

Original Research Article

Incidence and sero epidemiology of brucellosis from a tertiary care centre of rural Maharashtra

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	International Archives of Integrated Medicine, Vol. 2, Issue 8, August, 2015. Copy right © 2015, IAIM, All Rights Reserved. Available online at http://iaimjournal.com/ ISSN: 2394-0026 (P) ISSN: 2394-0034 (O)
	Received on: 29-07-2015 Accepted on: 05-08-2015 Source of support: Nil Conflict of interest: None declared.

Abstract

Background: It is usually difficult to diagnose brucellosis clinically in the absence of specific clinical features. Hence serological testing forms the mainstay of diagnosing the disease. Seroepidemiological determinants of brucellosis in rural western Maharashtra have not been closely investigated.

Aim: The present study was therefore conducted to determine the incidence and to analyze seroepidemiological determinants of Brucellosis in cases of pyrexia of unknown origin (PUO) in rural western Maharashtra.

Material and methods: The present hospital based cross sectional survey was carried out in Rural Medical College, Loni on 500 cases of PUO. SPSS version 20.0 was used for analysis. The serum samples were subjected to serological tests like Rose Bengal Plate Test (RBPT) and Standard Tube Agglutination Test (STAT). The inoculated agar plates were watched daily for the presence of growth *Brucella* on culture.

Results: Males (51.6%) outnumbered females (48.4%) in the study sample. Out of 500 cases 10 samples showed the presence of *Brucella* agglutinins. The male female ratio in the seropositive cases was 2.33:1. Headache and joint pain was observed in 5 and 3 cases respectively. 50% samples yielded the growth of *Brucella* on culture. All the culture positive samples had titer of 640 IU or more.

Conclusion: Agglutination test if properly performed can be used as a very dependable laboratory procedure for rapid diagnosis of Brucellosis.

Key words

Brucellosis, Incidence, Rose Bengal Plate Test, Standard tube agglutination test, Epidemiology.

Introduction

Brucellosis is one of the world's major zoonosis that continues to be of public health concern across the globe including India [1]. A marvellous characteristic of the disease is its protean presentations but it is not rare for the disease to present with nonspecific clinical features. It is usually difficult to diagnose clinically in such cases and serological testing forms the mainstay of diagnosing the disease [2].

It is well known fact that early recognition of brucellosis bears impact on patient management and prognosis. Early diagnosis is a key to improved outcome [3]. Brucellosis is diagnosed in the laboratory by means of blood culture or demonstration of elevated level of antibodies. Since one may fail to isolate *Brucella* from the blood even at the end of 6 weeks due to various reasons [3, 4], a positive diagnosis usually depends on clinical and serological data [5, 6].

The challenges encountered while assessing incidence of brucellosis vary from place to place depending on the geographic terrain and occupational milieu. Hardly any study has been undertaken in rural areas of Maharashtra on brucellosis and thus information on the same is patchy and scanty. According to best of our knowledge incidence and sero epidemiology of brucellosis in rural Maharashtra has not been closely investigated by the experts in the field. Therefore, it was planned to conduct a study with the objective of determining the incidence of Brucellosis in cases of pyrexia of unknown origin. An additional objective was to analyze sero epidemiology of Brucellosis.

Material and methods

The present hospital based survey was carried out in Rural Medical College, Loni targeting the 500 cases of PUO over a period of two years from June 2009 to May 2011 from rural western Maharashtra. Purposive sampling was adopted in the current study and a sample size of 500 was considered adequate to achieve the stated objectives.

Study strategy and proforma were framed under supervision and consultation with the experts. The proforma was designed in English initially and later translated in Marathi and retranslated to English to check validity of questions contained. Subjects were informed about the scope and nature of the study and were fully assured strict confidentiality. Minor changes were made in the proforma following the pilot study. All those who did not give informed consent for participating in the study were excluded from the study. Permission from Institutional Review Board was sought before the commencement of the present study.

Relevant information such as age, sex, occupation, religion of subjects was captured. Blood samples received in Central Clinical Laboratory for various serological test from adult patients with provisional diagnosis of PUO were included in the study. Blood samples were allowed to clot at room temperature for half an hour, after which serum was separated by dislodging the clot and centrifuged at 3000 rpm for 5 minutes. The serum samples were subjected to serological tests like Rose Bengal Plate Test (RBPT) and Standard Tube Agglutination Test

(STAT). RBPT antigen was obtained from Indian Veterinary Research Institute (IVRI) Izatnagar. 1:80 I.U. or above was considered diagnostically significant for brucellosis in human beings for STAT [7].

10 ml of blood was aseptically collected from ante cubital fossa of patients who showed the presence of anti-brucella antibodies by STAT. Subcultures were made from BHI broth and put on to BHI agar plates (Himedia) in duplicate every 4th day of incubation. At the same time smears were made from the broth and stained by modified cold ZN stain. Presence of acid-fast gram-negative coccobacillary forms was considered to be suggestive of *Brucella* organisms. Plates were incubated for at least six weeks before being discarded as negative [8].

The inoculated agar plates were watched daily for the presence of growth. When growth was observed on the plates the colony characters were noted. One mm, white, round, opaque, convex, raised, smooth colonies which showed Gram negative coccobacilli, oxidase, catalase and urease positive were identified as *Brucella spp.* These isolates were then sent to Indian Veterinary Research Institute (IVRI), Izatnagar for confirmation.

All the proformas were manually checked and edited for completeness and consistency and were then coded for computer entry. Finally they were compiled and summarized. The collected data was entered in Microsoft Excel. Coding of

the variables was done. SPSS version 20.0 was used for analysis. Interpretation of the collected data was done by using appropriate statistical methods.

Results

The present cross sectional study, carried out in the department of Microbiology, Rural Medical College, Loni included a total of 500 cases of PUO. Males (51.6%) outnumbered females (48.4%) in the study sample. Maximum, 14.4% males and 18.2% female cases were observed in the age group of 31-40 years. Occupation wise majority of patients were agricultural workers (39.4%) followed by home-makers (21.0%). Three-fourth of the study subjects (73.6%) were illiterate and out of those educated, majority (91.03%) were educated up to the primary level only.

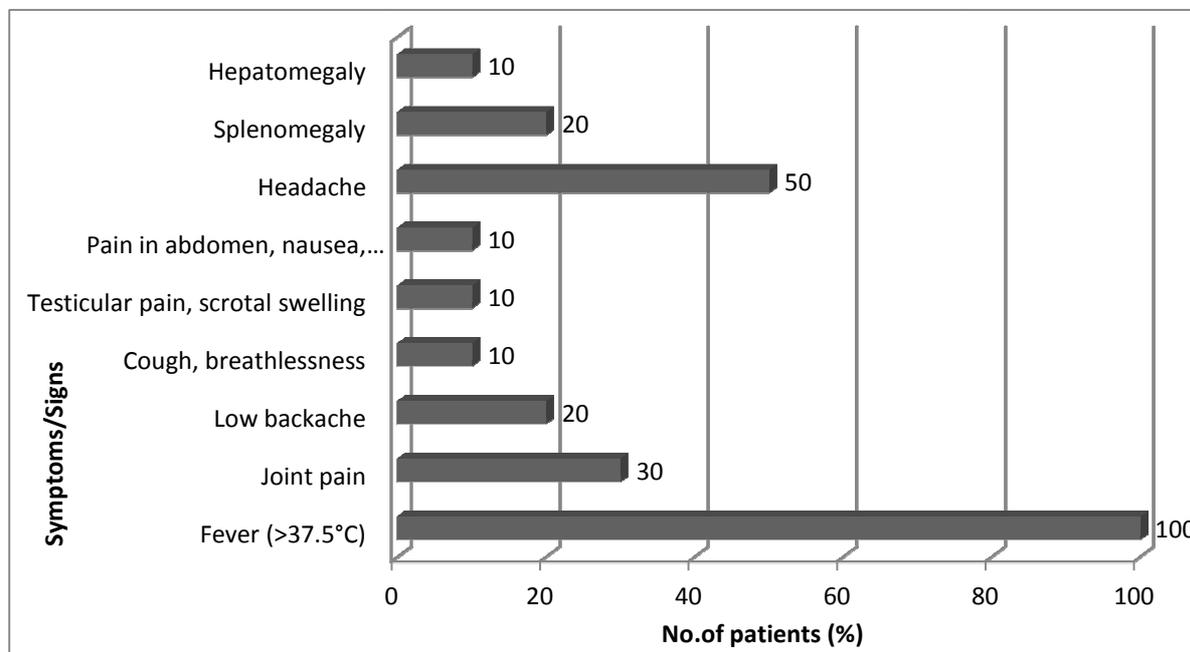
In the present study the prevalence of brucellosis among PUO cases was observed to be 2%. Out of 500 cases 10 samples showed the presence of brucella agglutinins. Maximum number of brucellosis cases found to be in the age group of 20-40 years. Maximum number of males was observed in the age groups of 21-30 years and 41-50 years whereas maximum number of females was seen in the age group of 31-40 years. The male: female ratio in the seropositive cases was 2.33:1. (**Table - 1**) Out of total, 60% of Brucellosis patients (6 cases) were agricultural workers whereas 30% (3 cases) were dairy workers.

Table - 1: Age and gender wise distribution of brucellosis cases.

Age (in years)	Male N (%)	Female N (%)	Total N (%)
>14-20	1 (10)	0 (0)	1 (10)
21-30	2 (20)	1 (10)	3 (30)
31-40	1 (10)	2 (20)	3 (30)
41-50	2 (20)	0 (0)	2 (20)
51 -60	1 (10)	0 (0)	1 (10)
>60	0 (0)	0 (0)	0 (0)
Total	7 (70)	3 (30)	10 (100)

Headache and joint pain was observed in 5 and 3 complaints were least reported by the patients. (Figure - 1) cases respectively. Respiratory illness, gastro-intestinal upset, hepatomegaly and urogenital

Figure - 1: Bar diagram showing clinical findings among diagnosed brucellosis cases.



Comparison among titers of STAT and 2-mercaptoethanol (2ME) was made in positive patients. Out of 10 seropositive cases only 1 patient showed equal STAT and 2ME titer which means only 1 patient had chronic disease, rest of the patients are in conversion phase from acute to chronic phase of the disease.

In this study 5 out of 10 seropositive samples yielded the growth of *Brucella* on culture. (Table - 2) STAT and 2ME titres are compared in positive patients i.e. titre equal to or more than 80 IU. (Table - 3) All the culture positive samples had titer of 640 IU or more. (Table - 4)

Table - 2: Comparison of serological test and bacteriological culture.

Number of patients N (%)	RBPT	STAT		Culture
		Phenol Saline	2-Mercaptoethanol	
05 (1%)	Positive	Positive	Positive	Negative
05 (1%)	Positive	Positive	Positive	Positive
490 (98%)	Negative	Negative	Negative	Negative

Discussion

Brucellosis is a disease of worldwide distribution. It has been reported from almost all the states of India [9]. Human beings are susceptible hosts and acquire the infection by consuming raw infected milk and unpasteurized

milk products. It is often mistaken for typhoid, malaria or may remain undiagnosed as PUO. Besides this, the disease may as well present in a wide range of signs and symptoms, which often lead the disease to go undiagnosed or misdiagnosed. It has been estimated that the true

incidence may be 25 times higher than the underreporting [10].
reported incidence due to misdiagnosis and

Table - 3: Comparison of titer of Standard Tube - Agglutination Test (STAT) and 2-Mercaptoethanol (2ME) in positive patients.

Sr. No	STAT TITER	2-ME TITER
1	80 I.U.	80 I.U.
2	160 I.U.	80 I.U.
3	160 I.U.	80 I.U.
4	320 I.U.	160 I.U.
5	320 I.U.	160 I.U.
6	640 I.U.	320 I.U.
7	640 I.U.	160 I.U.
8	640 I.U.	160 I.U.
9	1280 I.U.	320 I.U.
10	1280 I.U.	640 I.U.

Table - 4: Culture positivity in seropositive cases.

Titer	Seropositive N (%)	Culture Positive N (%)
80 IU	1 (10)	0 (00)
160 IU	2 (20)	0 (00)
320 IU	2 (20)	0 (00)
640 IU	3 (30)	3 (30)
1280 IU	2 (20)	2 (20)
Total	10 (100)	5 (50)

In the present study prevalence of brucellosis in PUO in the region of rural western Maharashtra by serology is 2%. Various studies have showed the seroprevalence of brucellosis in PUO in India to be from 0.8% to 6.8%. The results are comparable with the studies conducted by Balbir Singh with regard to seroprevalence of brucellosis [11]. Low percentage sero-positivity (1.4%) has been reported by Shukla R N, et al. [12]. Roy P B, et al. have reported high percentage (5.9%) sero-positivity [13]. Joshi et al from Delhi and Koshi, et al. from Vellore reported the culture positive results as low as 0.00% and 0.77% respectively [14, 15].

Though there are reports of brucellosis from various parts of India it is just the tip of the iceberg, as many cases remain undiagnosed. The

failure to see cases of Brucellosis in a region is due to the fact that sera of cases of PUO are not routinely screened for brucellosis, due to absence of proper laboratory facilities and lack of awareness among patients and physicians alike [14]. The true reason for under diagnosis is non suspicion of brucellosis by the clinical fraternity. The only way to diagnose the disease is to suspect it. A history of occupational or environmental exposure to possible sources of infection should always be sought.

Among 500 cases, 10 showed the presence of Brucella agglutinins. The Male-Female ratio of seropositive cases is 2.33:1. Vaishnavi, et al. (1.8:1), Thakur S.D., et al. (2:1), Kadri S.M., et al. (3:1), and Randhawa, et al. (3:2) also showed that prevalence is more among males than in

females. The increased incidence in males during the present study may be attributed to the fact that majority of the males are exposed to animals compared to females. This fact also explains the occupational hazard of the disease [16-19].

The prevalence in high risk groups, i.e. agricultural workers was 60%. This is in agreement with the observations of another study from northern India [20]. India is an agricultural country with dairy farming and animal husbandry as one of the major industries. Compared to that, very little work seems to have been done in India. The awareness of the disease in the people involved in veterinary and agriculture profession is also of prime importance. This would help to take necessary preventive measures. It is suggested that the individuals engaged in an occupation which brings them in constant contact with *brucella* infected animals and *brucella* cultures (laboratory workers), should be screened periodically for Brucella agglutinins [14].

Another author studied 250 human serum samples and reported seropositivity in four samples (1.6%), he also suggested that STAT and 2ME together increase the specificity of the test [21].

It was observed in this study that 5 out of 10 seropositive samples yielded the growth of *Brucella* on culture. The failure to isolate *Brucella* from the blood may be due to several reasons ranging from unavailability of organism in circulation at the time of blood collection to technical difficulties and contamination of culture [4, 14]. It is rare to have an insignificant agglutination titer when *Brucella* isolation has been made, though there are some reports in which isolations have been made even when the titers were low and even when serology was negative. This has been explained by presence of blocking antibodies in blood by various workers [3, 14].

Brucella is primarily an intracellular pathogen affecting the reticuloendothelial system.

Brucellae have a special predilection for intracellular growth and may be demonstrated inside phagocytic cells. This accounts for their refractoriness to chemotherapy and the coexistence of viable bacilli with high levels of circulating antibodies [14].

Conclusion

To conclude, findings of the present study demonstrate that the agglutination test if properly performed can be used as a very dependable laboratory procedure for rapid diagnosis of Brucellosis. Many cases of brucellosis are missed by the clinicians because it is not even considered as an alternative diagnosis. Performance of serological test for diagnosis of brucellosis in cases of PUO, low backache, joint pain etc. may increase the detection of the disease.

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