Original Research Article

Breaking the Diagnostic Enigma: Brucella Infection in the Spectrum of Pyrexia of unknown origin at a Tertiary Care Hospital in North India

Abstract

Background: Brucellosis is a neglected zoonotic infection that poses significant public health concerns. It is endemic in various regions of Asia, notably in India. It contributes to pyrexia of unknown origin and has the potential to cause life-threatening multisystem disease.

Objectives: The focus of this work was to diagnose brucellosis by IgM/IgG ELISA

Methods: A total of 94 serum samples were collected and processed for screening of *brucella* by Enzyme-Linked Immunosorbent Assay.

Results: Human brucellosis was prevalent in a study of 94 individuals with suspected Pyrexia of Unknown Origin (PUO), with diverse infectious causes identified. The diagnostic success rate was 61.7%, and Brucella-positive cases (mean age 34.08±15.14) were more common in males (69.2%) and rural individuals (61.5%), particularly farmers/unskilled laborers and housewives. The Orthopedics/Rheumatology ward exhibited a higher prevalence (53.8%), with clinical manifestations including pyrexia, arthralgia, anemia, backache, splenomegaly, hepatomegaly, and thrombocytopenia/thrombocytosis. PCR analysis identified one B. abortus case (1.1%), and serological/molecular analyses revealed distinct patterns, including solely IgG positive (3.2%), solely IgM positive (9.6%), and positivity for both IgM and PCR (1.1%).

Conclusion: The potential for unusual clinical presentation and the low titers of serologic reactivity remind us that brucellosis remains a diagnostic challenge that requires clinical suspicion and thorough evaluation.

Keywords: Brucella, Seroprevalence, IgM ELISA, IgG ELISA, Arthralgia, PUO,

Introduction

Brucellosis, a zoonotic disease, remains a formidable global public health concern impacting animals and humans. It is caused by various species of the genus Brucella *with B. melitensis, B. abortus, B. canis, and B. suis* (except biovar 2) being the primary contributors to human infections [1]. The prevalence of brucellosis varies significantly worldwide, with over 500,000 new cases are reported annually. The incidence ranges from <0.01 to > 200 per 100,000 population in India.[2] Human transmission occurs through direct contact with infected animals, contaminated secretions, and the consumption of unpasteurized dairy

products.[3] In humans, brucellosis manifests with symptoms such as fever and muscle and bone pain, often overlooked globally.

Brucella is an intracellular pathogen. During an infection, it survives and multiplies in macrophages; the bacteria adapt to the acidic pH, low levels of oxygen, and low levels of nutrients [15,16]. Brucellosis adopts a chronic and persistent course, evolving into a granulomatous condition that can impact any organ system. While brucellosis seldom proves fatal in humans, it can result in profound debilitation and long-term disability. Reports indicate that around 2% of untreated patients succumb to the disease.[4]. Clinicians face significant challenges in promptly and accurately diagnosing human brucellosis. The disease presents with non-specific clinical features, and its slow growth rate in blood cultures further complicates identification. Additionally, the complexity of serodiagnosis contributes to the diagnostic hurdles. Thus, this study aims to investigate the seroprevalence of human brucellosis in patients of pyrexia of unknown origin in northern U.P, India.

Material and Methods

Patients of all age groups fulfilling the case definition of pyrexia of unknown origin [5] attending the outpatient or inpatient departments of King George's Medical University, Lucknow, were included in the study. 94 blood samples, which were referred to the Department of Microbiology over a period of one year (2019-2020), along with all the relevant history, were duly noted and analyzed.

Laboratory procedure: All the samples were centrifuged at 3000g for 10 minutes, and then the serum samples were used to diagnose brucellosis. Firstly, ELISA was performed to qualitatively determine Brucella IgG and IgM class antibodies as per the manufacturer's protocol. (NOVATECH Immunodiagnostic GmbHDietzenbach, Germany) then, all the samples were subjected to molecular detection by performing conventional PCR for *Brucella abortus* and *Brucella melitensis.* DNA was extracted from serum samples (100µL) with the High yield DNA Purification kit (QIAGEN Gmbh, Germany), according to the supplier's manual. The known positive culture of *B.abortus and B.melitensis* brought from IVRI Bareilly were taken as positive controls, and their genomic DNA was extracted by boiling culture method as described by Shome et. al. [6]

For conventional PCR, oligonucleotide sequences taken for *B. abortus* & *B. melitensis* were as follows [7]

Forward primer: 5' -TGCCGATCACTTAAGGGCCTTCAT-3' (498bp)

Reverse primer: 5' -GAC GAACGGAATTTTTCCAATCCC-3'

Forward primer: 5' -TGCCGATCACTTAAGGGCCTTCAT-3' (731bp)

Reverse primer: 5' -AAA TCGCGTCCTTGCTGGTCTGA-3'

Conventional PCR

The amplification reaction was carried out in a total volume of 25 µl, which constitutes universal PCR master mix (12.5 µl) (Fischer Scientific Baltics UAB, Vilnius, Lithuania), forward and reverse primer (1.0 µl), nuclease-free water (5.5 µl) and 5 µl of template DNA of the isolates. It was taken in 0.2 ml thin-walled PCR tubes with one positive and one negative control. After sealing the tubes with caps, it was placed into a thermal cycler. The amplification process was started with an initial denaturation step 95°C for 5 min each PCR reaction consisted of 34 cycles, denaturation at 95°C for 1min (*Brucella.abortus*) & 94°C for 1min (*Brucella.melitensis*), annealing at 55°C for 1 minute (*Brucella.abortus*) & 64°C for 1 min (*Brucella.melitensis*), extension for both was done at 72°C for 1 min and final extension was done at 72°C for 7 min for both strains. All bands were detected in 1.5% agarose gel with 0.5 µl/ml of ethidium bromide (0.5mg/ml, Medox Biotech Pvt. Ltd.) with molecular weight marker (100 bp DNA ladder; Bangalore Ganei, India) and PCR products of negative and positive control electrophoretically. A constant current of 100 V was maintained for 1 hour, and amplified DNA was analyzed using UV transillumination at 264 nm wavelength.

Statistical Analysis

The data was analyzed using the Statistical Package for Social Sciences, version 21.0. The chi-square test and the Independent samples t-test were used to compare the data.

Results:

In the current investigation, we explored the seroprevalence of human brucellosis within a cohort of 94 individuals presenting with suspected Pyrexia of Unknown Origin (PUO). Diagnostic assessments were conducted through specific tests, revealing a diverse spectrum of infectious etiologies. Remarkably, the highest prevalence was attributed to Rheumatoid Factor (RF) positivity (19.1%), followed by Dengue (16%), Brucella (13%), tuberculosis (11.7%), Scrub typhus (8.9%), Chikungunya (5.6%), typhoid (3.3%), and Japanese Encephalitis (JEV) (1.4%). Interestingly, none of the cases tested positive for Leptospira, and all blood cultures remained sterile. Among the diagnosed cases, a subset fell under various categories, including endocarditis, blood cancer, brain tumor, chest pain with cough, encephalitis, and renal abnormalities. The diagnostic success rate reached 61.7%, with 58 cases conclusively identified.

Further exploration into the demographics of Brucella-positive cases revealed a mean age of 34.08±15.14, predominantly affecting males (69.2%) and individuals from rural areas (61.5%). Notably, farmers/unskilled laborers and housewives contributed significantly to the brucellosis cases, with 38.4% and 30.8%, respectively. as seen in Table 1

The distribution of brucellosis cases across medical wards indicated a higher prevalence in the Orthopaedics/Rheumatology ward (53.8%, $\chi 2$ =6.36; p=0.012). Clinical manifestations were diverse, with prominent features of pyrexia of unknown origin, arthralgia, anemia, backache, splenomegaly, hepatomegaly, and thrombocytopenia/thrombocytosis.

PCR analysis targeting *B. abortus* and *B. melitensis* species identified one positive case (1.1%) for *B. abortus*, while *B. melitensis* remained undetected. Interestingly, acute cases constituted the majority (76.9%), with 20% demonstrating coexistence with Japanese encephalitis. Serological and molecular analyses revealed distinct patterns, with 3.2% solely IgG positive, 9.6% solely IgM positive, and 1.1% demonstrating positivity for both IgM and PCR. as seen in (figure 1).

DISCUSSION

The outcomes of this study shed light on the intricate landscape of human brucellosis within a cohort presenting with Pyrexia of Unknown Origin (PUO). Our exploration into the prevalence of brucellosis among cases of pyrexia of unknown origin yielded intriguing findings, aligning closely with investigations conducted in North India.[8] The diagnostic success rate of 61.7% indicates the inherent difficulty in pinpointing the precise etiology of PUO, necessitating further refinement of diagnostic protocols. The age-specific vulnerability to brucellosis, particularly among individuals aged 30 to 35 years, underscores the occupational nature of exposure in this demographic. In our study, the higher prevalence among males, rural residents, and individuals engaged in specific occupations, such as farmers/unskilled laborers, suggests potential occupational and environmental risk factors associated with brucellosis. A study by Sharma H.K. et al [9] reported a similar age group prevalence of >20-35 years, with a higher seroprevalence rate (1.99%) in the 30–40-year age bracket, predominantly among males due to their direct livestock contact in rural settings. Our study aligns seamlessly, suggesting increased risks in young adults attributed to their heightened occupational interactions with animals. Another study resonates with the same findings. [10-11] Notably, our study focused on rural areas of Uttar Pradesh, mirroring the observations of D.K. Kochar et al. [12].

The increased prevalence in the Orthopaedics/Rheumatology ward implies a connection between brucellosis and musculoskeletal manifestations, warranting in-depth investigations into the mechanisms. The clinical manifestations observed in brucellosis cases, including pyrexia of unknown origin, arthralgia, anemia, and hepatosplenic involvement, align with classical presentations. Our study echoes the work of Julia E et a al [13] noting fever (83%) as the predominant clinical feature, followed by arthralgia (59.6%), and other manifestations. Overall clinical picture of brucellosis in our study was very similar to that reported by workers elsewhere in the world [14-15]. Serological techniques, despite their limitations, remain essential, with IgM and IgG ELISA assays aiding in distinguishing acute and chronic phases. The seroprevalence of brucellosis in our study was 10.6% and 3.2% for IgM and IgG ELISA, respectively. This corresponds with findings by Renu et al[14] Molecular analysis revealed a low incidence of *B. abortus,* 1 (1.1%) and the absence of *B. melitensis* highlights the need for further surveillance and understanding of regional strain variations. Furthermore, Many of the patients with prolonged pyrexia are empirically treated with antitubercular therapy or antibiotic therapy thus bringing down the sensitivity of PCR. The prevalence of acute cases, coupled with instances of coexistence with Japanese encephalitis, adds a layer of complexity to the clinical presentation, necessitating nuanced

management strategies, particularly in persons belonging to the high-risk category for brucellosis. In some cases, the coexistence of IgM and PCR positivity suggests ongoing active infection, necessitating timely intervention.

The limitation of the study was that it was performed in a tertiary care hospital. Therefore, the present scenario and data do not represent the entire community, and the incidence of brucellosis may be higher; maximum enrolment was done from the hospitalized patients who were likely to have a severe illness. Imaging studies (e.g., spine magnetic resonance imaging) were not routinely used. Patients on antibiotics were also included, which would reduce PCR sensitivity. Being a tertiary care hospital, complications of brucella cases may be overlooked.

Conclusion

This study enhances our understanding of the epidemiology, clinical presentations, and diagnostic challenges associated with human brucellosis in the context of PUO. The ongoing difficulty in pinpointing brucellosis is underscored by its elusive nature, necessitating a multifaceted approach from clinicians. This involves a careful evaluation of clinical symptoms, meticulous laboratory testing, and, in some instances, the use of imaging studies. Swift and precise diagnosis is crucial for initiating timely antibiotic treatment, improving prognosis, and preventing the development of chronic complications.

Ethical approval: Ethical approval was obtained from the Research Ethics Committee of King George's Medical University, Lucknow. University (Ref. No. 97th ECM II B-Thesis/P73).

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Table:1 Association of brucellosis positivity with demographic and clinical profile and outcome

S.No.	Characteristic	Brucellosis Positive (n=13)	Statistical Significance
1.	Mean age± SD (years)	34.08 ± 15.14	t=0.633; p=0.528
2.	**Sex**		
	Male	9 (69.2%)	χ^2 =0.191; p=0.662
	Female	4 (30.8%)	
3.	**Rural residence**	8 (61.5%)	χ^2 =0.298; p=0.585

4.	**Occupation**		
	Farmers/Unskilled labourers	5 (38.4%)	
	Housewives	4 (30.8%)	
	Students	2 (15.4%)	χ^2 =3.552; p=0.615
	Skilled labourers/Vendors	1 (7.7%)	
	Clerk/Shopkeeper	1 (7.7%)	
5.	Orthopaedics/Rheumatology ward	7 (53.8%)	χ^2 =6.36; p=0.012
6.	Pyrexia of Unknown origin	11 (84.6%)	χ^2 =0.029; p=0.866
7.	Arthralgia	8 (61.5%)	χ^2 =0.057; p=0.811
8.	Backache	5 (38.5%)	χ^2 =3.115; p=0.078
9.	Abdominal pain	1 (7.7%)	χ^2 =0.349; p=0.555
9.	Hepatomegaly	4 (30.8%)	χ^2 =3.633; p=0.057
10.	Splenomegaly	5 (38.5%)	χ^2 =6.611; p=0.010
11.	Anaemia	6 (46.2%)	χ^2 =2.239; p=0.135
12.	Thrombocytopenia/thrombocytosis	4 (30.8%)	χ^2 =5.308; p=0.021
13.	Elevated CRP	6 (46.2%)	χ^2 =0.514; p=0.474
14.	Diabetes	0 (0%)	χ^2 =1.403; p=0.236
15.	Thyroid disorder	1 (7.7%)	χ^2 =0.001; p=0.971
16.	RF positivity	3 (23.1%)	χ^2 =0.150; p=0.698
17.	Dengue	2 (15.4%)	χ^2 =0.004; p=0.952
18.	Tuberculosis	2 (15.4%)	χ^2 =0.198; p=0.656
19.	Moratality	1 (7.7%)	χ^2 =0.001; p=0.971

Figure 1: DNA amplified fragments of *B. abortus by* agarose gel electrophoresis and ethidium bromide staining.