is made by isolation of *Brucellae* from clinical specimens. Since *Brucella* culture is cumbersome, takes longer time, hazardous and most of the laboratories lack culture facilities, serological tests play a major role in the diagnosis of the disease. Commonly employed tests include RBPT, STAT and 2ME tests. In most of the private set ups brucellosis is generally diagnosed based on the results of RBPT or a similar test without confirmation by STAT and 2ME tests. RBPT being sensitive should be used only as a screening test and STAT as confirmatory. However interpretation of the serological tests is often difficult in endemic areas where a high proportion of the population have antibodies against *Brucella* [12]. Also cross reacting antibodies seen in *Francisella tularensis, Esch. coli* O:116 and O:157, *Salmonella urbana* O:30*, Yersinia enterocolitica* O:9, *Vibrio cholera* infections, pose a problem in the interpretation [13,14]. Estimation of baseline titers of *Brucella* agglutinins in healthy individuals residing in an area and cutoff values are necessary for diagnosis of brucellosis.

In our study, of the 321 samples screened, 19 (5.91%) samples showed positive reaction by RBPT method. Since the base line titre of 80 IU by STAT was demonstrated in healthy individuals, titres $\geq$ 160 IU should be considered indicative of infection which is one dilution higher than that mentioned in the IVRI literature [9]. Two samples showed positive reaction in 2ME test at the dilution of 40 IU, hence titres of $\geq$ 80 IU could be considered significant.

In the study by Vaishnavi et al. 16.8% of blood donor population from Chandigarh showed *Brucella* agglutinins at 40 IU dilution, where as a study by Nagarathna et al. showed very low positivity (1.1%) by STAT [15,16]. In the present study low titres in STAT up to 80 IU were seen in 52.63% of RBPT positive donors. The absence of symptoms in the population agrees with the observed titers of antibodies. The lower titres in these individuals could be due to previous subclinical exposure to the *Brucella* organisms as most of them were from semi rural background where animal exposure and raw milk ingestion are very common.

As with other serological tests, the sensitivity and specificity of the confirmatory agglutination tests for brucellosis depend on the cut-off value used and on the background level of antibodies in the healthy population. In the regions where brucellosis is endemic, the specificity and positive predictive value of a test result can be increased by selecting higher cut-off value. But if higher cut-off value is selected the sensitivity decreases, as some patients with acute/ persisting / relapsing brucellosis may have low antibody levels and chances of false negativity increases. Hence lower cut off titres should be selected. Also consistent clinical features, epidemiological history like animal exposure, consumption of raw milk and milk products should be taken into consideration before interpretation of the results. 42.67% of the population revealed contact with animals and occasional raw milk ingestion.

To conclude a prevalence rate of 5.92% by

RBPT was found amongst the blood donors of Satara District of Western Maharashtra, India. RBPT results should be confirmed by STAT and 2ME tests. Based on the results of this study, we recommend titres of 160 IU and above for STAT and 80 IU and above for 2ME test be considered significant along with consistent clinical features for this region of Maharashtra.

Acknowledgements:

We acknowledge the help of Late Dr. Phatak, blood bank officer for providing the samples and the data.

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