

Serodiagnosis of Brucellosis in Patients with Fever of Unknown Origin at a Tertiary Care Hospital in Bangalore: A Cross-sectional Study

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ABSTRACT

Introduction: Brucellosis is a zoonotic disease caused by bacteria of the genus *Brucella*. Although it is not commonly transmitted between humans, it can be transmitted through direct handling of cattle or their products, such as raw milk, unevenly heated milk, clotted cream, and cheese, for varying periods. Brucellosis is considered as one of the neglected zoonotic diseases worldwide, as it can cause debilitating acute infections and later become chronic with numerous complications.

Aim: To determine the prevalence of brucellosis among patients with Pyrexia of Unknown Origin (PUO).

Materials and Methods: This cross-sectional study was conducted in the Department of Microbiology at Ramaiah Medical College and Teaching Hospital in Bangalore, Karnataka, India, over a period of two years, from December 2019 to December 2021. The study included patients who visited the Outpatient Department (OPD) and those admitted to the hospital with fever persisting for more than 5-7 days and Fever of Unknown Origin (FUO). The total sample size was 180, and 5-10 ml of blood was collected from each patient under aseptic

precautions from the median cubital vein for serological tests. The blood samples were stored at 2-8°C for the Rose Bengal Test (RBT) assay and Enzyme-Linked Immunosorbent Assay (ELISA). The demographic parameters considered included clinical history, age, gender, residency, and history of animal exposure. Data analysis was performed using the statistical software Statistical Package for the Social Sciences (SPSS) version 18.0, employing the Chi-Square test, Fisher's-Exact test, and descriptive statistics. The significance level employed was set at p-value <0.05.

Results: Among 180 samples, six (i.e., 3.3%) were positive for the *Brucella* RBT (BRBT), while 11 (i.e., 6.1%) were positive for the (ELISA IgM), and 17 (i.e., 9.4%) were positive for the (ELISA IgG). Additionally, 22 samples (i.e., 12.22%) tested positive for either ELISA of IgG or IgM (ELISA IgG/IgM).

Conclusion: The present study identified 22 patients (12.22%) with *Brucella*-positive cases who presented with various clinical signs and symptoms, originating from different geographical locations and regardless of gender. These findings suggest that all cases of FUO presented to a clinician should be evaluated for *Brucella* infection.

Keywords: Enzyme linked immunosorbent assay, Immunoglobulin M and G, Pyrexia, Rose bengal test, Zoonosis

INTRODUCTION

Brucellosis is a neglected zoonotic disease of major public health concern, and its existence or prevalence often goes unnoticed and/or is under-reported [1]. It poses an important occupational hazard for livestock farmers, dairy workers, veterinarians, slaughterhouse workers, and laboratory personnel [1,2]. The clinical manifestations of brucellosis vary and may overlap with other bacterial infections. Therefore, it is crucial to establish an appropriate definitive diagnostic test.

Brucellosis is a disease that affects domestic and wild animals, and it can be transmitted to humans (zoonosis) [3]. The causative organisms belong to the genus *Brucella*, with the species involved in human disease being *Brucella melitensis* and *Brucella abortus*, *Brucella canis* and *Brucella suis*. Primarily, domestic animals such as sheep, goats, cattle, dogs, pigs, and camels are affected [3,4]. Transmission of *Brucella* can occur through direct handling of cattle or their products, including raw milk, inadequately heated milk, clotted cream, and cheese for varying periods [1]. *Brucella* is also present in various animal excretions and products of parturition, which contaminate the soil. Infection can be transmitted through ingestion, inhalation, or contamination of skin abrasions by these animal products [5].

Brucellosis is characterised by protean clinical manifestations, although it commonly presents as a FUO and can lead to

complications such as neurobrucellosis, arthritis, endocarditis, and respiratory tract infections [3].

Brucellosis can affect all body tissues and systems and is sometimes asymptomatic. If not promptly treated, it can cause debilitating conditions. Brucellosis is a clinically enigmatic disease and is difficult to diagnose based solely on clinical evaluation [6]. Brucellosis may co-exist with other infectious co-morbidities, including syphilis, Human Immunodeficiency Virus (HIV)/ Acquired Immunodeficiency Syndrome (AIDS), malaria, and tuberculosis [7].

Animal brucellosis is highly prevalent in India, but there is a lack of collaboration between veterinarians and clinicians. The lack of awareness among clinicians, low index of suspicion, and unavailability of diagnostic tests contribute to the underdiagnosis of the disease. Although Brucellosis is easily treatable and curable but failure to diagnose leads to chronic morbidity [8]. Additionally, successful treatment depends on selecting the appropriate regimen and duration of antimicrobial therapy based on the presence of focal disease and underlying conditions [9].

Moreover, the clinical manifestations of brucellosis are found to vary or overlap with those of other bacterial infections. Therefore, an appropriate definitive diagnostic test is needed. The aim of this study was to determine the prevalence of brucellosis among patients with PUO. The objective of the study was to compare the diagnosis of

brucellosis using the RBT and detection of IgM and IgG in serum samples by ELISA.

MATERIALS AND METHODS

The present study was a cross-sectional study conducted at the Department of Microbiology, Ramaiah Medical College and Teaching Hospital in Bangalore, Karnataka, India, for two years, from December 2019 to December 2021. Ethical clearance was obtained from the institutional Ethical Committee (IEC number: MSRMC/EC/2017) before initiating the study. Informed consent was obtained, and patient details were collected.

Inclusion criteria: Patients visiting OPD and those admitted to the hospital with a fever lasting for more than 5-7 days or with FUO were included in the study.

Exclusion criteria: Patients diagnosed with chronic conditions such as tuberculosis, HIV, syphilis, and malaria were excluded from the study.

Sample rationale: Based on a previous study by Agasthya AS et al., which reported a brucellosis prevalence of 2.14% with an absolute precision of 2.15% at a desired confidence level of 95%, the estimated sample size was 178 samples [6].

A total of 180 serum samples were collected from patients aged above 18 years, who presented to the hospital with a complaint of fever lasting for more than five to seven days or FUO.

Study methods: Detailed clinical history, along with demographic profiles including age, sex, residency, and history of animal exposure, was collected. Aseptically, 5-10 mL of blood was collected from each of the 180 patients through the median cubital vein for serological tests and stored at 2-8°C.

All blood samples underwent the RBT for the detection of anti-*Brucella* antibodies. Subsequently, *Brucella* ELISA IgM and *Brucella* ELISA IgG tests were performed.

Procedure for RBT: A 30 µL of plain serum was dispensed on a white glossy ceramic plate, and an equal volume of RBT colour antigen obtained from the Institute of Animal Health and Veterinary Biologicals, Bengaluru, was added. One positive and negative control was also included alongside the patient's sera. The plate was then gently shaken on a shaker at room temperature for four minutes [10,11]. Results were observed for agglutination within 2-3 minutes after proper mixing. Samples showing agglutination were considered positive for brucellosis.

Procedure for Brucella ELISA: Brucella IgM and IgG detection was performed using a commercial kit (CALBIOTECH) following the manufacturer's guidelines [12]. The reagents and wells provided in the kit were stored at 2-8°C until analysis. Each ELISA test included one positive control, one negative control, and a calibrator control. The obtained results were interpreted based on the instructions provided in the kit manual. The antibody index for each determination was calculated by dividing the Optical Density (OD) value of each sample by the cut-off value. The interpretations of the results are provided in [Table/Fig-1] [13].

Antibody index	Interpretation
<0.9	No detectable antibody to <i>Brucella</i> IgM/IgG by ELISA
0.9-1.1	Borderline positive.
>1.1	Detectable antibody to <i>Brucella</i> IgM/IgG by ELISA

[Table/Fig-1]: Antibody index interpretation of ELISA.

The interpretation steps are as follows:

1. Check the Calibrator Factor (CF) value on the calibrator bottle, as this value may vary between different kit lots. It is important to check the value for each kit.
2. Calculate the cut-off value: Calibrator OD x CF.
3. Calculate the Antibody (Ab) Index for each determination by dividing the OD value of each sample by the cut-off value.

STATISTICAL ANALYSIS

The data was analysed using the statistical software SPSS (SPSS Inc., Released 2009. PASW Statistics for Windows, Version 18.0, Chicago). The Chi-square test was employed to compare the occurrence of brucellosis among cases with PUO across different age groups and genders. In cases where the assumptions for the Chi-square test were not met, adjacent row values were combined, and the test was applied. If the Chi-square test assumptions failed in a 2x2 table, Fisher's-exact test was utilised. Descriptive statistics of the results for BRBT, ELISA IgM, ELISA IgG, as well as ELISA IgM or IgG, were summarised as percentages. A significance level of p-value<0.05 was considered statistically significant.

RESULTS

A total of 180 cases of FUO were included in the study. The number and percentage distribution of brucellosis-positive cases among all FUO cases are presented in [Table/Fig-2].

Distribution	Frequency		Percentage (%)	
	Number	Total	Number wise	Total
History				
Occupational exposure	1	22	4.55	100
Exposure not known	21		95.5	
Geographical habitat				
Rural	15	22	68.2	100
Urban	7		31.8	
General signs and symptoms				
Fever	22	22	100	100
Other general signs and symptoms				
Generalised weakness	1	4	4.55	18.18
Loss of appetite	1		4.55	
Rashes	1		4.55	
Lymphadenopathy	1		4.55	
Musculoskeletal				
Body ache	2	5	9.09	22.73
Body pains	3		13.64	
Neurological				
Headache	3	4	13.64	18.18
Reduced responsiveness	1		4.55	
Gastrointestinal				
Pain abdomen	2	7	9.09	31.82
Vomiting	4		18.18	
Loose stools	1		4.55	
Pulmonary: Cough/ Breathlessness	2	2	9.09	9.09
		22	100	100
Raised ESR	5 out of 22		22.72 %	

[Table/Fig-2]: Number and percentage distribution of FUO cases positive for brucellosis.

The 180 cases were divided into four age-based subgroups as follows: Group I (<30 years of age, n=59), Group II (30-45 years of age, n=56), Group III (>45-60 years of age, n=35), and Group IV (>60 years of age, n=30). Among the total cases, 103 were males (57.2%) and 77 were females (42.8%).

The number of cases found positive for BRBT in Group I, Group II, Group III, and Group IV were 2 (3.4%), 2 (3.6%), 2 (5.7%), and 0, respectively. While the number of cases found positive in males and females was 4 (3.9%) and 2 (2.6%), respectively [Table/Fig-3].

The total number of RBT positive cases was six, including four males and six females. Therefore, out of 180 cases, only six tested positive for Brucellosis (3.3%) [Table/Fig-3].

Variables		RBT		Total	p-value
		Positive n (%)	Negative n (%)		
Age	Group I	2 (3.4)	57 (96.6)	59	1.00
	Group II	2 (3.6)	54 (96.4)	56	
	Group III	2 (5.7)	33 (94.3)	35	
	Group IV	0	30 (100)	30	
Gender	Male	4 (3.9)	99 (96.1)	103	1.00
	Female	2 (2.6)	75 (97.4)	77	

[Table/Fig-3]: Age and sex-wise distribution of RBT positive among FUO.

Test applied-Chi-square/Fischer-exact, level of significance $p < 0.05$

Likewise, the number of cases found positive for ELISA IgM in Group I, Group II, Group III, and Group IV were 5 (8.5%), 4 (7.1%), 2 (5.7%), and 0, respectively. While the number of cases found positive in males and females was 8 (7.8%) and 3 (3.9 %), respectively [Table/Fig-4].

Variables		IgM		Total	p-value
		Positive n (%)	Negative n (%)		
Age	Group I	5 (8.5)	54 (91.5)	59	0.332
	Group II	4 (7.1)	52 (92.9)	56	
	Group III	2 (5.7)	33 (94.3)	35	
	Group IV	0	30 (100)	30	
Gender	Male	8 (7.8)	95 (92.2)	103	0.512
	Female	3 (3.9)	74 (96.1)	77	

[Table/Fig-4]: Age and sex-wise distribution of IgM positive among FUO.

Test applied-Chi-square/Fischer-exact, level of significance $p < 0.05$

The total number of IgM positive cases was 11, including eight males and three females. Therefore, out of 180 cases, only 11 tested positive for Brucellosis (6.1%) [Table/Fig-4].

Further, the number of cases found positive for ELISA IgG in Group I, Group II, Group III, and Group IV was 4 (6.8%), 4 (7.1%), 4 (11.4%), and 5 (16.7%), respectively. While the number of cases found positive in males and females was 11 (10.7%) and 6 (7.8 %), respectively [Table/Fig-5].

Variables		IgG		Total	p-value
		Positive n (%)	Negative n (%)		
Age	Group I	4 (6.8)	55 (93.2)	59	0.129
	Group II	4 (7.1)	52 (92.9)	56	
	Group III	4 (11.4%)	31 (88.6)	35	
	Group IV	5 (16.7)	25 (83.3)	30	
Gender	Male	11 (10.7)	92 (89.3)	103	0.512
	Female	6 (7.8)	71 (92.2)	77	

[Table/Fig-5]: Age and sex-wise distribution of IgG positive among FUO.

Test Applied: Chi-square/Fischer's-exact test, level of significance: $p < 0.05$

The total number of IgG positive cases was 17, including 11 males and six females. Therefore, out of 180 cases, only 17 tested positive for Brucellosis (9.4%) [Table/Fig-5].

Similarly, the number of cases found positive for ELISA IgM or IgG in Group I, Group II, Group III, and Group IV were 7 (11.9%), 6 (10.7%), 4 (11.4%) and 5 (16.7%), respectively. While the number of cases found positive in males and females was 15 (14.6 %) and 7 (9.1%), respectively [Table/Fig-6].

The total number of IgG/IgM positive cases was 22, including 15 males and seven females. Therefore, out of 180 cases, only 22 tested positive for Brucellosis (12.22%) [Table/Fig-6].

The results of the BRBT, ELISA IgM, ELISA IgG, as well as ELISA IgM or IgG, when compared between different age groups (Group I, Group II, Group III, or Group IV), did not show any statistical significance among the different age groups.

Variables		IgG or IgM		Total	p-value
		Positive n (%)	Negative n (%)		
Age	Group I	7 (11.9)	52 (88.1)	59	0.617
	Group II	6 (10.7)	50 (89.3)	56	
	Group III	4 (11.4)	31 (88.6)	35	
	Group IV	5 (16.7)	25 (83.3)	30	
Gender	Male	15 (14.6)	88 (85.4)	103	0.234
	Female	7 (9.1)	70 (90.9)	77	

[Table/Fig-6]: Age and sex-wise distribution of IgG or IgM positive cases among FUO patients.

The Chi-square/Fischer-exact test was applied with a significance level of $p < 0.05$

DISCUSSION

Brucellosis is a neglected zoonotic disease and one of the causes of prolonged fever in endemic areas and PUO. In developing countries like India, where backyard agriculture is the predominant occupation, people are exposed to livestock and are more prone to infection [14,15]. Since the clinical symptoms of brucellosis are not specific, the diagnosis should be based on the history of exposure, clinical signs, and specific laboratory evidence [4]. In this study, fever was found to be present in all reported cases of FUO. The clinical features of FUO-positive brucellosis varied in terms of occupational exposure, geographical habitat, general signs, and symptoms. Therefore, the frequency and percentage distribution of FUO-positive brucellosis cases in this study.

Brucellosis is endemic, necessitating the use of rapid, sensitive, and highly specific diagnostic methods for early detection and prevention of antibiotic resistance due to overlapping therapies [6,9]. In the present study, the occurrence of *Brucella* positivity was 22 cases (12.22%). Globally, the incidence of brucellosis is estimated to be 500,000 cases per year, with a higher prevalence rate in Western Asia, India, the Middle East, Southern Europe, and Latin American countries [16,17]. Similarly, the prevalence of brucellosis in Meghalaya is reported to be 11.37% according to Shukla JL et al., while the incidence in the Dharwad district of Karnataka was found to be 14.10% by Mohite RS et al., [18,19]. Studies conducted by Naik VR et al., [14] and Gunjal SP et al., [20] showed a seroprevalence of 19.8% and 15.06%, respectively, in patients with febrile illness. The present study revealed a prevalence of 12.22%. Furthermore, among these 22 positive cases, 15 (14.6%) were males, and 7 (9.1%) were females. The higher occurrence of brucellosis in males compared to females is consistent with the findings of Agasthya AS et al., and Kaur A et al., [21,22]. These results emphasise the importance of timely and accurate diagnosis of brucellosis.

In the present study, the results of BRBT, ELISA IgM, ELISA IgG, and ELISA IgG or IgM tests were positive in 6 (3.3%), 11 (6.1%), 17 (9.4%), and 22 (12.2%) cases, respectively, out of 180 cases.

It has been reported that BRBT tests, although rapid screening tests with high sensitivity, should be confirmed by other tests that detect both agglutinating and non agglutinating antibodies [5]. ELISA has demonstrated high specificity and sensitivity and is considered the test of choice for diagnosing patients with brucellosis, particularly those with chronic and Central Nervous System (CNS) infections [23,24]. In this study, all samples that were tested positive with BRBT were also found to be positive with ELISA, indicating that the BRBT test can continue to be used as a rapid screening test in routine practice.

Based on the above, it is also believed that timely and accurate diagnosis with appropriate tests will assist clinicians in alleviating the suffering of patients with prolonged fever and/or FUO by initiating therapy without delay.

Limitation(s)

Raising awareness about herd vaccination, the health hazards faced by animal handlers, timely and accurate diagnosis, and ensuring

that clinicians are knowledgeable about safe livestock practices for patients with prolonged illness and/or FUO are crucial steps in overcoming this zoonotic disease.

CONCLUSION(S)

The present study aimed to evaluate the prevalence of brucellosis among patients with prolonged fever and FUO by utilising *Brucella* RBT, *Brucella* ELISA IgM, and *Brucella* ELISA IgG tests. The findings of the present study indicate that brucellosis is a bacterial disease with diverse clinical signs and symptoms, originating from various geographical regions, and affecting individuals regardless of their gender. Therefore, it is recommended that all cases of FUO presented to clinicians should undergo diagnosis for *Brucella* infection using ELISA, although RBT serves as a rapid screening test.

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