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Original Article

SEROPREVALENCE OF HUMAN BRUCELLOSIS AMONG THE HIGH-RISK POPULATION

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ABSTRACT

Objective: Brucellosis is a zoonotic disease of worldwide distribution and has great economic concern. It is a contagious disease of ruminant animals but also effects human beings. The duration of the disease can vary from a few weeks to many months.

Methods: A total number of 200 samples tested for RBPT and STAT by using phenol saline as diluent to know the IgG titre and 2-mercaptoethanol was used as diluents to know the IgM titre. ELISA test performed for all positive samples in RBPT, to know the presence of IgM antibody. All the results were analyzed statistically.

Results: Of the 200 serum samples, highest proportion of positive cases were slaughter house worker 21.05% distribution according to positivity of RBPT and STST highest proportion in slaughter house workers 13.5% and lowest proportion in PUO cases 6.97%.

Conclusion: Prevention of human brucellosis focuses mainly on elimination of infection among farm animals. Cooperation is recommended between public health and veterinary officials to overcome the failure of controlling disease among both animals and humans.

Keywords: Human brucellosis, RBPT, STAT, ELISA, and IgM antibody

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INTRODUCTION

Brucellosis is one of the world's major zoonosis that continues to be public health and economic concern in many parts of the world [1]. It is transmitted directly or indirectly to humans from infected animals to humans by direct contact or by consumption of raw milk infected with Brucella [2]. Brucella organism shed in milk, urine and vaginal discharge, thereby contaminate environment. The infection occurs through the ingestion of raw milk of infected animals, contact with vaginal secretion, urine, faeces and blood of infected animals through un-breached skin and mucous membrane of conjunctiva and also by inhalation. Other modes of transmission are travel from or to endemic zones, person-to-person transmission through blood transfusion, organ transplant, and bone marrow transplant [3]. Brucellosis is an infectious zoonotic disease of various animals and humans caused by Brucella species. It is a contagious disease of ruminant animals but also affects human beings [4].

Brucellosis is also known as "undulant fever," "Mediterranean fever," or "Malta fever." David Bruce (1887) a pathologist working in the British army, first isolated the organism during Malta fever outbreak among British soldiers. He also established the linking of fever in brucellosis patients due to the consumption of unpasteurized goat milk. Alice Evans, an American microbiologist suggested the name "Brucellosis" in 1918 in honor of Bruce [5, 6].

About 69% of the Indian population live in approximately 649,481 villages where people are in close approximation with cattle. The development of agriculture and incorporation of animal husbandry is responsible for the proximity of man and animals even today in many developing countries, including India. This proximity has led to the transmission of various zoonoses, including brucellosis from animals to man [7]. The prevalent species involved in human disease are *Brucella melitensis* and *Brucella abortus*; the former species is responsible for severe and prolonged disease with resultant disability [8]. The duration of the disease can vary from a few weeks to many months. Veterinarians are usually infected by inadvertent inoculation of animal vaccines against Brucella melitensis and Brucella abortus [9]. Its clinical manifestations and focal complications are often troublesome in making a clinical diagnosis. Its diagnosis, therefore requires microbiological confirmation by means of isolation from blood or demonstration of the presence of specific antibodies by serological tests [10, 11].

Brucella acts as a potential agent of bioterrorism and remains on the category B biodefense research list of both the Centers for Disease Control and Prevention and the National Institute of Allergy and Infectious Diseases. Its propensity for airborne transmission and induction of chronic debilitating disease requiring combined antibiotic regimens for treatment, its abundance around the world and its vague clinical characteristics defying rapid clinical diagnosis are some of the characteristics that apply to the pathogen's weapons potential [12]. *B. melitensis* and *Brucella suis* are developed as biological weapons in several countries [12].

India is an agricultural country and exposure of human beings to animals is quite high [13]. Despite this, very limited studies on brucellosis have been undertaken in an occupationally exposed group. There are very few reports of Brucella in recent years even though it is discovered in 19th century.

AIM

The aim of present study was to know the seroprevalence of human brucellosis and evaluation of seroprevalence of human brucellosis and evaluation of serological tests for the diagnosis of brucellosis.

MATERIALS AND METHODS

Study design

The present study was done in the department of Microbiology, Andhra Medical College, Visakhapatnam over a period of 6 mo from July 2023 to December 2023. The study was approved by Institutional Ethics Committee.

Statistical analysis

Data collected was entered into Microsoft Excel-2010 version. Descriptive variables will be expressed in numbers and percentages. Continuous variables will be expressed as means± standard deviation. Statistical test-Chi square test will used for analyzing qualitative variable and student 't' test for quantitative variable. For all statistical purposes, P value<0.05 was considered statistically significant.

Methodology

Blood samples were collected from different study groups like veterinary doctors, veterinary staff, slaughter house workers, dairy farm workers, patients with pyrexia of unknown origin and blood donors. Consent was taken from the entire study group.

Processing of samples

The cross-sectional study was carried out in Serology section of the Department of Microbiology. A total number of 200 blood samples were collected. Among them 119 were veterinarians, 38 were slaughter houses and dairy farm workers and 43 were PUO cases. A detailed history of individuals was taken which included the name, age, history of consumption of raw milk, history of fever in the past and complaints of joint pains, if any.

For all the blood samples classical Rose Bengal test was performed. Standard tube agglutination test was done for all positives in RBPT by using phenol saline as diluent to know the IgG titre and 2-mercapto ethanol was used as diluents to know the IgM titre. ELISA test was performed for all positive samples in RBPT, to know the presence of IgM antibody [14]. All the results were analyzed statistically.

RESULTS

A total number of 200 patients were recruited during the study, of which they are categorized into three groups like: veterinarians 119, slaughter house workers and dairy farm workers 38 and patients with Pyrexia of unknown origin 43. The age group ranges from 21-60 years. Majority of positive samples are seen in age group of 31-40 y with male preponderance

Distribution of cases according to positivity of RBPT and STAT, highest proportion of positive cases in slaughter house and dairy farm workers (13.5%) and lowest proportion in PUO cases (6.97%). The prevalence of brucellosis by 2 Mercaptoethanol Standard tube agglutination test was highest in slaughter house and dairy farm workers (7.89%) and lowest in PUO cases (2.32%). Distribution of cases according to results of IgM ELISA, highest proportion of positive cases was present in slaughterhouse and dairy farm workers (21.05%) and lowest prevalence rate in PUO cases (6.97%).

Results are analyzed by using chi-square test. P value> 0.05 for RBPT, STAT, STAT with 2 ME. So, the difference in the positivity in all these is not significant among the occupational groups. P value<0.05 for IgM ELISA. So, IgM ELISA test positivity is significant among occupational groups.

Table 1: Positivity of serological tests among patient groups

Group	Number (n= 200)	RBPT+ve	%	STAT with phenol saline	%
Group I: Veterinarians	119 (59.5%)	10	8.4%	10	8.4%
Group II: Slaughter house workers	38 (19%)	5	13.5%	5	13.5%
Group II: PUO's	43 (21.5%)	3	6.97%	3	6.97%

Table shows highest positivity of RBPT (13.5%), STAT (13.5%) in slaughter house workers.

Table 2: Positivity of serological tests among patient groups

Group	Number (n= 200)	STAT with 2ME+ve	%	IgM ELISA	%
Group I: Veterinarians	119 (59.5%)	8	6.72	11	9.24
Group II: Slaughter house workers	38 (19%)	3	7.89	8	21.05
Group II: PUO's	43 (21.5%)	1	2.32	3	6.79

Table shows highest positivity of STAT with 2ME (7.89%) and IgM ELISA (21.05%) in slaughter house workers.

DISCUSSION

Worldwide millions of persons are at risk of acquiring brucellosis, especially in developing countries, where the infection in animals has not been under control, and mismanagement of animal quarantine eradication of infected animal [15]. It has been estimated that the incidence in humans ranges widely between different regions, with values of up to 200 cases per 1 lakh populations. Clinical picture of brucellosis in man is very heterogenous and nonspecific which may be represented in both subclinical and atypical infection either in acute or chronic stage [16, 17]. So, the diagnosis of brucellosis requires laboratory confirmation or isolation of the pathogen or determination of specific antibodies [18].

Furthermore, handling of these microorganisms represents a high risk for laboratory personnel [19]. The most widely used serological tests for diagnosis of brucellosis are agglutination tests; however, indirect enzyme-linked immune sorbent assay (iELISA) was documented as the most sensitive test [20]. The highest incidence of brucellosis was reported in this study by IgM ELISA (11%) may be due to occupational exposure among veterinary staff and handling of animals among the rural group. Higher prevalence rates were reported by Modak D., *et al.* 2024 (15.8%) [21]. However lower rates were detected by Mousa *et al.* (0.08%) [22] Dajani *et al.* (0.04%) [23] and Shome R *et al.* (0.92%) [24].

The detection of specific IgM antibody is important to diagnose brucellosis in the early phase. IgM antibodies were estimated in 200 screened cases (11%). These findings are similar to that reported by Shukla *et al.* 2022 (11.37%) [25] Soliman *et al.* 1998 (10.9%) [2] Mrunalini N *et al.* 2004 (11.5%) [26]. The present study was divided into 3 groups among them veterinary staff are in high proportion and PUO cases are in lower proportion. In this study age distribution of group 1 veterinarians, group 2 slaughterhouse workers and dairy farm workers, group 3 pyrexia of unknown origin cases were from 21 to above 60 y. Regarding the prevalence of brucellosis among different age groups, there is highest percentage of patients with age 31-40 y (35.5%) and the lowest percentage of patients with age 51-60 y (11.5%) by agglutination tests. However, the same prevalence by ELISA in both the age groups of 31-40 y correlated with studies of Shukla *et al.* (2022) [25]. In study conducted by Agasthya *et al.* (2007) [15]. The highest prevalence was found among 41-50 y age group (45.36%) and the lowest prevalence was found among 21-30 y (7.21%) and Study conducted by Modak D., *et al.* (2024) [21] found that highest prevalence rates was found among 51-60 y (23.5%) and lowest prevalence was seen in age group of 21-30 y (8.8%) 22 patients were brucella positive by IgM ELISA. In that (12.28%) were males and (6.89%) were females. The seropositivity is higher in males compared to females in this study due to higher exposure to risk factors. But the study conducted by Modak. D. *et al.* (2024) [21] reported high positivity in females (14.8%) than males (10.9%). In this study, a smaller number of females was taken than males. So, difference in seropositivity between males and females is statistically not significant.

In this study 200 patients were tested by RBPT, 18 Samples (9%) were positive. The study was correlated with study of Sharma H. K. *et al.* (9.91%) [27]. Study conducted by Modak D. *et al.*, [21] reported higher positivity of RBPT test results (18.5%). Cifti C. *et al.* (2005) [10] compared slide agglutination, standard tube agglutination test and comb tube agglutination test. Kumar P *et al.* (1997) [28] compared the serum samples by using slide agglutination test and standard tube agglutination test. He stated that slide agglutination was positive in 12.75%.

In our study the Rose Bengal plate test was positive in 9%. It is less than that of IgM ELISA (11%) used in our study. This was consistent with the findings of Sharma H. K. *et al.* [27] (9.91%, 16.52%). 200 serum samples were tested and a significant antibody titre of>160 IU was detected in 18 samples (9%). In kumar P *et al.* (1997) [28] study the standard tube agglutination test was positive in 50.30% samples. In other studies, like that of Modak D. *et al.* (2024) [21] showed that STAT was positive in 15.5% among the serum samples. Our study used STAT and detected lesser number of positive cases when compared to ELISA IgG and IgM. This conquers agreement with the results of Pathak A. *et al.* (2014) [29] and Sharma HK *et al.* (2016) [27].

IgM antibodies were estimated in 200 screened cases (11%). These findings agree with that reported by Diaz *et al.* (1991) [11] and Ariza *et al.* (1992) [30]. Annapurna SA *et al.* (2012) [14]. Agasthya *et al.* (2011) [15] compared Brucella indirect ELISA test with RBPT and STAT. In this study, by indirect ELISA detected 20 samples positive (3.6%), which are negative by RBPT and STAT. M. O. Gad EL-Rab *et al.* (1998) [31] compared Brucella ELISA test with Brucella culture and STAT. In his study IgM ELISA detected lesser number than other serological techniques.

Our results revealed that the prevalence of brucellosis was significantly higher in rural areas (13.07%) than in urban areas (7.14%). These findings coincide with that reported by Kumar A. *et al.* (2010) [32], Kavi A. *et al.* (2015) [33] and Mangalgi SS. *et al.* (2015) [34] who concluded that the higher prevalence in rural areas may be due to close contact of individuals with livestock. This is a concordance between that results of IgM ELISA with RBPT and STAT with only insignificant difference of 2%.

In controls among 50 individuals 1(2%) had RBPT positive>160IU in standard tube agglutination test. 22 were positive by IgM ELISA. Of these, 18 had significant antibody titre of>160 IU by standard tube agglutination test also. Geographical variation was found between different regions.

CONCLUSION

This study was done to diagnose the brucellosis in high-risk groups. Persistence of animal reservoir of infection, low physician awareness, poor availability of diagnostic facilities and non-existence of regional databases contributes towards the perpetuation of the zoonosis in India. The cases of brucellosis may be easily misdiagnosed because of the deceptive nature of the clinical signs and symptoms. High degree of cure rate can be achieved by treatment, which is otherwise having high degree of mortality and morbidity. Prevention of human brucellosis focuses mainly on elimination of infection among farm animals. Cooperation is recommended between public health and veterinary officials to overcome the failure of controlling disease among both animals and humans. IgM ELISA antibody detection is the sensitive and specific test of choice in the diagnosis of patients with acute brucellosis.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

The author(s) have no competing interests for financial support, publication of this research, patents and royalties through this collaborative research. All authors were equally involved in discussed research work. There is no financial conflict with the subject matter discussed in the manuscript.

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