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Detection of Seroprevalence and Baseline Titre of Brucella Agglutinins in Blood Donors of Dhule District

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Abstract

Introduction: Brucellosis is a major zoonosis in the developing countries including India. The reported incidence of human brucellosis in endemic areas varies widely, from <0.01 to >200 per lakh population. Despite being endemic in many developing countries, brucellosis remains under-diagnosed and under-reported. Interpretation of the serological tests is often difficult in endemic areas due to presence of antibodies against *Brucella in* high proportion of the population . 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. *AIM:* To determine the baseline titre of brucella agglutinins, amongst apparently healthy blood donors in Dhule, Maharashtra, India. *Materials and Methods:* A total of 480 serum samples were analyzed by RBPT and STAT method for detection of *Brucella* Agglutinins over period of 6 months from July 2016 to December 2016. These agglutination tests were performed according to manufacturer's guidelines by using commercial kits containing stained *Brucella abortus* Antigen suspension. *Result:* Of these 480 samples 32 (6.7%) were positive for *Brucella* agglutinins by RBPT. Of these 32 RBPT positive samples 22(4.6%) showed agglutination by STAT. Amongst 22 STAT positive samples 15 samples were found positive for *Brucella* agglutinins at 1:40 and 7 samples were positive at 1:80. *Conclusion:* As RBPT is very sensitive test and its results should be confirmed by STAT. Since the base line titre of 1:80 by STAT was demonstrated in healthy individuals, titres 1:160 and above should be considered significant along with consistent clinical features.

Keywords: Brucellosis; Baseline Titre; RBPT; STAT; Agglutinins.

Introduction

Brucellosis is one of the most important bacterial zoonotic disease caused by various species of genus *Brucella,* a Gram negative, facultative intracellular, coccobacillary organism. It is acquired by direct or indirect contact with infected animals or animal products and through the consumptions of unpasteurised milk and dairy products [1]. In addition, it is an occupational hazard to persons involved in certain professions such as farming, ranchers, veterinarians and slaughterhouse [2, 3]. Brucellosis is also known by Malta fever, Mediterranean fever, Gibraltar or Rock fever, and undulant fever often relating to localities in which it was particularly prevalent [4]. It continues to be a public health hazard due to expansion of animal industries and urbanization, and the lack of hygienic measures in animal husbandry and in food handling [5]. Brucellosis has major economic ramifications due to time lost by patients from normal daily activities and losses in animal production [6]. Human brucellosis is endemic in countries like Mediterranean basin, the Arabian Gulf, the Indian subcontinent and parts of Mexico as well as Central and South America [7]. The reported incidence of human brucellosis worldwide in endemic areas varies widely, from <0.01 to >200 per lakh population .The true incidence however, is unknown and it has been estimated that it may be 25 times higher than the reported incidence due to

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misdiagnosis and underreporting [8]. Also it remains a constant threat to humans due to lack of facilities for effective diagnosis and treatment in certain regions.

Human brucellosis manifests as an acute, subacute and chronic disease involving any organ or organ system with sometimes serious complications. The disease usually remains undiagnosed or misdiagnosed due to no specific signs. Though Brucellosis is diagnosed in the laboratory by using various techniques , the timely and accurate diagnosis of human brucellosis continues to challenge clinicians because of its non-specific clinical features, slow growth rate in blood cultures, and the complexity of its serodiagnosis.

In most laboratories serodiagnosis is usually done by Rose Bengal Plate Test (RBPT) which is a screening test and positive results are confirmed by the Standard Tube Agglutination Test (STAT) [7].

Interpretation of the serological tests is often difficult in endemic areas due to presence of antibodies against *Brucella* in high proportion of the population . 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[9].

Since there are no documented reports on human brucellosis from Dhule district of North Maharashtra, a study is undertaken at to determine the baseline antibody titers for *Brucella* in healthy individuals in this area.

Material and Methods

This study was conducted over a period of 6 months from July 2016 to December 2016 in the department of microbiology. A total of 486 samples were collected from healthy blood donors, attending blood bank department as well as through camps organized by Blood Bank Department.

A detailed pre-donation questionnaires for health screening were included in the donor registration form. Blood samples were collected and serum separated. After separating serum compulsory serological tests were performed for Transfusion Transmissible Infections and the samples positive for Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Human Immunodeficiency Virus (HIV), Syphilis and malaria infections were excluded.

Serum samples were analyzed by RBPT and STAT method for detection of *Brucella* Agglutinins. These agglutination tests were performed according to manufacturer's guidelines by using commercial kits containing stained *Brucella abortus* Antigen suspension, procured from Tulip Diagnostics LTD. RBPT was used as screening test and positive results showing visible agglutination were confirmed by STAT. STAT is the most wildely used test for the detection of *Brucella* Antibodies in humans. Doubling dilution of test serum sample was done with isotonic saline from 1:10 to 1:1280 . A drop of *B. abortus* antigen suspension was added to each tube. The contents of the tube were mixed and tubes were incubated at 37^0C overnight (approximately 18 hours). The titre of the sample was the highest dilution of serum sample that gave a visible agglutination.

Results

Of the 486 samples collected from blood donors 480 samples screened for detection of *Brucella* agglutinins as 5 samples were positive for HBsAg and 1 sample was reactive for HIV Antibody. Of these 480 samples 32 (6.7%) were positive for *Brucella* agglutinins by RBPT. Of these 32 RBPT positive samples 22(4.6%) showed agglutination by STAT (Table 1). Amongst 22 STAT positive samples 15 samples were found positive for *Brucella* agglutinins at 1:40 and 7 samples were positive at 1:80. No sample was found positive at dilution of 1:160 and above (Table 2)

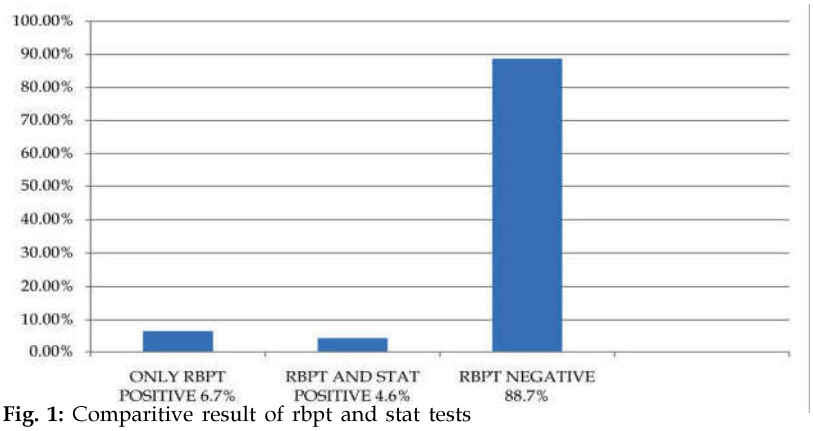

Fig. 1: Comparitive result of rbpt and stat tests

Table 1: Number of positive samples for *Brucella* agglutinins

Total Samples	RBPT Positive	STAT Positive
480	32(6.7%)	22(4.6%)

Table 2: Titre of *Brucella* agglutinins by STAT method

Method	Positive Samples	TITRE 1:40	TITRE 1:80
STAT	22	15	7

Discussion

Dhule district has predominantly agriculture based economy; so milk production , cattle sheep rearing practices are common in rural areas. Incidence of brucellosis is higher in rural areas where agriculture is the main occupation . Non occupational exposure may also occur due to consumption of raw milk or milk products or handling of contaminated meat. Also the disease is initially confused with tuberculosis, malaria or typhoid and many cases go unrecognized, undiagnosed or unreported [10].

Interpretation of the serological tests is often difficult in endemic areas due to presence of antibodies against *Brucella* in high proportion of the population and also due to cross reactivity with other bacteria such as *Yersinia enterocolitica* O:9, Salmonella urbana group N, *Vibrio cholerae, Escheichia coli* O:157 etc [11,12]

Several studies have been performed to determine seroprevalence of Brucellosis among slaughterhouse workers,veterinary personnel,dairy workers but there is paucity of published literature on *Brucella* agglutinins in blood donors . To the best of our knowledge there are no documented reports to determine the base line antibody titer of healthy individuals that is blood donors in this area.

In our study out of 480 samples screened for *Brucella* agglutinins 32(6.7%) shown positive reaction by RBPT method and 22(4.6%) shown positive reaction by STAT method. Reported incidence and prevalence varies from country to country and state to state. There is reported prevalence of 3.6% by Torres-Padilla et al [13] and 0.057% by Rabbani Khorasgani et al [14] in blood donors from Mexico and Boushehr province of Iran respectively. Masoomeh Sofian et al also reported a very low prevalence of 0.33% in healthy individuals from central province of Iran[15]. A range of prevalence of 1-3.2% from Turkey and 4.5% from Saudi Arabia, has been recorded[16]. In India seroprevalence of 16.8% by Vaishnavi et al [17] and 3.11% has been reported by Smita Mangalgi et al [9] from Chandigarh and Satara respectively. A very low prevalence of 1.1% has been reported by S.Nagarathna et al. from Manipal [8].

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 [9]. Since the base line titre of 80 IU by STAT was demonstrated in healthy individuals, titres > 160 IU should be considered indicative of infection which is only one dilution higher than the base line titre.

Conclusion

As RBPT is very sensitive test its results should be confirmed by STAT. Since the base line titre of 1:80 by STAT was demonstrated in healthy individuals, titres 1:160 and above should be considered significant along with consistent clinical features. Seroprevalence of Brucella agglutinins is low (4.6%) in this study. However in order to assess status of the disease in this area, further wide prospective study on a large cluster of samples on long-term basis, needs to be conducted .

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