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Seroprevalence of Human Brucellosis among Patients Attending a Tertiary Care Hospital

Jagadevi, D. S. Shubha* and K. S. Sudhindra

Department of Microbiology, Basaveshwara Medical College and Hospital, Chitradurga -577502, Karnataka, India

**Corresponding author*

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A B S T R A C T

Brucellosis is zoonotic disease, more contagious and chronic disease with absence of specific clinical features which is difficult to diagnose clinically. Important test routinely used for diagnosing this disease is serological test although isolation of organism and Polymerase chain reaction used for definitive diagnosis. The present study was done to know the seroprevalence of human brucellosis in and around Chitradurga. Out of 154 Blood samples referred by clinicians for serodiagnosis of brucellosis to Microbiology laboratory 69 samples were included for evaluation of Brucella. Demographic, clinical and other required data of patients extracted from medical case records. Serum was separated from samples and Rose Bengal plate test (RBPT) carried out by both qualitative and semiquantitative method. The overall seroprevalence of brucellosis in the study was 11.59%. Disease was more commonly seen among 23-39 years age group. Slightly higher cases identifies among males than females. Fever with no apparent clinical features was present in 8/13(61.5%) patients. 69.2% cases had history of animal contact and living in rural areas. RBPT semiquantitative test Titres of 1:2 were detected in majority (38.4%) of samples. Conclusion: Though culture is gold standard, serology remains simple, cost effective, best diagnostic method for Brucellosis. Clinicians should have high degree of suspicion of brucellosis with history of animal contact, or living in rural areas. Use of the rose Bengal test as the sole technique for the diagnosis of brucellosis can be considered.

Introduction

Brucellosis is a disease of domestic and wild animals that can be transmitted to humans (zoonosis). The occurrence in humans is closely related to their prevalence in various domestic animals (sheep, goat, cattle). The disease exists worldwide, particularly in the Mediterranean zone, Eastern Europe, the Indian subcontinent, and in parts of Mexico and South America. Brucellosis is a recognized public health problem and one of the major causes of morbidity. It is recognized as a re-emerging neglected disease of considerable economic and social importance (Annapurna S. Agasthya *et al.*, 2012; WHO, 2006). Human brucellosis (undulant fever) is a common febrile

illness caused by Gram-negative coccobacillus belonging to genus Brucella, which currently includes 12 species, of which only four are particularly pathogenic for humans: *Brucella abortus*, *Brucella canis*, *Brucella suis* and *Brucella melitensis*. *Brucella melitensis* and *Brucella suis* are potential agents of biological terrorism (Smita S. Mangalgi *et al.,* 2016). Brucellosis in humans is acquired by direct contact with infected animal tissues, blood, urine, vaginal discharge or placenta with abraded skin or mucosa or conjunctiva of men. By ingestion of contaminated raw milk, meat or dairy products or rarely vegetables or water contaminated with animal excreta. By inhalation of infectious aerosols or dust especially in occupationally exposed persons.

Common symptoms of brucellosis are fever, chills, sweats, weakness, loss of weight and abdominal pains, but it is not rare for the disease to present as respiratory illness, central nervous system infections, heart disease, urogenital infection or as chronic localized lesions (WHO, 2006).

Brucellosis is usually diagnosed in the laboratory by means of blood culture or demonstration of elevated level of antibodies or Polymerase chain reaction (PCR).Though isolation of Brucella from clinical specimens remains as the gold standard test, it is a very tedious, time consuming and is often unsuccessful process. Many diagnostic laboratories in the developing countries do not have facilities for PCR for routine diagnosis. (Smita S. Mangalgi *et al.,* 2016) An early diagnosis is necessary for a prompt and effective treatment and one has to relay on serological diagnosis, hence it is the most widely used diagnostic means.

There is presently a large number of serological tests, which can be used for diagnosis of human brucellosis, although they each have important limitations. Rose Bengal plate test (RBT), standard tube agglutination test (STAT) are commonly used tests for diagnosis human brucellosis other tests includes Complement fixation test (CFT) and the enzyme-linked immunosorbent assay (ELISA).

The Rose Bengal plate test is a rapid agglutination test which was designed originally for screening use in veterinary medicine, but is now often used for the diagnosis of human brucellosis. Brucel-RB slide screening test is a type of RBPT test. Because of its simplicity, low cost & rapid results it is used as a screening test in human brucellosis and would be optimal for small laboratories with limited means. (Avinash Reddy *et al.,* 2014) Because of occurrence of few false positive results STAT is preferred over RBT. However STAT is relatively complicated, time consuming, and requires qualified personals to perform. Hence the present study is carried out to assess the diagnostic yield of the RBT for the diagnosis of human brucellosis. Objective of this study is to estimate the seroprevalence of human brucellosis in and around Chitradurga.

Materials and Methods

This cross-sectional study was done on 62 blood samples received at Microbiology Department, Basaveshwara medical college and hospital, Chitradurga over a period of three years (January 2017- December 2019). Age, sex, occupation and other demographic details of patients and clinical features were extracted from medical records.

Inclusion criteria

Blood samples collected from clinically suspected of brucellosis and various high risk group individuals.

Exclusion criteria

Patient's sera which were positive for various tests like WIDAL, VDRL, RA Factor and ASLO were excluded from the study.

Serum was separated from blood sample and subjected to the Rose Bengal test(RBT) with commercially available Brucel-RB test kit manufactured by Tulip diagnostics(P)LTD. India, a slide screening test for brucella antibodies. (according to manufacturer's instructions. Brucel-RB reagent contains smooth, killed buffered

suspensions of strain 99, coloured with rose bengal, standardized against the 2nd Intenational preparation of anti *Brucella abortus* from NIBS (UK) (WHO). Because of its formulation in an acid buffer, the test reagent is reactive with both IgM & IgG antibodies & very useful for the diagnosis of chronic individuals, which present with a high level of IgG antibody.

Procedure

Qualitative method

For this test 50 µL of plain serum is dispensed on a clean glass slide and mixed with an equal volume of RBT antigen (previously equilibrated at room temperature and shaken to resuspend any bacterial sediment) using mixing sticks. The slide is then rotated gently back and forth observed for agglutination macroscopically at 4 minutes against a white background. Any degree of visible agglutination was taken as positive and no agglutination was taken as negative. With each set of test sera, a known positive and negative control sera were also included. Positive test result indicates the presence of antibodies of Brucella in concentration ≥25 IU/ml in the patient serum.

Semiquantitative method

Positive sera are tested further by semiquantitative method. Titrations were made by serial two fold dilutions with saline solution. 50 µL of saline are dispensed on the tile and the first one is mixed with an equal volume of the positive plain serum (1/2 serum dilution). Then, 50 µL of this first dilution are transferred to the second drop with the help of a micropipette and mixed to obtain the 1/4 dilution. From this, the 1/8 to 1/128 dilutions are obtained by successive transfers and mixings taking care of rinsing the pipette tip between transfers.

Finally, each drop is tested with an equal volume (50 µL) of the RBT reagent. Using separate mixing sticks, contents of each dilution was mixed and observed for agglutination. With each set of test sera, a known positive and negative were also included. The titre is defined as the highest dilution showing agglutination. The approximate antibody concentration in the patient sample is calculated as follows : 25 X anti-Brucella titre= IU/ml

Statistical analysis

Data analysis was carried out using Statistical Package for the Social Sciences and Microsoft Excel.

Results and Discussion

Out of 154 blood samples received for serological evaluation of Brucella 89 serum samples included in the study. Thirteen samples were found to be positive for RBT slide screen test and 76 samples were found to be negative. The overall seroprevalence of human brucellosis in our study was 14.6 % (13/89).

Majority of the patients were in the age group of 21-39 years. Seroprevalence was slightly higher among males 10.11% than females 4.49%. RBPT semiquantitative test titres of 1:2 were detected in majority of samples i.e38.4% (5/13) followed by 1:4 & 1:8 titre in 3 each samples and 1:16 titre in two samples.

Brucellosis is one of the important zoonotic diseases in India and continues to be a major public health concern globally. Clinical diagnosis is difficult due to misleading nonspecific manifestations and unusual presentation and requires laboratory testing for confirmation.

Although culture is considered gold standard test for diagnosis it requires long time for isolation, associated with false negative results and has a great risk for the laboratory staff (Dıaz *et al.,* 2011; Gupte and Kaur, 2015). Serodiagnosis remains an accessible and inexpensive method for the detection of brucellosis. As a first-line method we can adopt serological tests routinenly for screening and confirmation of brucellosis.

In the current study 89 samples were tested by Rose Bengal test for brucella antibody, 13 were positive with this qualitative slide agglutination method and the seroprevalence of brucellosis 14.6%. Slight higher seroprevalence observed in our study as compared with the studies conducted by Avinash Reddy *et al.,* (2014).

Although many studies have confirmed the high sensitivity of the rose Bengal test, information about its specificity is scarce (Geresu and Kassa, 2016; Mantur *et al.,* 2014; Aicha Qasmaoui *et al.,* 2021).

False positive reaction in RBT may occur due to Cross reacting antibodies which are seen in Francisellatularensis, E.coli O :116 and O :157, Salmonella urbana O:30, Yersinia enterocolitica O:9, Pseudomonas matophilia and Vibrio cholera infections (Corbel *et al.,* 1984). The true prevalence of human brucellosis is difficult to estimate.

Many cases are under-reported because many times they remain undiagnosed or misdiagnosed as pyrexia of unknown origin because either they are inapparent or of their atypical manifestations. (Thakar and Thapliyar, 2002).

In our study positive cases were present in all age groups and disease was more commonly seen among 21-39 years age group.

Demographics of positive brucellosis show a slight difference among two sexes. We noticed 10.1% of males and 4.4% of females diagnosed positive for Brucella.

The present study correlates well with studies done by Metri Basavaraj *et al.,* (2011); Avinash Reddy *et al.,* (2014) and Thakar and Thapliyar (2002) which show that prevalence is more among males than in females.

Fever with no apparent clinical features was present in 8/13(61.5%) patients. Ruiz-Mesa *et al.,* (2005) also reported 66.7% fever, comparable with our study. RBPT semiquantitative method titres of 1:2 were detected in majority (38.4%) of samples. Quantitative RBPT demonstrates sensitivity & specificity equivalent to that achievable by performing STAT. RBT is therefore, recommended for routine use in laboratories as an accurate & speedy diagnostic tool (Aicha Qasmaoui *et al.,* 2021).

In humans, consumption of contaminated food and occupational contact are the main risks of infection (WHO, 2006; Aicha Qasmaoui *et al.,* 2021).

In our study, Out of 13 positive cases 69.2 %(9/13) known to have history of animal contact and living in rural areas. Our results seem to be consistent with other similar investigations (Smita S. Mangalgi *et al.,* 2016; Metri Basavaraj *et al.,* 2011; Avinash Reddy *et al.,* 2014). Shepherds and farmers were found to be largely affected. This has also been reported in several studies (Satyajeet K Pawar *et al.,* 2012; Abel B. Ekiri *et al.,* 2020).

Therefore, the best way to prevent brucellosis infection is to avoid eating undercooked meat and unpasteurized dairy products, including milk, cheese and ice cream. Pasteurization of dairy products for human consumption is important to prevent disease.

Vaccines for use in humans are not yet available, so the eradication and control of human brucellosis depends on the eradication of Brucella species from cattle, goats, pigs and other animals (Aicha Qasmaoui *et al.,* 2021).

Methods of control and prevention of this disease in humans depend heavily on the accuracy of diagnostic tools and on the use of diagnostic methods that are robust, reliable, sensitive, specific, and rapid. And the implementation of effective and safe vaccination programs and improved hygienic level and require sustainable collaborations for a single health, collaboration between the animal and human sectors with the sensitization of the population (WHO, 2006; Aicha Qasmaoui *et al.,* 2021; Abel B. Ekiri *et al.,* 2020)

Table.1 Age distribution and RBT test results of serum samples

Total no of samples tested	RBT Positives n (%)	RBT Negatives n (%)
Male- 68	9(10.11)	59(66.23)
Female – 21	4(4.49)	17(19.1)
Total- 89	13(14.6)	76(85.4)

Table.2 Age-wise distribution of Brucella patients

Age Group years	Positive Cases (13)	Percentage %
<20	1	7.6
21 – 39	7	53.8
40 – 60	3	23.1
>60	1	15.3

Table.3 Results of RBT test and titre and age of patients

S. No.	Age years	Qualitative RBT	Semi-quantitative RBT (titre)
1	19	Positive	1:2
2	24	Positive	1:2
3	27	Positive	1:4
4	29	Positive	1:8
5	29	Positive	1:2
6	32	Positive	1:8
7	34	Positive	1:4
8	34	Positive	1:8
9	38	Positive	1:16
10	42	Positive	1:4
11	46	Positive	1:16
12	51	Positive	1:2
13	62	Positive	1:2

Limitations

In our study we noticed under-reporting of cases that remained undiagnosed which results in an underestimation of reported cases. It is therefore necessary to establish a close collaboration between the laboratory and the clinician for a good diagnosis.

The present study included RBPT screening test only addition of confirmatory tests is essential for definitive diagnosis. In view of high seroprevalence among high risk health care workers, nonavailability of effective human vaccines, risk of bio-war threat, so it should develop effective public health strategies.

Regular screenings for brucellosis and awareness programs are necessary to control brucellosis in occupationally exposed groups.

Alertness of clinicians and close collaboration with microbiologists are essential to diagnose and treat brucellosis. Serodiagnosis is an accessible and inexpensive method in the detection of brucellosis whose sensitivity and specificity vary.

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