Prevalence of Brucellosis in Patients with Fever of Unknown Origin: A Crossectional Study in a Tertiary Care Hospital

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Abstract: Background: *Brucellosis is an important re-emerging zoonosis with a worldwide distribution. It is a common but often neglected disease in India.*
Objectives: *To detect prevalence of brucellosis in clinically suspected cases of Fever of Unknown Origin and to compare the diagnostic efficacy of ELISA and Conventional blood culture.*
Material And Methods: *Blood samples from 100 suspected cases of fever of unknown origin (FUO) were subjected to Conventional blood culture and ELISA.*
Results: *Out of 100 suspected cases, 6% cases were positive for Brucella Isolates by ELISA whereas none of the blood cultures by Conventional Castaneda technique yielded any isolates. Males in the middle age-group constituted majority (66.66%) of the patients and maximum (83.33%) were from rural background. Risk factor such as contact with Livestock (83.33%) followed by ingestion of raw milk (16.66%) was found to be more significantly associated with Brucellosis. Animal attendants (50%) followed by veterinarians(33.33%) including both doctors and Para veterinarian's staff and dairy farmer (16.6%) dealing with milking and handling of milk products were found to be more prone to brucellosis.*
Conclusion: *ELISA proved as a reliable and fast method to identify the Brucella isolates in suspected patients of fever of unknown origin as compared to conventional blood culture method .*
Keywords: *ELISA- Enzyyme linked immunosorbent assay , FUO- Fever of unknown origin*

I.   Introduction

Brucellosis is the most common zoonotic infection worldwide. The reported incidence of human brucellosis worldwide in endemic areas varies widely, from <0.01 to >200 per lakh population.[1] The true incidence of human brucellosis however, is unknown for most countries including India. The global burden of human brucellosis remains to be more than 500,000 infections per year .Worldwide, brucellosis remains a major source of disease in humans and domesticated animals. In case of FUO, infectious diseases are the major causes of unexplained fever (46.4%) of which enteric fever is the commonest (63.9%), followed by malarial fever (19.4%) and tuberculosis fever (11.1%).[2]The incidence rate of brucellosis in patients of FUO is 132.4 per 100,000cases.[3]

Brucellosis is a professional hazard in agricultural and rural farm workers, veterinarians and slaughter house workers which may affect any organ or system of the body, causing osteomyelitis, hepatomegaly, splenomegaly, lymphadenopathy, meningitis and other central nervous system symptoms, epididymoorchitis and endocarditis. The case fatality rate of untreated brucellosis may be up to 2% & usually result from endocarditis.[4]The disease is accidentally transmitted to humans during occupational contact with infected animals or by consumption of contaminated animal products.[5]The clinical picture of brucellosis is very nonspecific and may, moreover, show great variability.[6] The most common presenting symptom is fever. The intermittent or remittent fever may be accompanied by fatigue, malaise, anorexia, chills, sweats, headache, backache, myalgia, and arthralgia and weight loss. Brucellosis remains an uncontrolled public health problem worldwide. In many developing countries, the problem is compounded by the absence of national surveillance programmes, diagnostic facilities and reliable data.

Blood cultures remain the gold standard test for detecting patients with bacteraemia. Isolation of the organism from blood confirms the diagnosis and enables identification of the cause of infection and administration of adequate antimicrobial therapy from antimicrobial susceptibility testing. Prompt diagnosis and effective treatment is necessary to prevent death and complications. A variety of serological tests have been developed for detection of brucellosis of which Rose Bengal plate test and Standard tube agglutination test are the most widely used. More recently the Brucella ELISA (enzyme linked immunosorbent assay) test has been introduced for detection of Brucella specific IgG and IgM antibodies. ELISA has also become increasingly popular as a well standardized assay for brucellosis. Hence our study is aimed to detect prevalence of brucellosis

in clinically suspected cases of Fever of Unknown Origin and to compare the diagnostic efficacy of ELISA and conventional blood culture.

II.   Material and Methods

This cross-sectional study was conducted over a period of one year from January 2011 to December 2011 at M.M.Institute of Medical Sciences and Research , Mullana. Blood samples from 100 suspected cases of fever of unknown origin (FUO) attending the OPD and indoor were included in the study and investigated in the department of microbiology for brucellosis. Diagnosed cases of fever were excluded.Patients were interviewed using a structured questionnaire to collect demographic and risk factor information. The diagnosis of brucellosis was based on clinical findings and on either positive blood cultures for brucella or the presence of serum antibodies.

2.1 Sample collection and Processing

7ml blood sample was collected aseptically from adult patients and 5ml was injected into Castaneda blood culture bottle containing 50ml of brain heart infusion broth (Dilution 1:10) and 2ml of the remaining sample was collected in a plain bulb. For paediatric patients, 1ml of blood was collected in Castaneda blood culture bottle containing 10ml of brain heart infusion broth and 1ml was collected in a plain bulb and transported to the laboratory immediately. After inoculation, the air in the bottle was replaced by a mixture of air with added 5% CO_2 and the bottles were tilted so that the liquid flows over the solid medium, and then incubated in the upright position in the incubator at 37 ^{o} C and examined every 3 days. Any colonies that appeared in the solid media were subcultured and identified. If no colonies are observed, bottles were tilted again and reincubated, repeating the 3-day cycle for at least 35 days (1, 24, and 25). Futher processing was done by standard laboratory procedures. The negative blood samples were discarded after 35 days of incubation. Serum separated from clotted blood of the patients was used for serological investigations and Brucella IgM ELISA Kit was used for the detection of IgM antibody to Brucella in human serum or plasma.

III.   Results

This cross sectional study was conducted over a period of one year from January 2011 to December 2011. A total of 100 suspected cases of fever of unknown origin were included in this study and investigated for brucellosis by conventional blood culture method and ELISA. Out of 100 cases, Brucella species was found to be positive in 6(6%) cases. None of the blood cultures by Conventional Castaneda method yielded any isolate of Brucella species.

Table 1: Recovery rate of Brucella isolates by Conventional blood culture and ELISA

Total (n)	Conventional blood culture	ELISA
6	0	6

Out of 100 samples of fever of unknown origin, brucella was recovered in only 6(6%) cases and highest rate of Brucella recovery was by ELISA i.e. 6 (100%) whereas no Brucella was isolated by Conventional blood culture.

Table 2: Distribution of Brucella isolates according to Gender, Age and Residence

Parameters	Brucella (n=6)	Percentage (%)
Gender		
Male	4	66.66
Female	2	33.33
Age		
<20	1	16.66
21-30	1	16.66
31-40	1	16.66
41-50	2	33.33
>50	1	16.66
Residence		
Urban	1	16.66
Rural	5	83.33

Males constituted majority (66.66%) of the patients and maximum (83.33%) were from rural background. Maximum patients were found in the middle age group in this study. 33.33% were between 41-50 years, followed by 16.66% in the range of 21- 30 years. 16.66% in 31- 40 years and 16.66% were > 50 years of age.

. **Table 3: Correlation of risk factors in Brucella**

Risk Factors	Brucella (n=6)	Percentage (%)
Contact with livestock	5	83.33%
Ingestion of raw milk	1	16.66%

Risk factor such as contact with Livestock was found to be more significantly associated with Brucellosis i.e. 5 out of 6 (83.33%) cases followed by ingestion of raw milk 1 (16.66%).

Table 4: Occupation wise distribution of Brucella isolates

Occupation	Brucella(n=6)	Percentage(%)
Animal attendants	3	50%
Dairy farmers	1	16.6%
Veterinarians	2	33.33%

Out of 6 Brucella positive cases, 3 (50%) were animal attendants followed by 2 (33.33%) veterinarians including both doctors and Para veterinarian's staff. 1 (16.6%) patient was dairy farmer dealing with milking and handling of milk products.

Table 5: Symptomwise distribution of Brucella positive patients

Symptoms	Brucella (n=6)	Percentage (%)
Fever	6	100
Chills and Rigor	3	50
Myalgia and Arthalgia	2	33.33
Low backache	4	66.66
Joint pain	5	83.33
Night Sweats	3	50
Dry Cough	-	-
Breathlessness	-	-
Nausea, Vomiting	3	50
Anorexia	2	33.33
Headache	3	50
Fatigue	2	33.33
Convulsions	-	-
Weight Loss	2	33.33
Weakness	1	16.66
Malaise	1	16.66

Majority of patients presented with symptoms of Fever (100%) followed by low backache (66.66%) and joint pains (66.66%). 50% patients presented with symptoms of Chills and rigor, Night sweats, Nausea, vomiting and Headache. Rest of the patients had complaints of myalgia (33.33%), arthralgia (33.33%), Anorexia (33.33%), fatigue (33.33%), weight loss (33.33%), weakness (16.66%) and malaise (16.66%).

IV. Discussion

This study shows the recovery rate of Brucella isolates by ELISA and Conventional blood culture method in clinically suspected cases of Fever of unknown origin. Out of 100 cases, Brucella species was found to be positive in 6(6%) cases.The highest rate of Brucella recovery was by ELISA i.e. 6 (100%) and none of the blood cultures by Conventional Castaneda technique yielded any isolates. Similar findings have been reported by Joshi D.V and Prakash O from Delhi and Stephen S et al from Manipal.[7,8] This has been attributed to the empirical antibiotic treatment given to the patients, which could have suppressed the bacteriological evidence.

Males are more prone due to their professional activities[9].Our study showed that the male constituted the majority (66.66%) of the patients followed by females (33.33%). However, children also constitute an important age group for infection of brucellosis in countries where raw goat milk is consumed. Man acquires brucellosis between 20 to 45 years; the active years of life. Maximum patients were found in the middle age group in this study. 33.33% were between 41-50 years, followed by 16.66% in the range of 21- 30 years. 16.66% in 31- 40 years and 16.66% were > 50 years of age.

In our study the risk factor such as contact with Livestock was found to be more significantly associated with Brucellosis i.e 5 out of 6 (83.33%) followed by ingestion of raw milk 1 (16.66%).This is probably because Indians boil the milk before use (even the pasteurized milk is boiled), and if at all raw milk is consumed: it is consumed occasionally once in a way which may not favor the chances of brucella infection However, our findings are in contrast to a study from Bikaner in which consumption of raw milk was associated with brucellosis.[10]

Out of 6 Brucella positive cases, 3 (50%) were animal attendants followed by 2 (33.33%) veterinarians including both doctors and Para veterinarian's staff. 1 (16.6%) patient was dairy farmer dealing with milking and handling of milk products. These findings were similar to the studies done by A.Nazir et al (2006)[11] and Prabhu Prakash et al (2012)[12] who also reported maximum positivity rate in patients with direct contact with infected livestock or with contaminated animal products.

Majority of patients presented with symptoms of Fever (100%) followed by low backache (66.66%) and joint pains (66.66%). 50% patients presented with symptoms of Chills and rigor, Night sweats, Nausea, vomiting and Headache. Rest of the patients had complaints of myalgia (33.33%), arthralgia (33.33%), Anorexia (33.33%), fatigue (33.33%), weight loss (33.33%), weakness (16.66%) and malaise (16.66%). These results are consistent with the studies done by BG Mantur (2007)[6] and Prabhu Prakash (2012)[12] who also reported fever to be the most presenting symptom in patients with brucellosis.

V. Conclusion

In the end, it is concluded that ELISA test offered a significant diagnostic advantage over conventional Castaneda blood culture method in diagnosis of brucellosis in clinically suspected cases of Fever of Unknown origin. Therefore IgM ELISA could be of value in developing countries where diagnostic capabilities for culture including automated culture systems and PCR are poor. Similarly Brucellosis as a possible cause of fever of unknown origin , joint pains or any atypical presentation should be included in differential diagnosis in the clinical practice and immunisation should be mandatory for all meat handlers, milk men and veterinarians.

References

[1]. Corbel MJ. Brucellosis: an overview. Emerg Infect Dis.1997; 3: 213-221
[2]. Refai, M. Incidence and control of brucellosis in the Near East region. Vet. Microbiol.2002; 90: 81-110.
[3]. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos E. The new global map of human brucellosis. Lancet Infect Dis.2006: 6: 91-99
[4]. Yagupsky P. Detection of Brucella melitensis by BACTEC NR660 blood culture system. J. Clin. Microbiol.1994; 32: 1899-1901.
[5]. Colmenero JD et al. Complications associated with Brucella melitensis infection: a study of 530 Medicine cases. Medicine(Baltimore).1996;75:195–211.
[6]. Mantur BG, Biradar MS, Bidri RC, Mulimani MS, Veerappa K, Kariholu P et al. Protean clinical manifestations and diagnostic challenges of human brucellosis in adults: 16 years' experience in an endemic area. J Med Microbiol.2006.; 55: 897-903.
[7]. Jorgensen JH et al. Controlled clinical laboratory comparison of BACTEC Plus Aerobic/F resin medium with BacT/Alert aerobic FAN medium for detection of bacteremia and fungemia. J. Clin. Microbiol. 1997; 35:53-58.
[8]. Agarwal PK, Gogia. A Fever of Unknown Origin.JAPI.2004;52:314-18.
[9]. Wong SY, Lam MS. Pyrexia of unknown origin- approach to management. Singapore Med J.1995;36:204-208
[10]. Kochar DK, Gupta BK, Gupta A, Kalla A, Nayak KC, Purohit SK. Hospital-Based Case Series of 175 Cases of Serologically Confirmed Brucellosis in Bikaner. J Assoc Physician India 2007; 55:271-275.
[11]. Nazir A et al. Diagnosis of brucellosis in patients with pyrexia of unknown origin.Bacteriology CLI Dec 2006;8:19-21.
[12]. Prabhu Prakash et al. Epidemiology of Brucellosis in high risk group & PUO patients of Western- Rajasthan. National Journal of Community Medicine 2012; 3 :61-65.